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TECHNIQUES FOR STUDYING THE BIOGEOCHEMISTRY OF NUTRIENTS IN THE TAMAR CATCHMENT

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TECHNIQUES FOR STUDYING THE BIOGEOCHEMISTRY OF NUTRIENTS IN THE TAMAR CATCHMENT

by

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ABSTRACT

TECHNIQUES FOR STUDYING THE BIOGEOCHEMISTRY OF NUTRIENTS IN THE TAMAR CATCHMENT

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Chapter One describes nitrogen and phosphorus species in the aquatic environment, their role in eutrophication, current legislation relevant to nutrient water quality and catchment management and the role of predictive modelling of nutrient export with respect to the management of river catchments. It also summarises analytical techniques for the determination of nitrogen and phosphorus species in natural waters and the use of generic ecotoxicological assays to link nutrient water quality and organism health.

Data integrity is essential to biogeochemical studies that inform scientific research and environmental management. Reliable, accurate data permit valid conclusions to be drawn. The quality assurance and quality control measures undertaken to ensure good analytical data in this study, including participation in the certification of a seawater certified reference material for nutrients (MOOS-1), are discussed in Chapter Two. In Chapter Three, the nutrient biogeochemistry of the waters leaving the Tamar catchment and entering the Tamar estuary is discussed. Historical nutrient and physico-chemical data for the Tamar River at Gunnislake were used to identify long-term environmental trends (1974 to 2004) and nutrient and physico-chemical data collected in this study between May 2003 and May 2004 was used to identify short-term trends over the study period.

The nutrient export coefficient modelling approach was used to model phosphorus and nitrogen export from the Tamar catchment (Chapter Four). A TP export model from the Tamar catchment was successfully constructed using historical land use data and catchment demographics, calibrated with hindcasted water quality data, and validated with TP field data (May 2003 and May 2004) collected in this study. Modelled P (43. 5 tonnes P y^{-1}) export agreed within 8 % with the measured P load (40.1 tonnes P y^{-1}). An annual TN model was also constructed and calibrated for the Tamar catchment using the May 2003 to May 2004 field data. The calibrated model agreed within 1 % of the measured TN export (2053 tonnes N y^{-1}).

The development and deployment of a portable FI analyser for continuous, real-time monitoring of FRP in the Tamar catchment is discussed in Chapter Five. The optimised method can be used for the determination of FRP in freshwater systems (4-150 μg L^{-1} P) and in coastal waters (10-150 μg L^{-1} P) and is capable of sampling with high temporal resolution (up to 15 samples h^{-1}). The analyser was used *in situ* (bank-side and shipboard deployment) to provide real-time FRP data and in the laboratory to determine FRP in freshwater samples. All data were in good agreement with values obtained using a validated air-segmented, continuous flow laboratory reference method

The acute toxicity of nitrate and nitrite on the freshwater swan mussel, *Anodonta cygnea*, was investigated (Chapter Six). A 96 h LC50 value of 222 mg L^{-1} N for the exposure of *A. cygnea* to nitrite was established in this study. Toxicity studies indicated that nitrate was not toxic to *A. cygnea*. Established indicators of physiological stress were used to determine the effect of environmentally high and extreme levels of nitrite on *A. cygnea*. There was no significant difference in cardiac activity, condition index or lysosomal stability between control organisms (0 mg L^{-1} N) and organisms exposed to sub-lethal nitrite concentrations (0.1, 1.0, 22.2 mg L^{-1} N). Therefore, nitrite concentrations encountered in typical freshwater catchments such as the Tamar catchment are unlikely to induce physiological stress in *A.cygnea*.

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GLOSSARY

Course fish – freshwater fish found along a fast running watercourse that are not salmonid fish

Diffuse pollution – pollution from widespread activities with no single discrete source, also called non-point sources of pollution.

LC50 – the amount of toxicant present per litre of aqueous solution that is lethal to 50% of the test organism population within the stated study time.

Limit of detection (LOD) – the lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated level of probability (3 std. deviations represents 99 % confidence limit).

Limit of quantitation (LOQ) – the limit of quantitation (LOQ), determined from 10 standard deviations of the slope, is regarded as the lower limit for precise quantitative measurements.

Mean – the value obtained by dividing the sum of a set of quantities by the number of quantities in the set, also called *average*.

Median – relating to or constituting the middle value of a set of numbers or data points in a group of numbers: as many numbers of the group are larger than the median as are smaller; when there is an even number of numerals in the group, the median is usually defined as the number halfway between the middle pair.

Point source – pollution from a discrete source.

Riparian zone – transition zone between land and water environments; they typically consist of vegetated corridors adjacent to stream channels or rivers.

Salmonid fish – large bony fish with two dorsal fins (the second being adipose (without rays)) and body covered with small scales; includes salmon, sea trout and relatives.

Surface waters – water that remains on the earth's surface; all waters whose surface is naturally exposed to the atmosphere, such as rivers, lakes, reservoirs, ponds, streams, impoundments, seas and estuaries.

t-value – a measure of how extreme a statistical estimate is: the hypothesised value is reasonable when the t-value is close to zero; the hypothesised value is not large enough when the t- value is large positive; the hypothesised value is too large when the t-statistic is large negative. Also called the *t-statistic.*

z-score – a comparison of the bias estimate for each analyte, calculated from the difference between the mean analyte concentration.

List of Abbreviations

ATP	adenosine triphosphate
ADP	adenosine diphosphate
AMP	adenosine monophosphate
BSA	bovine serum albumin
EA	Environment Agency of England and Wales
EDTA	ethylenediaminetetraacetic acid
DOP	dissolved organic phosphorus (<0.45 µm) also known as FOP
FOP	filterable organic phosphorus (<0.45 µm) also known as DOP
FRP	filterable reactive phosphorus
GIS	Geographical Information Systems
GQA	General Quality Assessment (Environment Agency of England and Wales)
HELCOM	Helsinki Commission, the governing body of the "Convention on the Protection of the Marine Environment of the Baltic Sea Area"
KHP	potassium hydrogen orthophosphate
MEDPOL	the Mediterranean Research and Pollution Monitoring Plan of the Barcelona Convention
NED	naphythlyethylenediamine
OECD	Organisation for Economic Co-operation and Development
OSPAR	Oslo and Paris Commissions
PN	particulate nitrogen
PP	particulate phosphorus
PTA	phytic acid
QUASIMEME	Quality Assurance of Information for Marine Environmental Monitoring in Europe
STP	sodium tripolyphosphate
STW	sewage treatment works
TDN	total dissolved nitrogen
TDP	total dissolved phosphorus
TN	total nitrogen
TP	total phosphorus
UWWD	Urban Wastewater Directive
WFD	Water Framework Directive

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AUTHOR'S DECLARATION

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EMEC, European Meeting for Environmental Chemistry, Plymouth, 10th to 13th December, 2003. Poster presentation: Integrating analytical chemistry and ecotoxicology for the assessment of water quality.

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CHAPTER 1

Introduction

1.1 Nutrients in natural waters

Nutrient availability to autotrophs (vascular plants, macroalgae, microphytobenthos, phytoplankton) regulates aquatic primary productivity in natural waters (Schlesinger, 1997; Vitousek and Howarth, 1991; Havens et al., 2001). Nitrogen (N) and phosphorus (P), in particular, are most limiting to growth and productivity in aquatic ecosystems (Halstead et al., 1999; Vollenweider, 1968; Hecky and Kilham, 1988, Seitzinger 1988; Smith et al., 1999). Although essential to productivity, excessive nutrient inputs to waterbodies generally lead to increased biomass and can cause alterations in habitat, species composition and, ultimately, ecosystem dynamics (Havens et al., 2001). Surface waters are the main route for transport of nutrients from land-based sources to coastal waters but they may also exhibit symptoms of excessive nutrient inputs (Chapman, 1998; Withers and Muscutt, 1996). As a result, monitoring and managing nutrient inputs to surface waters and their export from river catchments has become a critical part of sustainable resource management and environmental quality. This thesis critically appraises methods of measuring and predicting the N and P species in a freshwater catchment and their export.

1.1.1 Phosphorus

Phosphorus, the eleventh most abundant element in the earth's crust, is classed as a trace element in natural waters (McKelvie, 2000) and is essential for all living organisms. It plays a vital role in the biogeochemical cycles, in the building of nucleic acids and cell membranes, and is contained in the energy-transferring molecules adenosine triphosphate (ATP) and associated compounds of the energy cycle (O'Neill, 1998; Nebel and Wright, 1998).

In the natural waters, phosphorus exists in a variety of forms many of which are highly mobile within aquatic systems (Yaqoob et al., 2004; McKelvie, 2000). The total phosphorus (TP) content of aquatic systems may be in the particulate, dissolved or

colloidal phases. Only part of the total phosphorus pool is biologically available, either immediately available for biological utilisation or in a form that can be transformed by natural processes to a biologically utilisable form (Denison et al., 1998). Orthophosphates ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) are the most biologically available form of P and also the most abundant P species in natural waters (Maher and Woo, 1998; Worsfold et al., 2005). Other dissolved P species include inorganic condensed phosphates (eg. tripolyphosphate and pyrophosphate), organic condensed phosphates (eg. ATP) and organic species (eg. phytic acid and glucose phosphates) (Maher and Woo, 1998; Worsfold et al., 2005; Yaqoob et al., 2004). Organic P is found in both the particulate and dissolved phase.

Particulate P fraction can be biotic or abiotic in nature (McKelvie, 2000). Phosphorus bound in minerals (eg. apatite [$Ca_5(PO_4)_3OH$], brushite [$CaHPO_4 \cdot 2H_2O$] and wavellite [$Al_3(PO_4)2(OH)_3 \cdot 5H_2O$] and in mixed phases (eg. P adsorded onto clay particles and clay-metal complexes) are all abiotic in nature (Mc Kelvie, 2000; Maher and Woo, 1998; Scheslinger, 1997). Particulate P compounds held within the cellular structure of bacteria and plankton are biotic. A portion of particulate P (eg. the mixed phases) remains exchangeable and can escape to overlying water when fine particles are put back into suspension by turbulence (Andrieux-Loyer and Aminot, 2001).

The separation of dissolved and particulate P phases is operationally defined based on filtration using 0.45 or 0.2 μm membrane filters (Worsfold et al., 2005; Yaqoob et al., 2004; Murphy and Riley, 1962) (Figure 1.1). The exchange of P between dissolved (<0.45 μm) and particulate (>0.45 μm) fractions is governed by biologically mediated processes, physico-chemical complexation and release reactions (Hanrahan et al, 2002). The term 'total' is always associated with all parameters determined from the whole or unfiltered sample. Parameters with the word 'filterable' indicate parameters that have been determined from the filtered sample. With respect to P determination, 'reactive' refers to the P species which react with molybdate to form the coloured product

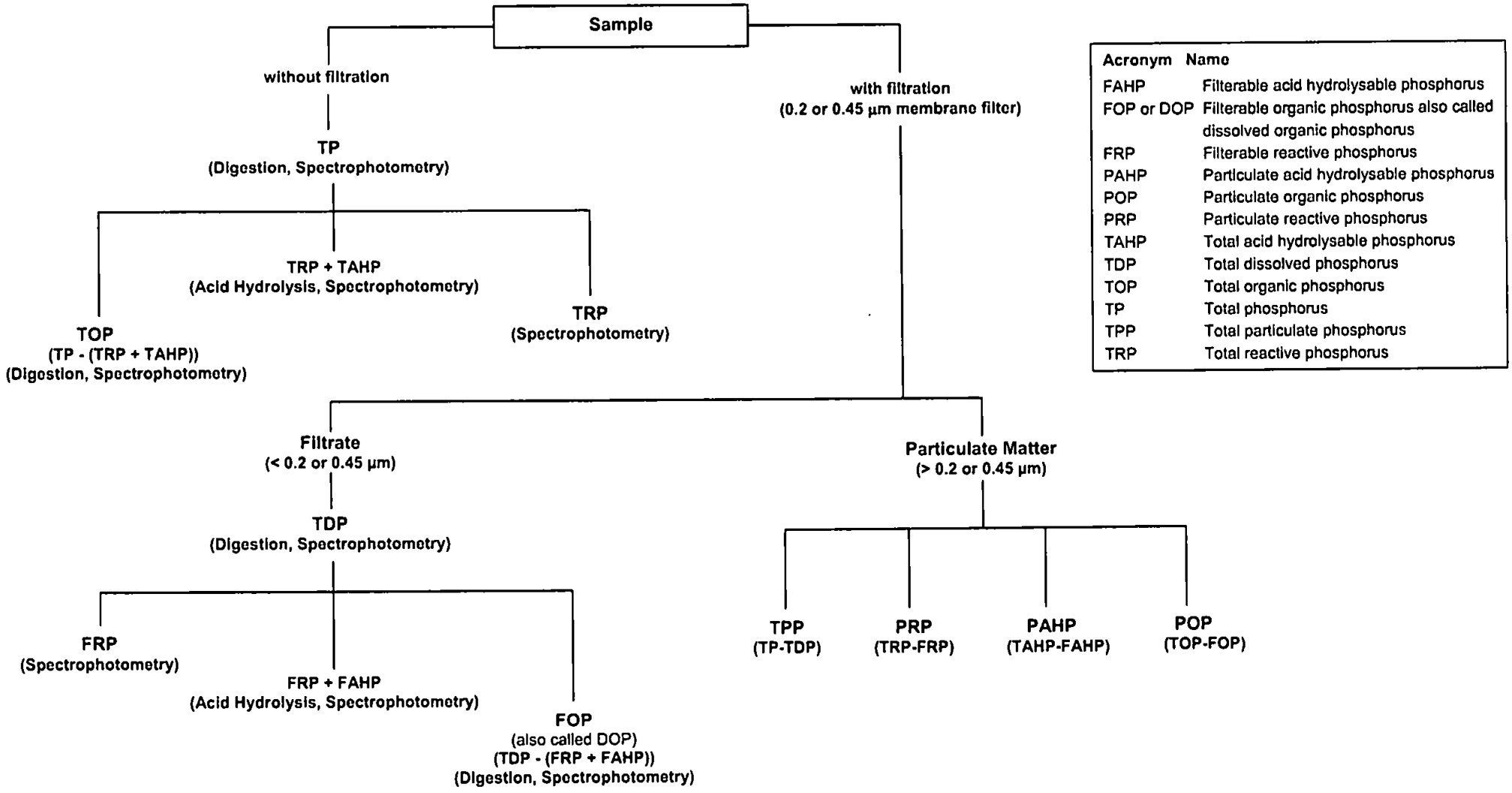


Figure 1.1: Operationally defined aquatic P fractions (adapted from Worsfold et al., 2005)

phosphomolybdenum blue (reduced form). Numerical treatment of parameters determined from filtered and whole samples also allows the calculation of other parameters (Estela and Cerdà, 2005).

Each operationally defined fraction contains a range of P species (Jarvie et al., 2002). Filterable reactive phosphorus (FRP), which comprises of a combination of dissolved monomeric inorganic P species (orthophosphate P), is often referred to as soluble (SRP) or dissolved (DRP) reactive P, and may be also be referred to as molybdate reactive P (MRP) within the filtered sample. MRP can also refer to FRP plus the portion of the PP that reacts with molybdate in unfiltered samples (Jarvie et al., 2002). Total filterable phosphorus, referred to as total dissolved phosphorus (TDP) is a combination of all the monomeric inorganic P compounds and hydrolysable P compounds (polymeric and organic) in the filtered fraction. Colloidal P compounds generally range from 0.001 to 1 μm in size and therefore they are usually determined as part of the TP or the TDP fractions.

Sources of P: Phosphorus in freshwater systems is derived from natural sources such as weathering of rocks, decomposition of plant and animal remains and excreta, and from anthropogenic sources such as agriculture, fertilisers and sewage (Figure 1.2). P is transported from the terrestrial environment to aquatic systems by factors such as rainfall and groundwater transport. Surface waters are also often used as receiving environments for wastewaters from sewage treatment plants, landfills and industry. The contribution of P through aerial transport such as adsorption onto dust particles is negligible.

The rate of P input to freshwater systems has been increasing due to human impacts from agriculture, urban development and population growth (Smith, 1999). The UK Environment Agency estimates that agriculture (47 %) is the largest contributor of P entering surface waters in the UK, followed by human and domestic wastes in sewage (24 %) and detergents (19 %) (EA, 1998a). The amount of P transported from agricultural lands has become more significant in the past 50 years (Sharpley and Withers, 1994;

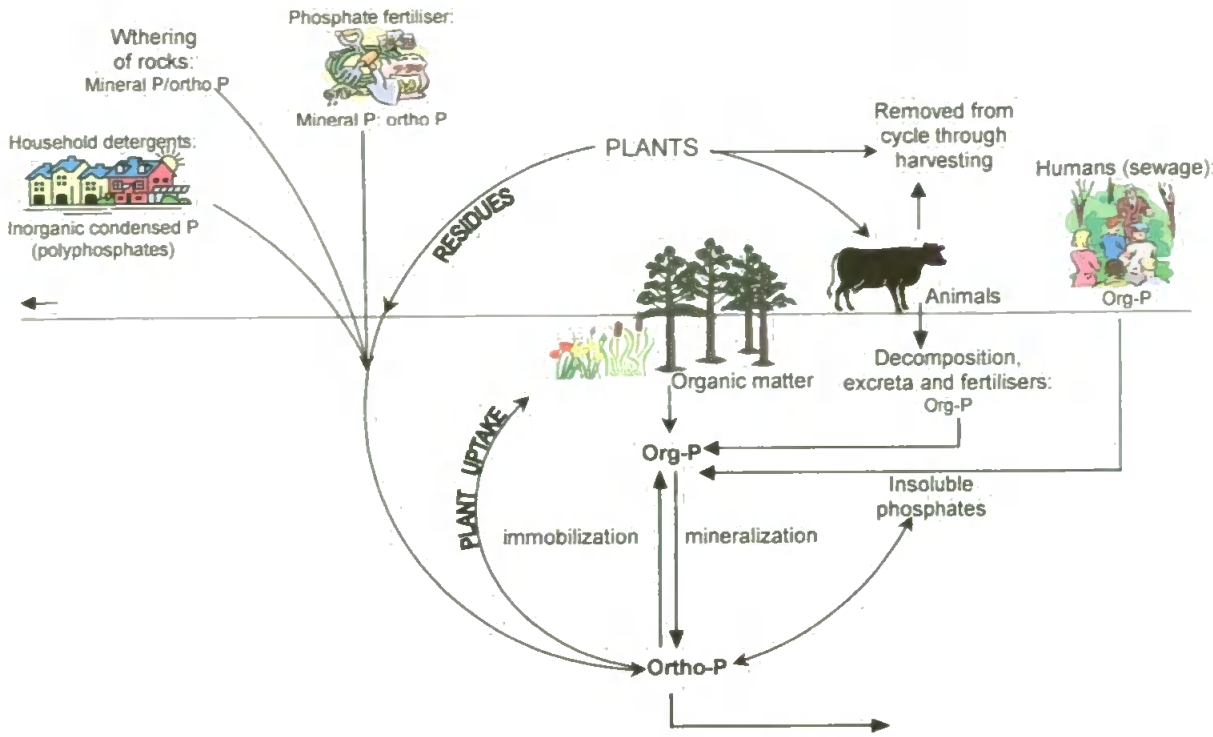


Figure 1.2: The phosphorus cycle. (adapted from Matakalli, 1993).
Ortho-P – orthophosphates; Org-P – organic P compounds.

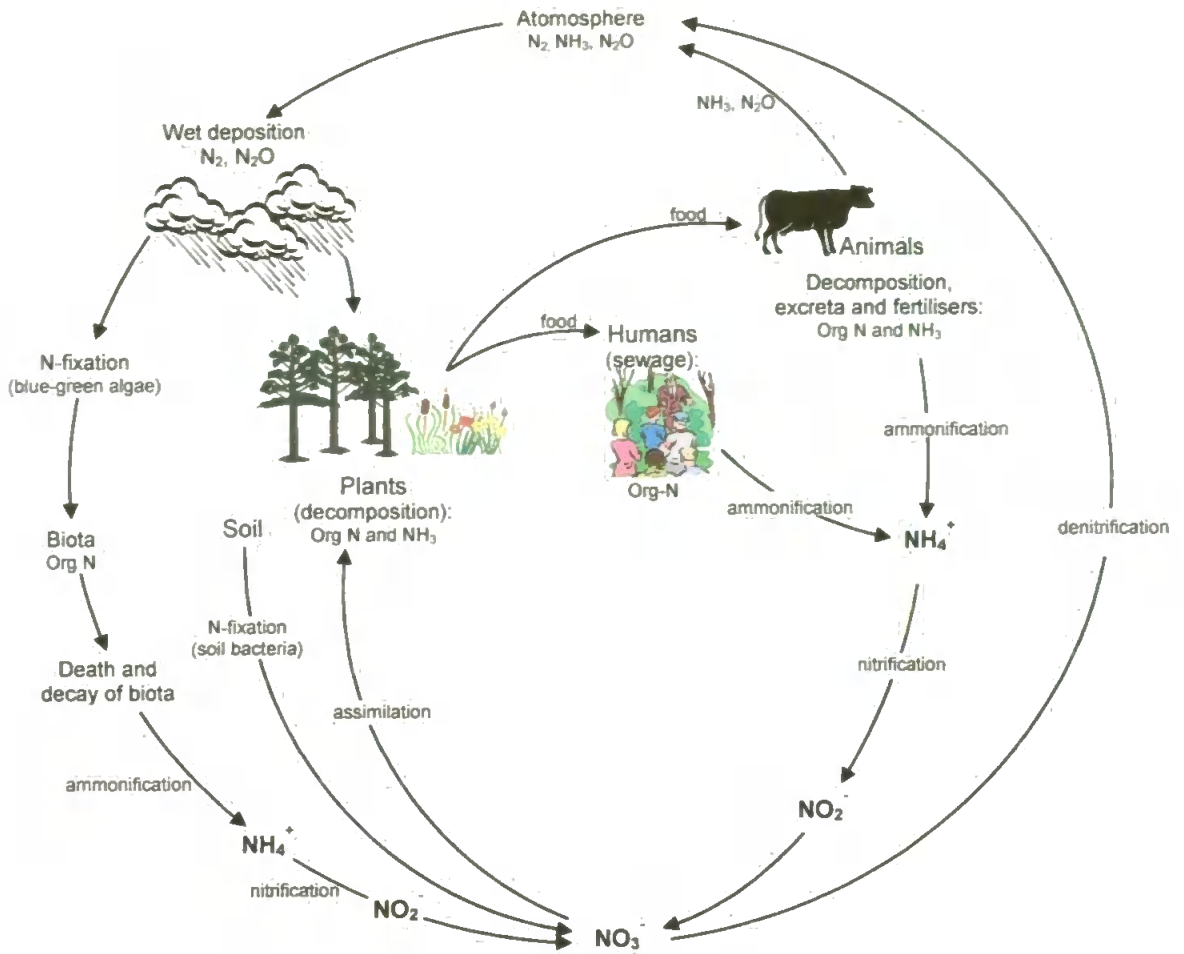
Withers and Muscatt, 1996). P inputs from fertilisers and manures can sometimes exceed P outputs in farm produce, causing P build up in soils (Withers et al., 2000; Carpenter et al., 1998; Smith et al. 1999). The weathering of rocks, such as apatite, builds up P in soil reserves and increases P availability for uptake by biota. Orthophosphate P release from soils is controlled by desorption kinetics (Johnes and Hodgkinson, 1998). Soils with low adsorption capacities, or where these capacities have been exceeded, are particularly prone to the leaching of additional inputs of soluble P from fertilisers. Research has also established that TP export from land to surface waters increases linearly with soil P content (Pote et al., 1996; Sharpley et al., 1995). Climatic conditions such as the amount of rainfall, acidity of natural waters and the presence of other minerals also influence the availability of P.

Sewage disposal and the use of polyphosphates in detergents are viewed as the two major causes of increased dissolved P loadings in the aquatic environment. Effluent from sewage treatment works is considered the major point source of phosphorus in UK surface waters (Environment Agency, 1998 a and b). Changes in detergent formulas to reduce P content have resulted in lower concentrations of dissolved P in sewage effluent in the UK (O'Neill, 1998). Population growth is therefore implicated in increasing P concentrations in aquatic systems.

1.1.2 Nitrogen

Nitrogen in the environment occurs in several forms and oxidation states. The presence of these nitrogen species in atmospheric, aquatic and terrestrial ecosystems is interlinked through biological transformations and abiotic processes and is known as the N cycle (Figure 1.3). Inorganic nitrogen species, excluding molecular nitrogen gas (N_2), dominate the environment as nitrate (NO_3^-), nitrite (NO_2^-) and ammonia (NH_3)/ammonium (NH_4^+). These N species are thought to be the primary bioavailable N species and take part in several important transformations involving nitrogen (Figure 1.3). N_2, the largest reservoir of nitrogen (approximately 78 % of the Earth's atmosphere), is relatively unreactive but enters the food chain through N-fixation. Nitrogen is an important constituent of amino acids, the building blocks of all cellular proteins and genetic material.

As with P, the distinction between particulate and dissolved phases is operationally defined and numerical treatment of parameters determined from filtered and whole samples also allows the calculation of other parameters (Estela and Cerdà, 2005) (Figure 1.4). The filtered fraction, however, is usually referred to as 'dissolved', and the component inorganic N species referred to by their chemical speciation (nitrate [NO_3^-], nitrite [NO_2^-] and ammonia/ammonium [NH_3/NH_4^+]). Dissolved organic nitrogen compounds include a number of naturally occurring compounds such as urea, vitamins and peptides. Gaseous



Nitrogen fixation – N-fixing bacteria convert N_2 to ammonia which dissolves to form ammonium

Assimilation – organic nitrogen is formed in plants and then in animals

Ammonification – bacterial conversion of nitrogenous residues to ammonia which dissolves to form ammonium

Nitrification – bacterial conversion of ammonium to nitrite, or nitrite to nitrate

Denitrification – nitrate loss as denitrifying bacteria convert nitrate to nitrogen dioxide and then to molecular nitrogen

Figure 1.3: The nitrogen cycle (adapted from Higson, 2003)

phase N compounds such as NH_3 and N_2O (nitrous oxide) may become adsorbed onto particulates in natural waters via wet or dry deposition processes. Organic particulate N compounds such proteins, peptides and nucleic acids held within plankton and bacteria are biotic in nature. Abiotic particulate N compounds include synthetic organic nitrogen compounds such EDTA (ethylenediaminetetraacetic acid) and pharmaceutical compounds (eg. bronchodilators such as noradrenaline or dietary supplements such as phosphopeptides) as well as naturally occurring compounds such as humic substances that

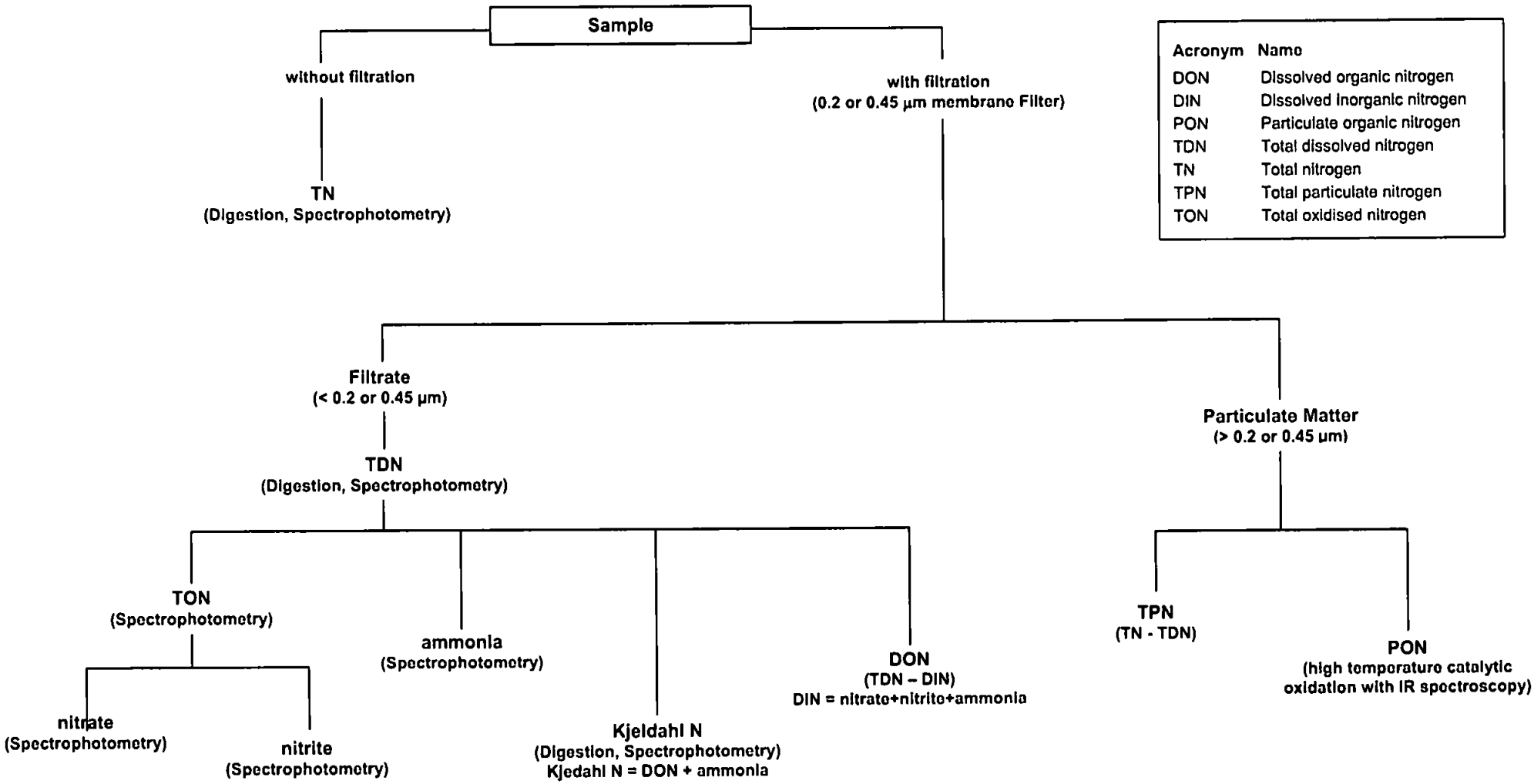


Figure 1.4: Operationally defined aquatic N fractions (Adapted from Robards et al., 1994)

become adsorbed on particulates. Nitrate is the common form of combined N found in oxic natural waters and can be biochemically reduced to nitrite, which can be rapidly oxidised to nitrate once more (denitrification/nitrification). Nitrates have a high solubility and are easily leached and therefore readily make their way to aquatic systems via surface and sub-surface flow and to groundwaters as a result of soil leaching. Natural concentrations of nitrates in rivers are usually less than 0.1 mg L^{-1} but may be enhanced by anthropogenic sources. Nitrate and nitrite concentrations typically ranges between 0–20 mg L^{-1} N (Robards et al, 1994) and 1-100 µg L^{-1} N (Chapman, 1998), respectively. However, in cases of extreme pollution, concentrations may be as high as 1 mg L^{-1} NO_2-N such as in waters strongly influenced by industrial effluent, or 500 mg L^{-1} NO_3-N in areas of high N fertiliser application (Chapman, 1998).

Sources of N: Nitrate is the predominant form of nitrogen transported in rivers (Jarvie et al, 2002). It is derived from a variety of sources within catchments such as fertilisers, sewage, industrial effluents, atmospheric inputs and mineralisation and nitrification of organic nitrogen in soils (Jarvie et al., 2002; Heathwaite et al., 1996).

Agriculture is the main diffuse anthropogenic source of N to the environment. It has been estimated to contribute 37 to 82 % of all N emissions to surface waters in Europe (Smith et al., 2003). Sharp increases in nitrate concentrations in many traditionally agricultural regions in Europe have been linked to fertiliser application (Hagebro et al., 1983). Global production of nitrogen fertilisers has increased almost ten-fold since the 1950s to 80 million metric tonnes N in 1990, and a further 50 % increase in production is predicted by 2035 (Vitousek et al., 1997). A substantial quantity of N is added through the application of manure to crops, however, this type of fertiliser application is not as strictly regulated as is the application of commercially produced fertilisers.

Nitrate leaching from unfertilised grasslands or natural vegetation is normally minimal although soils but have the potential to be a large source of nitrate (due to the activity of

nitrifying bacteria in the soil). Some agricultural activities, such as clearing and ploughing for cultivation, increase soil aeration and enhance the action of the nitrifying bacteria, thereby increasing the production of soil nitrate.

Point sources of N are largely from domestic sewage and industrial wastes, although there are agricultural point sources from bacterial decomposition of manure and silages wastes (Coles, 1999). Approximately 70 % of all sewage waste is discharged to inland running waters and the rest to estuaries and nearshore coastal waters. Untreated sewage contains as much as 55 mg L^{-1} TN and 25 mg L^{-1} NH_3-N (Coles, 1999). It is estimated that each human produces approximately 10.8 g N per day (Burt et al., 1993). Wastes that undergo secondary treatment contain 20 to 50 mg L^{-1} TN (Moss et al., 1998).

The transfer of atmospheric N products such as nitrous oxides and ammonia is via wet and dry deposition. Nitrous oxide emissions from industry is a key air quality issue because of its role as a greenhouse gas and as a precursor to acid rain. Water-soluble compounds such as acids, anhydrides, peroxides and ammonia are particularly susceptible to wet deposition. It is estimated that in the UK, 40 kg N is received per hectare of land on an annual basis from atmospheric sources, whether or not the land is used for agriculture (Golding, 1990).

1.1.3 Eutrophication

There are numerous definitions of eutrophication generally based on overnutrification of waters or based on the occurrence of algal blooms. In this thesis, eutrophication refers to '*the enrichment of waters by inorganic plant nutrients which results in the stimulation of an array of symptomatic changes...*' as defined by the Environment Agency of England and Wales (Environment Agency, 1998 a). The most common effects of the increased primary production associated with eutrophication in natural waters are shown in Figure 1.5 and include algal blooms; fish kills; change in species composition; loss of use of water resources and general degradation of water quality (Laane, 2005; Postel and Carpenter,

1995). These consequences are far-reaching and can compromise ecological, social and economic functions of the involved waterbodies (Chapman et al., 1998; Smith et al., 1999; Withers and Muscutt, 1996). Therefore, the eutrophication of water bodies is of universal concern (Maher and Woo, 1998).

The symptoms of eutrophication in freshwater systems may vary with stream or river characteristics. Eutrophication is more likely to be promoted in stream with low flows and extended exposure to sunlight than streams with higher flows or less light exposure, under identical nutrient inputs. Also, in smaller rivers, eutrophication promotes growth of vascular plants whereas in larger rivers phytoplankton growth is promoted due to difference in flow rates (Chapman, 1998). Construction of locks or reservoirs along a river course can reduce river flow and promote eutrophication in the associated rivers (Chapman, 1998).

In freshwater lakes and rivers, the availability of phosphorus usually limits primary productivity whilst in marine waters N is usually in short supply (Ricklefs and Miller, 2000) although exceptions may occur. N and P inputs to surface waters derive from a range of sources including groundwater, fluvial, and atmospheric inputs (Smith et al., 1999). However, present literature suggests that the occurrence of eutrophication has increased in frequency due to the accumulation of nutrient rich effluent from anthropogenic sources of N and P (Jones et al., 1990; Elofsson et al., 2003) causing nutrients to accumulate at a rate greater than can be recycled by decomposition or used by photosynthesis (Jones et al., 1990). Although no internationally accepted 'critical P concentrations' have been defined, guidelines for N and P concentrations in various waterbodies have been proposed. The Environment Agency (England and Wales) has developed interim guidelines for TP and FRP in surface waters as a eutrophication management tool (Scope Newsletter, 1999; EA, 1998 a) (Table 1.1) and Smith et al. (1999) in a review of the impact of eutrophication on all types of water bodies cited a classification of ecosystems in terms of both N and P supply rates (Table 1.1), with

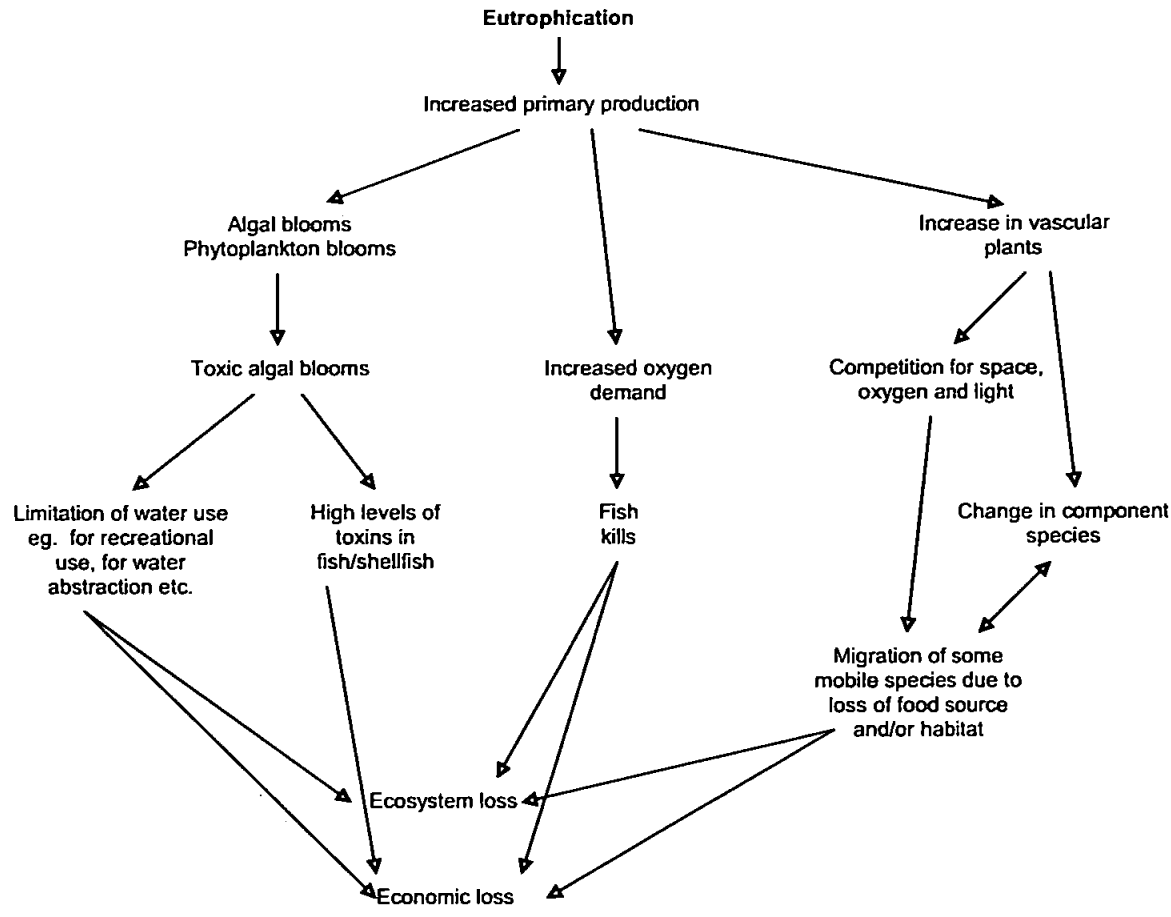


Figure 1.5: Common environmental, ecological and economic effects of eutrophication in freshwater systems

stream characteristics sourced from Dodds et al. (1998). Oligotrophic waters are defined as poorly nourished; eutrophic as well nourished or receiving a large supply of nutrients, and mesotrophic waters are between the former two states, thus receiving an adequate supply of nutrients (Smith et al., 1999).

Eutrophication in the UK: Water quality in the UK is of national concern as needs for freshwater increase and as changing land use affects water quality (Environment Agency, 2000). Eutrophication has been identified as one of the 10 major freshwater water quality issues in England and Wales (Environment Agency, 1998 b). The economic costs of eutrophication resulting from environmental damage to freshwater systems in England and Wales were calculated to be between 105 and 160 million euros per year (Pretty et al.; 2003, Laane et al., 2005).

Table 1.1: Classification of trophic states.

Trophic State	TN ($mg\ m^{-3}$)	TP ($mg\ m^{-3}$)	FRP[a] ($\mu g\ L^{-1}\ P$)	Suspended chlorophyll a ($mg\ m^{-3}$)	Benthic chlorophyll a ($mg\ m^{-2}$)	Reference
Oligotrophic	< 700	< 25	-	< 10	< 20	Smith et al., 1999; Dodds et al., 1998
Mesotrophic	700 - 1500	25 - 75	-	10 - 30	20 - 70	Smith et al., 1999; Dodds et al., 1998
Eutrophic	> 1500	> 75	-	> 30	> 70	Smith et al., 1999; Dodds et al., 1998
Oligotrophic	-	-	20	-	-	Environment Agency, 1998 a
Mesotrophic	-	-	60	-	-	Environment Agency, 1998 a
Meso-eutrophic	-	-	100	-	-	Environment Agency, 1998 a
Eutrophic	-	-	200	-	-	Environment Agency, 1998 a

a based on annual mean concentration (boundary concentrations)

Effluent from sewage treatment works is considered the major point source of phosphorus in the UK. Agriculture is the main diffuse source of both nitrogen and phosphorus (Johnes, 1996). Approximately 75 % of UK land area is used for agriculture which dominates the nitrogen cycle in the UK (Royal Society, 1983) and contributes approximately 47 % of phosphorus reaching UK surface waters (European Environmental Agency, 1999; Environement Agency, 1998 a).

Eutrophication is considered the most common problem in UK estuaries (Smith et al., 1999) and the eutrophication of coastal waters has been linked to variations in river nutrient loads (House et al., 1997). As nutrients move from freshwater to saline water in an estuary, they pass along a salinity gradient and may undergo physical (adsorption/desorption), chemical or biological processes (e.g. uptake/excretion), which essentially regulate the nutrient load that will reach coastal waters. Coastal waters have naturally low nutrient concentrations and are particularly susceptible to over-enrichment of nutrients leading to eutrophication.

1.2 Legislation for nutrients in natural waters

Eutrophication of natural waters is a worldwide problem (Maher and Woo, 1998), including the UK (Mainstone and Parr, 2002). The economic and environmental implications of eutrophication mean that there are statutory obligations and political pressures for the control of nutrients in UK freshwater systems (Mainstone and Parr, 2002). Several strategies are used in the control of N and P inputs to rivers at the national (UK) and European levels (Jarvie et al., 2002). The EC Water Framework Directive (WFD), which came into being in December 2000; provides a management structure for the future of European water policy (Environment Agency, 2002). The European Commission Water Framework Directive aims to maintain and improve the quality of aquatic ecosystems in the EU by using an integrated approach to the protection, improvement and sustainable use of the water environment (European Commission, 2000). It encompasses and incorporates existing legislation pertaining to water quality, including the Nitrates Directive (Directive 91/676/EEC) and Urban Wastewater Directive (UWWD, Directive 91/271/EEC). The WFD aims to maintain and improve the quality of aquatic ecosystems within the EU and sets a deadline for 'good status' for all waters (freshwater including surface and groundwaters, estuarine, and coastal) (Figure 1.6). This status is based on ecological, physico-chemical and hydromorphological criteria. Within the WFD, the physico-chemical conditions influencing the biological quality are related mostly to eutrophic processes (Borja et al., 2004, Bricker et al., 2003; Nielson et al., 2003). Water management of surface waters is based on river catchments and will use a combined approach of emission limits and quality standards.

Introduction of the UWWD to regulate water quality has resulted in an apparent decrease in phosphorus concentrations in EU rivers since the mid-80s, with an overall decrease of approximately 25 % over a decade (European Environmental Agency, 1999) because of improved wastewater treatment (industries and sewage) (Wolff et al., 1997). According to the EC UWWD, P concentrations in sewage and industrial effluent must be controlled;

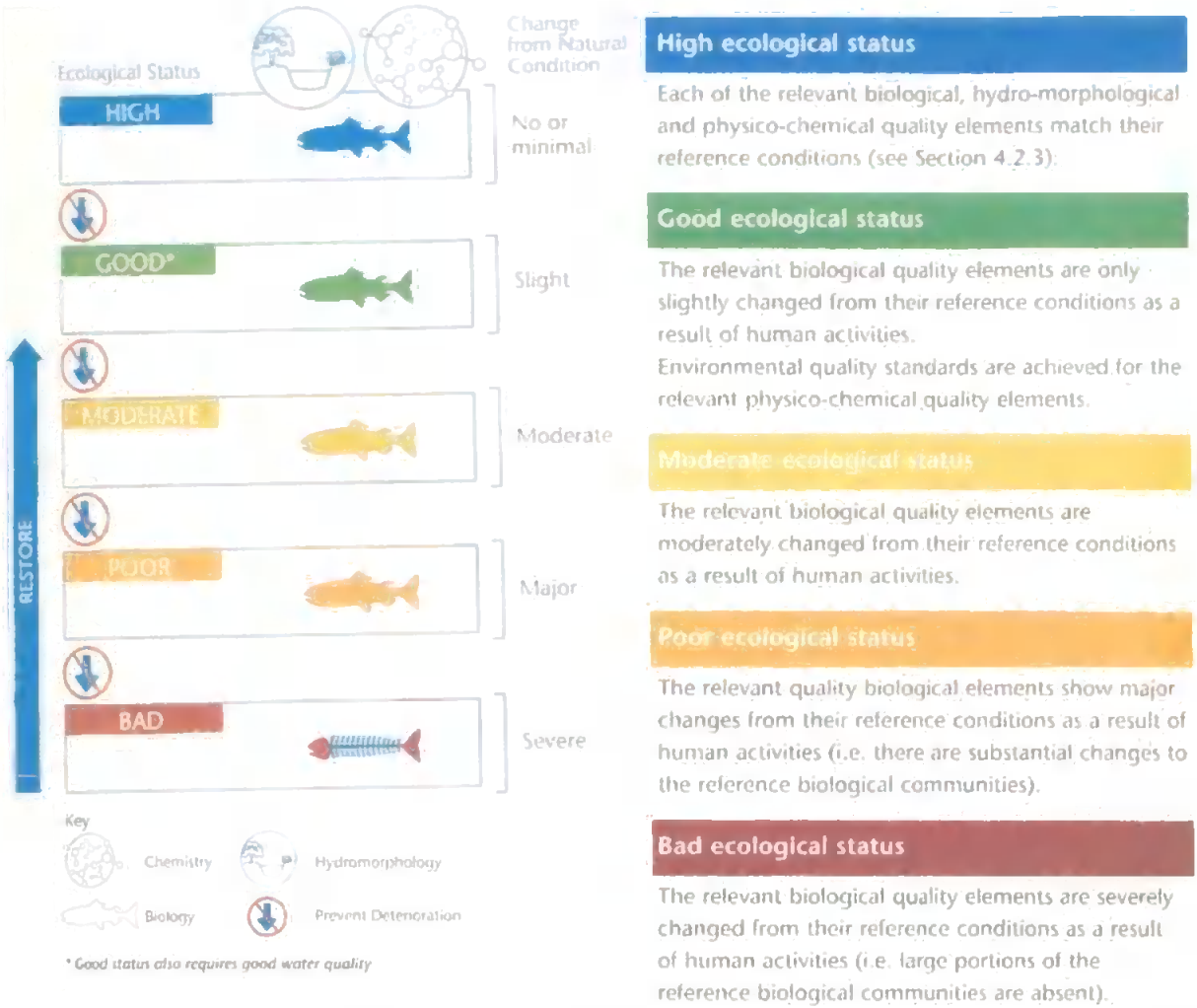


Figure 1.6: Status classification and environemental objectives as defined by the WFD. (Illustration reproduced from *The Water Framework Directive. Guiding principles on the technical requirements* (Environment Agency, 2002).

control concentrations (1-2mg L^{-1}) are determined by population density (Withers and Muscatt, 1996). The Nitrates Directive, valid from 31 December 1991, focuses on the prevention of N losses at source and outlines recommendations for changes in agricultural practice and land use (Jarvie et al., 2002). However, the extent of nitrate in surface waters has not decreased, despite reduced applications of nitrogen fertilisers (Wolff et al., 1997). The decrease in phosphorus has lead to increased N:P ratios over the past 15 years. Excess nitrogen with respect to phosphorus in surface waters discharged to receiving coastal

waters can cause an increase in undesirable toxic algal species and therefore negative effects on the food chain. Restoration of this equilibrium is desirable for both water and fish management, although no reduction in nitrogen is expected in the near future (Environment Agency, 1998 b; Wolff et al., 1997).

The WFD has set targets for reaching or maintaining at least 'good' ecological status, including nutrient status, in all surface waters and to comply with any standards/objectives for EU 'Protected Areas' by the year 2010 (Mainstone and Parr, 2002). Regular monitoring of the nutrient species important to eutrophication, such as FRP, TP, nitrate/nitrite, ammonia and TN, along with other physico-chemical characteristics, is necessary to establish baseline conditions and to allow regular assessment and classification of water quality.

Nitrate and nitrite concentrations in natural waters have maximum admissible concentrations set by the EU for surface waters intended for abstraction for drinking water (11.3 mg L^{-1} NO_3-N; 0.03 mg L^{-1} NO_2-N) and for the protection of course freshwater fish (9 μg L^{-1} NO_2-N) with a guide level of 5.65 mg L^{-1} NO_3-N for surface waters intended for abstraction for drinking water (Robards et al., 1994).

1.3 Monitoring nutrients in natural waters

The ability to measure nutrients is a key aspect of monitoring water quality. Analysis of bioavailable N and P species and the measurement of N:P ratios in natural waters is largely driven by the need to assess the potential for eutrophication (Neal et al., 2000; McKelvie, 2000), to determine bioavailable nutrients and to characterise nutrient dynamics (Estela and Cerdà, 2005; Maher et al., 2002). Nutrient data, especially total N and total P, are often used to calculate nutrient loads entering or leaving waterbodies, eg. the nutrient export from a freshwater catchments to an estuary or from estuarine waters to the sea. Reliable measurements are necessary if the analytical data from water quality

surveys are to be used to inform environmental management and research (Jarvie et al., 2002; Kramer, 1998).

TP, FRP, TN and nitrate/nitrite are the most frequently measured nutrient species in natural waters. TN and TP represent the maximum possible bioavailable N and P respectively, while FRP represents the most readily bioavailable P and nitrate/nitrite the most readily bioavailable N species. The determination of TN and TP in natural waters requires the use of digestive or oxidative processes to convert particulate, organic and condensed species to easily measurable species (nitrate and reactive phosphate, respectively) (Maher and Woo, 1998). Filtration to extract the dissolved phase (e.g. for measurement of FRP, DIN, nitrate/nitrite) should be carried out immediately or as soon as possible after sample collection as exchange on and off particulates may occur in sample container (Maher and Woo, 1998; Gardolinski et al., 2001; McKelvie, 2000; Lambert et al., 1992). Halliwell (1996, 2001) demonstrated that samples with high concentrations of condensed phosphates from detergents may undergo rapid hydrolysis and analysis should be performed within 24 hours of collection, even if filtered and/or refrigerated.

FRP and TP have been most frequently measured using spectrophotometric detection of phosphomolybdenum blue (McKelvie, 2000) based on the reaction of FRP with molybdate under acidic conditions to produce phosphomolybdic acid (Baldwin, 1998; Drummond and Maher, 1995), which is then reduced to form the coloured complex. The most common reductants are ascorbic acid, tin(II) chloride and tin(II) chloride/hydrazine sulphate (McKelvie, 2000). Spectrophotometric detection of the yellow product of the reaction of FRP and ammonium molybdate in the presence of ammonium metavanadate is less sensitive but is frequently used (Chen et al., 1991). Other methods of quantifying orthophosphate P include atomic spectroscopy techniques (such as ICP-AES), electrochemical techniques (such as potentiometry, voltammetry and enzyme electrodes) and separation techniques (such as IC, HPLC and capillary electrophoresis). Table 1.2 lists some commonly used techniques for the determination of P species in natural waters.

Table 1.2: Examples of methods for determination of P species. (adapted from McKelvie, 2000). The units used in this table are the same as in McKelvie (2000)

Technique	Matrix	Species detected	Method	detection range (μM P)	FIA sample throughput h^{-1}	Reference
Phosphomolybdenum blue Spectrophotometric determination	Natural waters and wastewaters	FRP	Reduction to phosphomolybdenum blue using stannous chloride Detection range dependant on light path length: (i) 0.5 cm; (ii) 2 cm; (iii) 5 cm;	(i) 9.7-64.5 (ii) 3.2-32.3 (iii) 0.2-6.5	n/a	APHA/AWWA, 1992
Phosphomolybdenum blue Spectrophotometric determination	Natural waters and wastewaters	FRP	Reduction to phosphomolybdenum blue using ascorbic acid Detection range dependant on light path length: (i) 0.5 cm; (ii) 2 cm; (iii) 5 cm;	(i) 9.7-64.5 (ii) 4.8-41.9 (iii) 0.3-8.1	n/a	APHA/AWWA, 1992
Phosphomolybdenum blue Spectrophotometric determination/FIA	River water	FRP	Portable field monitor; tangential filtration at sample inlet; phosphomolybdenum blue detection using a miniature CCD spectrometer;	0.26-16.3	38	Hanrahan et al., 2001 a
Phosphomolybdenum blue Spectrophotometric determination/ SIA	Freshwater	FRP	Sequential determination of phosphate and silicate; oxalic acid used to improve selectivity towards phosphate	6.5-226	75	Galhardo and Massini, 2000
Ion selective electrode	Natural waters	HPO_4^{2-}	Phosphate ISE based on rubbery membranes containing heterocyclic macrocycles; pH range 6-8; response time ≤2 min	1-3900	n/a	Le Goff, 2004
Amperometry/FIA	Synthetic wastewater	orthophosphate	Phosphate biosensor based hydrogel immobilized pyruvate oxidase on a thick-film Pt electrode	20-500-	24	Mak et al., 2003
Chemiluminescence/FIA	Freshwater	molybdate reactive P	luminol oxidized by the phosphomolybdic acid, formed in the presence of molybdate reactive P, to generate chemiluminescence	0.001-0.105	180	Yacoob et al., 2004
Phosphomolybdenum blue HPLC/ Spectrophotometric determination	Stream and estuarine waters	FRP	Off-line complexation of FRP (< 0.2 μm) as phosphomolybdenum blue and pre-concentration using solid phase extraction cartridges, then separation using reverse phase HPLC	0.001–0.015	n/a	Haberer and Brandes, 2003

Nitrate and nitrite are the most abundant N species found in natural waters. The most common way to detect nitrate is by conversion to the more reactive nitrite ion and simultaneous detection of nitrate and nitrite as a single measurable species (nitrite). The simultaneous determination of nitrate/nitrite in natural waters is most frequently carried out using spectrophotometric determination of the coloured product of the diazotization reaction between the resultant nitrite and a selected aromatic amine. Other methods of analysis include electrochemical detection and capillary electrophoresis (NO_2^- and NO_3^- detected sequentially in a single analysis). Examples of techniques commonly used to monitor nitrate/nitrite in natural waters and other environmental matrices are listed in Table 1.3.

Reduction of nitrate to nitrite is most commonly achieved using copper-coated cadmium (Cu/Cd) and usually 100 % conversion is achieved (Moorcroft et al., 2001). Other

Table 1.3: Examples of techniques for the simultaneous detection of nitrate/nitrite in environmental matrices.

Technique	Matrix	Method	detection range (μM N)	FIA sample throughput h^{-1}	Reference
Spectrophotometric detection/SFA	Pond water	Cd/Cu reduction then diazotization of sulfanilamide and N-1-naphthylethylenediamine; nitrate/nitrite and nitrite determined in same measuring cycle	36-3571	14	Legnerová et al., 2002
Spectrophotometric detection/FIA	Spring, river and sea water	Cd/Cu reduction of nitrate to nitrite then diazotization reaction of 3-nitroaniline and N-1-naphthyl-ethylenediamine	1.6-56	30	Ahmed et al., 1996
Spectrophotometric detection/FIA	Natural waters, soil	Samples acidified (5M HCl) then reduction using a zinc coated polyurethane foam column then reaction with 4,4'-methylene-bis-*m*-nitroaniline (detection at 495 nm)	1.9-63.9	15	Suvardhan et al., 2005
Spectrophotometric detection/SFA	Seawater	Cd/Cu reduction then diazotization of sulfanilamide and N-1-naphthylethylenediamine	0.004-0.250	60	Zhang, 2000
Spectrophotometric detection	River water; groundwater	Preconcentration of samples followed by Cd/Cu reduction then diazotization reaction of (i) sulfanilic acid and 1-naphthol-4-sulphonate (ii) sulfanilic acid and 1-naphthol and (iii) sulfanilic acid and phenol	(i) 0.11-2.14 (ii) 0.54-14.00 (iii) 0.18-5.38	n/a	Horita et al., 1997
Spectrophotometric detection/FIA	Wastewater, seawater	Cd/Cu reduction of nitrate to nitrite then reaction with 3,6-diamino acridine	0.001-0.4	68	Guerrero et al., 1996
Spectrophotometric detection/SIA	Natural waters; wastewaters	Cd/Cu reduction then diazotization of sulfanilamide and N-1-naphthylethylenediamine;	1.4-141.4	20	Galhardo and Massini, 2001
Electrochemical detection	Sewage (wastewater)	reagentless electrode system based upon the deposition of a macroporous Cu deposit	10-200	n/a	Davis et al., 2000
Bamperometry/FIA	Groundwater, spring water, wastewater	Photoreduction of nitrate to nitrite; nitrite oxidation of iodide ion to tri-iodide, which is detected by biamperometry	0.4 (LOD)	25	Torró et al., 1998
solid phase spectrophotometric detection	River water	Cd/Cu reduction then diazotization with 4-nitroaniline and 1-naphthol, then sorption of the azo dye onto thin-layer polyurethane foam (preconcentration factor >140) and direct spectrophotometric measurement of net absorbance at 625 nm	0.6-9.0	n/a	Abbas and Mostafa, 2000
Fluorescence/reverse FIA	seawater	Cd/Cu reduction of nitrate to nitrite then diazotization reaction with aniline, fluorescence if the electrons of the triple bond of the diazonium ion detected	0.69 x 10^{-3}-2.00	18	Masserini and Fanning, 2000
Chemiluminescence/FIA	River water, well water, rain water	Online photolytic conversion of nitrate to nitrite, then oxidation of nitrite to peroxynitrous acid (by H_2O_2) and subsequent chemiluminescent reaction with luminol	0.002-2.25	24	Mikuška and Večeřa, 2003
Ion chromatography	River, dam and storm waters	Cu/Cd reduction of sample with suppressed ion chromatographic detection of nitrite	0.1-19.4	12*	Thabano et al., 2004
Isotachophoresis-capillary zone electrophoresis	seawater	Artificial seawater used as background electrolyte; method applied to nitrate/nitrite analysis in MOOS-1 (seawater CRM); good agreement with tolerance levels was observed	0.75 (LOD)	n/a	Fukushi et al., 2003
Capillary electrophoresis	Surface water, wastewater	Capillary electrophoretic separation of NO_3^- and NO_2^- ions from other anions in aqueous solution. Baseline separation NO_3^- and NO_2^- up to 1613 μM N achieved in less than 5 min	2 (LOD)	n/a	Kubáň et al., 1999

reducing agents have also been used such as zinc, amalgamated Cd and hydrazine-Cu. The general mechanism of reduction is:

$$NO_3^- + Cd + 2H^+ \longrightarrow NO_2^- + Cd^{2+} + H_2O$$

Detection methods based on chemiluminescence require further reduction to nitric oxide, using reducing agents such as Ti (III), V (III), Mo (IV) and Fe (II) and Cr (III). Takeda

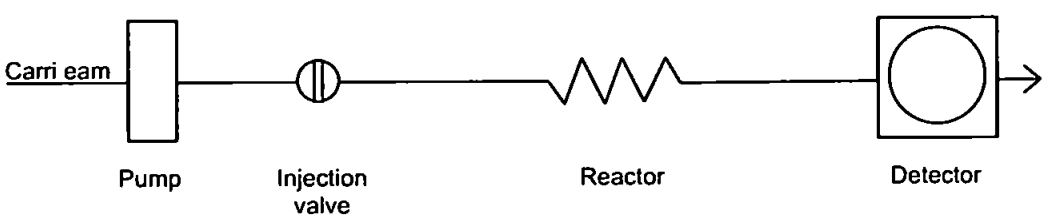
and Fujiwara (1993) reported a novel photo-induction method, using UV irradiation (wavelengths between 200-300 nm) to convert nitrate to nitrite and oxygen. Direct detection of the nitrate ion in natural waters is also possible using techniques such as UV spectrometry (Mou et al, 1993), and electrochemical detection (Davis et al., 2000). Although the nitrate ion is relatively inert, long-term storage of samples prior to analysis is not encouraged due to the propensity for bacterial spoilage (Moorcroft et al., 2001). This is also true of the more reactive nitrite ion. Also, nitrite can be oxidised in the presence of a ions such as Cr(III) and Fe (III), or reduced by compounds such as ascorbic acid and urea iodide, thus resulting in an underestimation of nitrate/nitrite (Moorcroft et al., 2001).

Many laboratories involved with routine analysis of natural waters use flow techniques for N and P determination. These include flow injection analysis (FIA); segmented flow analysis (SFA), also called air segmented continuous flow analysis (ASCFA); continuous flow analysis (CFA); sequential injection analysis (SIA); multicommutated FIA (Rocha and Reis, 2000) and multisyringe FIA (Miró et al., 2003). FIA and ASCFA are the most frequently used flow techniques in nutrient analysis and are discussed further.

Air segmented flow continuous analysis: Air segmented continuous flow analysis, introduced in the late 1950's, was a widely used technique in the clinical field (under the tradename 'Technicon') (Miró and Frenzel, 2004). In this technique, the sample/carrier stream is segmented by regular introduction of air bubbles (or an inert gas such as nitrogen in SFA) then the reagent is added to each discrete segment in order to reduce dispersion of the sample plug during mixing. The stream is debubbled prior to detection. The presence of the air bubble can have drawbacks (Miró and Frenzel, 2004). The air segmentation systems are technically complicated, making minaturisation difficult; the compressability of the gas phase can lead to pulsing which may affect the signal profile; also the flow velocity may not be easy to control. ASCFA is currently still in use and several companies such as Skalar (Gardolinski et al., 2001; Kotlash and Chessman, 1998), Technicon (Aminot and Kérouel, 2001; Kotlash and Chessman, 1998) and Alpkem (Zhang et al., 1999)

produce commercially available systems that can be used for the determination of nitrate/nitrite and FRP.

Flow injection analysis: Flow injection analysis (FIA) was introduced in the mid-seventies by Růžička and Hansen (1988) as a non-segmented alternative to ASCFA/SFA. Flow injection analysis (FIA) can be defined as the sequential insertion of a discrete sample solution into an unsegmented continuously flowing stream before continuous reagent mixing and subsequent detection of the analyte (Dănet et al., 2001; Růžička and Hansen, 1988). The simplest FI set-up consists of a pump to propel the carrier stream through a narrow tube, an injection valve to introduce sample into the system, a microreator in which the sample zone disperses and reacts with the components of the carrier stream, and a detector to sense the product of the reaction:



The FIA setup is 'monoparametric' although some methods, using multichannel FIA analysers and multianalyte detectors, allow simultaneous determination of several parameters (Dănet et al., 2001; Miró and Frenzel, 2004). This allows FIA to be an excellent tool for speciation studies or multiparameter determination in water analysis. Such methods are often used by laboratories conducting multiparameter analysis on each of a large number of samples (Miró and Frenzel, 2004). Injection techniques have evolved to the use of rotation valves and the injection can be remotely controlled via a digital interface to allow automated sample injection. Flow injection analysis has advantages of ease of operation, high precision, and high sample throughput, and therefore has been extensively used for the determination of nitrate and phosphate in natural waters. FIA systems are relatively easy to automate and allow the benefits of FIA to be achieved

without the operator needing to be there at all times. This allows cost-effective use of skilled personnel.

FI analysis coupled with spectrophotometric determination has been used by several researchers to monitor dissolved nutrients such as reactive phosphorus (Table 1.2) and nitrate/nitrite (Table 1.3) in natural waters (Lyddy-Meaney et al., 2002; Hanrahan et al, 2001 a; Auflitsch et al., 1997; McKelvie et al., 1997; Andrew et al., 1994; Robards et al., 1994). Many of these are laboratory-based methods. However, some methods are portable and may be taken to the field. Sample collection can be the largest potential source of error in monitoring natural waters (Dănet et al., 2001). Shipboard or 'bank-side' analysers can reduce possible errors from sample degradation and contamination between collection and analysis of samples. Field methods that are also submersible and can be deployed *in situ* have the added advantage of providing real-time monitoring data.

In situ *FI analysis*: The need for *in situ* real-time continuous monitoring of nutrients has been recognised (Dănet et al., 2001) and the development of *in situ* nutrient analysers to determine bioavailable nutrients in various water bodies can provide a valuable contribution to the process of characterising chemical water quality. *In situ* nutrient analysers have the potential to provide high temporal and spatial resolution water quality data for scientists and managers involved the process of assessing and ensuring aquatic ecosystem health. *In situ* nutrient analysis may be on-site or submersible.

Several submersible FI analysers have been developed for the determination of nitrate/nitrite in natural waters. All are based on conversion of nitrate to nitrite followed by spectrophotometric detection of the coloured product formed from the diazotisation reaction of nitrite and a selected aromatic amine. Analysers reported by Daniel et al. (1995 a and 1995 b), David et al. (1998, 1999) and by Le Bris et al. (2000) all used Cu/Cd reduction to convert nitrate in samples to nitrite while Sun et al. (2000) used photo-induced conversion of nitrate to nitrite. Daniel et al. (1995 a and b) reported an submersible FI

analyser capable of analysing 40 samples h^{-1} over the liner range 0.5-40 μM N, submersible up to 300 m and operational from 2 to 35 °C in natural waters with salinity varying from 0 to 35. This system was further modified to improve the working range to 0.5-100 μM N with a sample throughput of 45 samples h^{-1}. The *in situ* FI analyser developed by David et al. (1998 and 1999) is submersible up a to depth of 50 m, and could be configured to operate over a linear range of 2.8-100 μg L^{-1} N with a 20 mm path length flow cell, or at 85 to 2000 μg L^{-1} N with a 10 mm path length flow cell. This analyser was suitable for determining seawater concentrations of nitrate/nitrite as well as higher nitrate/nitrite concentrations encountered in rivers and estuaries and was used by Gardolinski et al. (2002) for nutrient surveys in the North Sea and for estuarine surveys (Gardolinski, 2002). Method performance in this study was assessed by participation in interlaboratory calibration exercises (Gardolinski et al., 2002). A submersible FI analyser, linear range of 0-40 μM N, capable of measuring 22 samples h^{-1} in deep sea conditions was developed by Le Bris et al. (2000) and was attached to a remotely operated vehicle (ROV) and used to monitor nitrate/nitrite over a hydrothermal vent at a depth of 1650 m. Sun et al. (2000) developed a FI analyser for continuous, unattended determination of nitrite/nitrate which was linear over the range 0.01-14 mg L^{-1} N. The analyser was operated automatically and unattended for a period of 30 days and was also capable of simultaneously determining ammonia (0.01-5 mg L^{-1} N) via chemical oxidation to nitrite.

Several *in situ* FRP analysers, including a submersible analyser, have been developed for the determination of FRP in freshwaters, marine waters and wastewaters. These are discussed further in Section 5.3.1.

1.4 Catchment management

1.4.1 River catchments

A river catchment is considered to be the area of land that is drained by a particular river. Catchment characteristics influence the hydrological regime and the chemical

characteristics of the main river within the catchment. Catchment size, form, geological features and climatic conditions influence the quantity of water drained by a particular river network, while water quality is further influenced by land management and land use practices within the catchment (Chapman, 1998).

River systems represent a complex drainage flow from surface waters, groundwaters and fluvial systems. River discharge rates are the single most important hydrological feature of a river as it provides a direct measurement of water quantity and hence availability, and it allows for the calculation of loads of specific water quality variables such as nutrient loads. It also provides a basis for understanding river processes and water quality. Generally, small catchments result in low median discharge with large variations between peak and low discharges. Larger catchments, however, tend to have more uniform flow. Behrendt (1996) concluded that although nutrient retention may not be significant for small river basins, they may be considerable in larger basins. From a management perspective, it is important to design facilities with low flows in mind, especially when designing treatment facilities or trying to control maximum permissible levels. At higher river discharge rates there is more 'dilution of pollution' and therefore potentially harmful ecological effects of polluted effluents can be significantly reduced. For this reason drought conditions can negatively affect water quality in terms of ecological health as well as in terms of amenity usage.

The determination of nutrient fluxes is important when studying the pollutant mass balance within the catchment and it is also necessary in the assessment of catchment outputs to lakes, estuaries and nearshore water waters. Environmental managers have recognised the need to address problems associated with increasing nutrient and sediment loads delivered to waterbodies (Johnes and Heathewaite, 1997).

Traditional methods of assessing the impact of land use change on water quality have been based on the collection of detailed physical and chemical water quality measurements to

predict environmental trends (Johnes, 1996). However, this can only be used for small catchments as prohibitive costs would be incurred for larger catchments.

1.4.2 Catchment models

Watershed or catchment models can provide information on nutrient sources, total nutrient input and the effects of individual parameters. These models are particularly useful for estimating diffuse sources of nutrient inputs (Ryding and Rast, 1989). In fact, since point sources can be measured and quantified more easily, non-point sources (diffuse) are of more concern in water quality (Beaulac and Recknow, 1982). Watershed models vary considerably in complexity, data requirements and output. Empirical models, including nutrient coefficient models, are easy of use to estimate annual nutrient outputs from a particular catchment. This type of model, however, is usually based on water quality measurements gathered under a particular set of climatic conditions. Hence it is often difficult to extrapolate to other watersheds. However, these empirical models do allow estimates of runoff and baseflow nutrient loading, and are therefore a valuable management tool for the estimation of nutrient inputs and sources.

The most reliable method of estimating nutrient output from a catchment is direct water quality measurements at the point where the river exits the catchment. The nutrient load calculated from each discrete sample includes point and non-point sources within the catchment, as a function of the river discharge properties at the time of sampling. These discrete measurements can then be used to calculate annual loads. It is obvious then that the accuracy of the load estimates is limited by the frequency of water quality sampling (House et al., 1997). Nutrient export coefficient models use this type of data for calibration. Alternatively, the unit area loads based on export coefficients may be used to estimate catchment export of nutrients. This approach is based on the observation that under average hydrological conditions in a watershed over and annual cycle, a given land

use practice within the catchment will export a relatively constant nutrient load per unit area, to the receiving waters. The export coefficient represents the rate of transport of a particular nutrient from its source within the catchment.

1.4.3 The export coefficient model

The export coefficient modelling approach allows nutrient exports to be predicted for a range of management systems and environmental conditions on a catchment scale (Johnes and Hodgkinson, 1998). As a management tool, the export coefficient model has several advantages over detailed process-based models (Johnes, 1996). The model is in the simple format of a numerical box model; it can be operated using simple spreadsheet computer packages, and it relies on archival data (Johnes, 1996).

An export co-efficient model developed for and adopted by the Organisation for Economic Development and Co-operation (USA) to predict nutrient loading on eutrophic, standing waters (Beaulac and Recknow, 1982) was used by Johnes and O'Sullivan (1989) to model nutrient export from an agricultural catchment in South West England. The export coefficient represents the rate of transport of a particular nutrient from its source within the catchment. This simple box model effectively treated the catchment under study as a single unit with one export coefficient for all land use type although previous work indicated the magnitude of nutrient fluxes is influenced by land use, soil, climate and land cover management practices (Beaulac and Recknow, 1982). Johnes (1990) later modified the export co-efficient modelling approach to better reflect spatial variations in land use, land use intensity and management practices. This allowed modelling of intensive systems (Johnes and Heathwaite, 1997) and the determination of nutrient loadings delivered to lakes in England and Wales in the development of a new Lake Classification and Monitoring Scheme for the National Rivers Authority (Johnes et al., 1996). This allowed the model to be applied to large-scale catchments. Therefore, the export coefficient expresses the rate at which nitrogen or phosphorus is exported from each land use type in

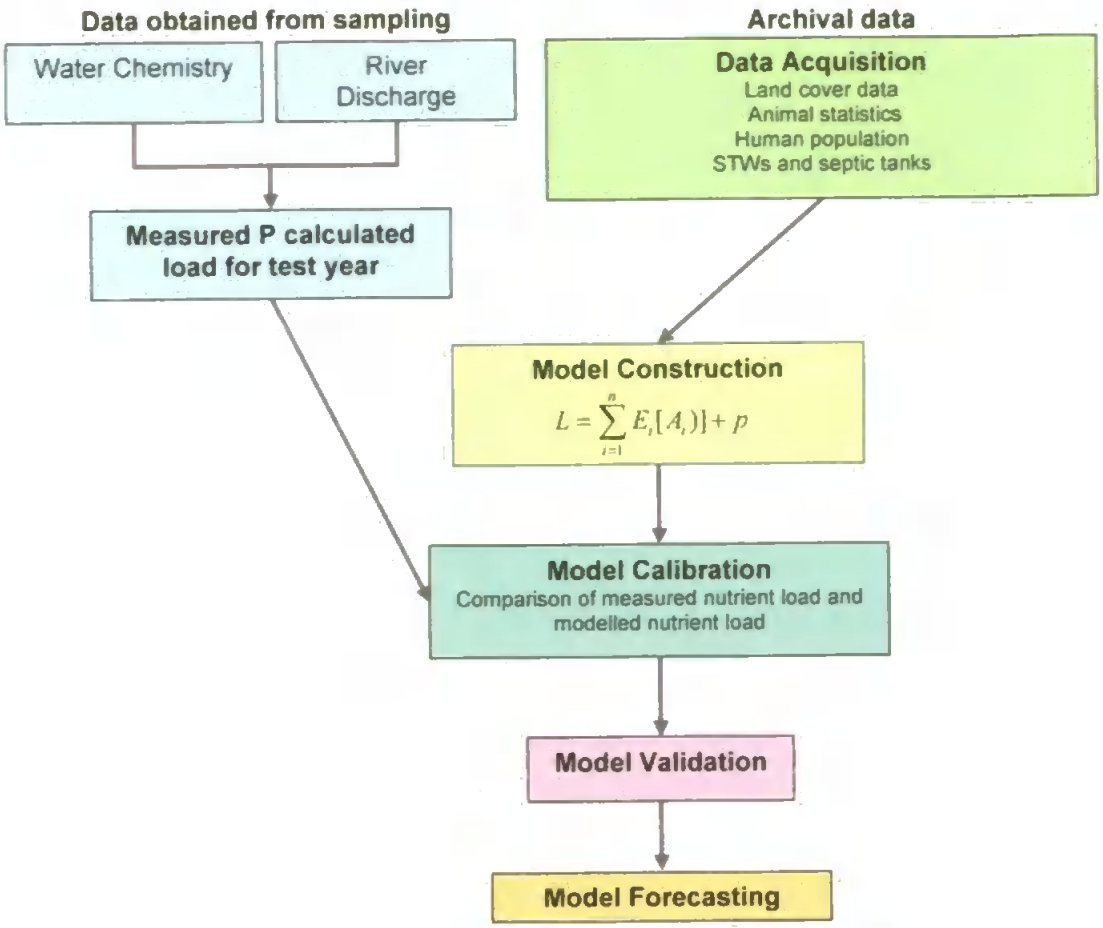


Figure 1.7: Schematic representation of the export coefficient modelling procedure

the catchment.

This approach has been used to model nutrient export for small agricultural catchments such as the Slapton catchment, South Devon and the Windrush catchment (Johnes, 1996; Johnes and Heathewaite, 1997). The former is dominated by livestock farming while the latter is charcterised by mixed arable farming and sheep farming. In a comparison of these catchments Johnes and Heathewaite (1997) were able to demonstrate the importance of basin hydrology on the export of nutrients. Their research demonstrated that for well-drained catchments, geographical distribution of nutrient exports was less significant that the actual input of nutrients. However, in catchments with shallow subsurface flows and

impermeable bedrock, land use practices in close proximity to rivers became more significant as potential nutrient export sources. For such catchments a distance-decay function was developed such that greater rates of nutrient exports for sources located within the riparian zone were modelled to allow more accurate prediction of nutrient loading. This approach was used to study TN and TP exports from hillside plots (<0.5 km^2), sub-catchments (approximately 10 km^2) within the Windrush catchment (363 km^2). Johnes and Heathewaite (1997) were able to show that the export coefficient modelling approach allowed scaling up from plot scale to the catchment scale. This approach therefore allows the identification, location and distribution of key nutrient sources.

Uses and limitations: Refinement of this approach for a particular watershed can result in a model that is an excellent predictive tool for nutrient export and eutrophication management within a specific catchment. Current research in this modelling approach is attempting to develop a more generic model that may potentially be applicable to more than one catchment (Hanrahan et al., 2001 b). Since export coefficients are selected for specific land use types and the geological characteristics of a catchment, it could be possible to apply a single model to catchments with similar characteristics and dominant land use practices and to expect good agreement with measured data. However, this approach should be treated with caution. Climatic conditions and land management practices vary globally. To date, the export coefficient applied in this approach has been largely investigated for temperate regions and the majority of coefficients that have been reported were researched for temperate regions only. Therefore this approach could not be applied outside this zone without considerable research being carried out to investigate export rates in regions with drastically different climatic, hydrological and geological characteristics.

Seasonal model: The export coefficient modelling approach has been primarily used to predict nutrient transfer from land to water on an annual basis (May et al., 2001).

Reynolds (1984) suggested that TP is an important ecological control on ecosystem development during the plant-growing season in spring and summer. In recognition of the varying ecological response to rivers over the period of a year, May et al. (2001) attempted to further refine the export coefficient approach to produce a model which would predict seasonal variations, using export coefficients that were normalised with respect to the monthly hydraulic runoff to predict monthly export of P from a small South UK agricultural catchment. Their results suggest and support a strong seasonal dependence of TP exports, with P retention in late spring to early autumn. This approach allows the export coefficient approach to potentially predict P fluxes on a seasonal basis such as during plant-growing season when the effects of eutrophication are often most pronounced.

1.4.4 Assessment of ecotoxicological status

Chemical monitoring can provide specific chemical data which allows characterisation of nutrient water quality. However, this type of data is often difficult to relate to bioavailability, toxicity and ecological status (Bloxham et al., 1999). Biological monitors can provide information that is potentially ecologically relevant and which indicate changes in water quality, but they do not provide sufficiently specific information to allow the chemical perturbation to be identified. Biomarkers of physiological stress have been shown to be useful indicators of environments stressful to aquatic organisms and have been used to relate environmental stress to organism health (Robillard et al., 2003; Riffeser and Hock, 2002; Baker and Hornbach, 2001; Nicholson, 1999; Bloxham et al., 1999; Lowe et al., 1995)

Cardiac activity, cell viability and condition index of mussels have been used as general indicators of organism health. Changes in cardiac activity have been related to many environmental, physiological and behavioural factors (Nicholson, 1999). The cardiac activity of mussels can be used as an indicator of water quality since heartrate is relatively

constant whilst the animal is immersed and little fluctuation in rate occurs, other than during valve closure (Helm and Truman, 1967; Coleman and Trueman, 1971). Any major perturbation of heartrate is therefore likely to be attributed to alterations in environmental conditions such as exposure to toxicants (Nicholson, 1999). Lysosomal stability is a useful indicator of cellular damage and cell viability (Shepard and Bradley, 2000) and therefore of organism health. Lysosomes are organelles important in nutrition, tissue repair and turnover of cellular components and physiological stress can impair function of these organelles and therefore adversely affect cell stability. The neutral red retention assay can be used to determine lysosomal stability and, therefore, cell viability (Nicholson, 1999). Condition index is a commonly used measure of gross nutrient reserves (Baker and Hornbach, 2001).

1.5 Project aims and objectives

This project aims to identify and validate reliable analytical technique for the quantitation of nitrogen and phosphorus species in natural waters, which will allow temporal and spatial resolution of nutrient data to carry out process-based studies. It also aims to use ectoxicological tests to assess ecosystem health with respect to nutrient water quality. The selected study area is the freshwater Tamar catchment, South West England (Section 3.2.1). The specific objectives of the project are as follows:

1. Evaluate selected laboratory methods for the measurement of nitrogen and phosphorus species in environmental waters.

2. Conduct bi-monthly discrete sampling of the Tamar catchment at Gunnislake to obtain N and P data including TP, TDP, FRP, TN, TDN and nitrate/nitrite.

3. Describe environmental trends with respect to nutrient water quality and nutrient biogeochemistry of the waters leaving the catchment and entering the Tamar Estuary

using historical physico-chemical data for the catchment. Field data collected in this study will also be used to identify short-term trends.

4. Use the nutrient coefficient modelling approach to predict nitrogen and phosphorus export from the Tamar catchment, South West England. Archival data available for the catchment area will be used to construct the N and P models. The P model will be using historical data available for the catchment and validated using field data. The N model will be calibrated using field data.

5. Develop an FI manifold for use with an *in situ* portable nutrient analyser, which will allow development of a robust, sensitive and reproducible *in situ* and bench top determination of FRP in freshwater. The detection limit should be 10 μg L^{-1} P and the upper limit of the linear range should be at least 100 μg L^{-1} P, based classification of trophic states (Section 1.1.3)

6. Conduct deployments of the *in situ* portable FRP analyser to determine analytical performance under field conditions. The analytical performance of the portable analyser will be validated through comparison with a validated laboratory reference method

7. Determine, under controlled laboratory conditions, the 96 h median lethal concentration (LC50) of selected nitrogen species (nitrate and nitrite) which may cause mortality to selected freshwater bioindicator species (*Anodonta cygnea*) as a means of determining short-term acute toxicity to these selected nutrients.

8. Use established ecotoxicological tests to investigate the effect of sub-lethal nitrite concentrations and high environmental concentrations of nitrite on the physiological health of a selected freshwater invertebrate (*Anodonta cygnea*).

CHAPTER 2

Laboratory analytical techniques for quantifying nutrients in the Tamar catchment

2.1 INTRODUCTION

Phosphorus and nitrogen are key determinands in most environmental monitoring and research programmes (Kramer, 1998) because of their importance in the eutrophication of aquatic systems (Neal et al., 2000). FRP and nitrate/nitrite are frequently monitored to determine trophic status with respect to water quality, while total nitrogen and total phosphorus data are frequently used in nutrient load predictions from catchments and from the discharge of wastewaters into natural waters. Therefore, reliable analytical methodologies are critical to obtaining accurate analytical results. They are an essential component of field studies that inform environmental management, environmental conservation (Aoyagi et al., 1988) and the scientific community and permit valid conclusions to be drawn about the status of environmental matrices and the associated impacts. Adequate quality assurance/quality control (QA/QC) schemes are necessary to ensure data integrity (Aminot and Kérouel, 1996 a).

Sensitive methods are desirable for determining low or limiting concentrations, while rapid methods are time efficient and allow a higher sample throughput, which is necessary for intensive field monitoring. This chapter describes analytical techniques used to quantify phosphorus and nitrogen species in water samples from the Tamar River catchment. It discusses the QA/QC measures undertaken to ensure good analytical data in this study, including participation in the certification of a seawater certified reference material for nutrients (MOOS-1), are also discussed.

2.2 EXPERIMENTAL

2.2.1 Reagents

All reagents used were of AnalaR grade and purchased from BDH Chemicals (Merck), Poole, unless otherwise specified. The surfactants Brij 35 (30 %) and FFD6 were obtained from Skalar UK Limited. Ultra pure water (Elga Maxima, 18.2 M Ω cm^{-1}) was used to

make up all solutions unless otherwise stated.

Ammonium molybdate solution: 0.23 g L^{-1} potassium antimony tartrate was dissolved in approximately 800 mL of ultra pure water and 69.4 mL H_2SO_4 (97 %) was carefully added, while swirling cooling. Then 6 g L^{-1} ammonium molybdate was added and the resultant solution made up to 1 L using ultra pure water. 2 mL FFD6 surfactant was then added to the solution and mixed thoroughly.

Ascorbic acid solution: 11 g ascorbic acid was dissolved in approximately 800 mL of ultra pure water, then 60 mL acetone was added and the resultant solution made up to 1 L using ultra pure water. 2 mL FFD6 was then added to the solution and thoroughly mixed.

Ammonium chloride buffer: 25g ammonium chloride was dissolved in approximately 800 mL of ultra pure water. The pH of the solution was then adjusted to 8.2 with ammonia (ca. 1 mL, NH_4OH, 25 %) and made up to 1 L using ultra pure water. 3 mL Brij 35 (30 %) surfactant was then added and thoroughly mixed.

Colour reagent: 150 mL *o*-phosphoric acid was carefully diluted in approximately 800 mL of ultra pure water, then 10g sulphanilamide and 0.5g α-naphthylethylenediamine dihydrochloride (NED) were added and dissolved. The solution was made up to 1 L using ultra pure water and stored in a dark-coloured bottle.

Oxidising reagent: 3.0 g boric acid and 5.0 g potassium persulphate were dissolved in 100 mL 0.375 M sodium hydroxide.

Phosphate standards: Potassium dihydrogen orthophosphate was dried at 105 °C for 3 h, cooled in a desiccator, then 0.4394 g of the dried salt was made up to 1 L with ultra pure water to give a 100 mg L^{-1} P stock solution. The working standards (0.01 – 0.15 mg L^{-1} P) were prepared by serial dilution of the 100 mg L^{-1} P stock solution with ultra pure water.

Nitrate standards: Sodium nitrate was dried at 105 °C for three hours, cooled in a

desiccator, then 6.0681 g of the dried salt was made up to 1 L with ultra pure water to give a 1000 mg L^{-1} N stock solution. Nitrate standards (0.2 – 5.0 mg L^{-1} N) were prepared by serial dilution of the 1000 mg L^{-1} N stock solution with ultra pure water.

Nitrite standards: Sodium nitrite was dried at 105 °C for three hours, cooled in a desiccator, then 0.4926 g of the dried salt was made up to 1 L with ultra pure water to give a 100 mg L^{-1} N stock solution. Nitrate standards (0.2 – 5.0 mg L^{-1} N) were prepared by serial dilution of the 100 mg L^{-1} N stock solution with ultra pure water.

2.2.2 Cleaning protocol

All laboratory glassware, bottles and containers used in experiments, sampling and sample storage were soaked in nutrient-free detergent (10 % Decon®-Neutracon) for 24 h, then rinsed 3 times with ultra pure water. They were then soaked in 10 % v/v HCl for a minimum of 24 h and subsequently rinsed 3 times with ultra pure water, dried at room temperature and stored in resealable plastic bags until required for use.

2.2.3 Determination of FRP and nitrate/nitrite using the Skalar SAN Plus Flow Analyser

2.2.3.1 Instrumentation

The Skalar SAN Plus flow analyser (Skalar Analytical B.V., Breda, Netherlands) is an air-segmented continuous flow analyser which consists of an autosampler, a chemistry unit, a matrix photometer and a digital interface which allows data transfer to a computer (Figure 2.1). The programming and operation of the Skalar SAN Plus flow analyser is carried out using the Skalar Flow Access software. The autosampler (SA 1050) was programmed with an injection time of 60 s and a wash time of 120 s and has the capacity to house up to 140 sample tubes at a time. Sample was pumped from the autosampler to the chemistry unit (SA 4000 segmented flow analyser). The chemistry unit consisted of two 16-channel

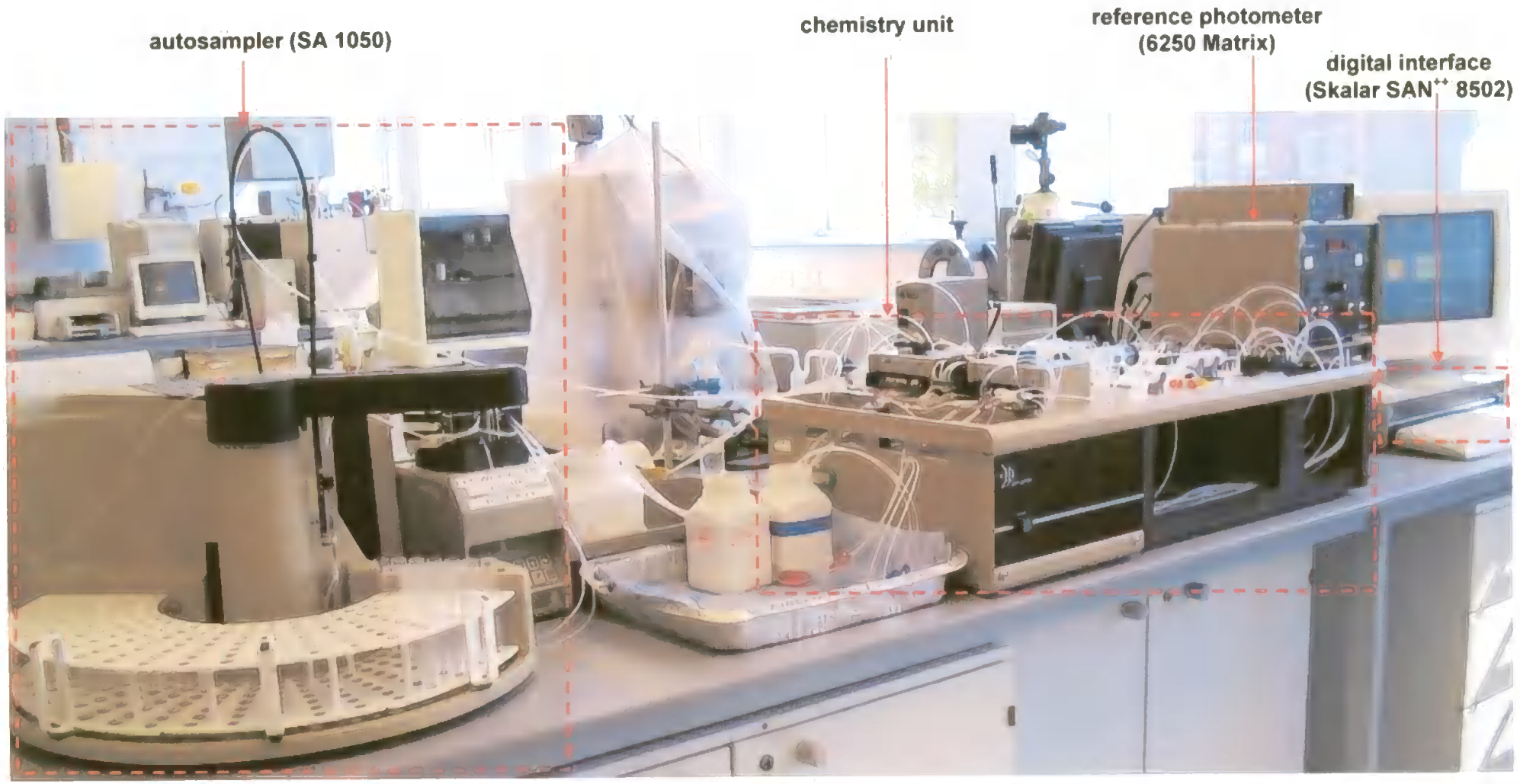


Figure 2.1: Photograph showing the sub-units that make up the Skalar SAN Plus air-segmented continuous flow analyser

peristaltic pumps (to propel sample and the reagent through the manifold), an air pump (30 bubbles per minute to segment the sample and reagent streams) and four independent manifolds (FRP, nitrate/nitrite, ammonia and silicate), with a 50 mm flow cell and a flow-through dual channel single beam colorimetric detectors for each manifold. The sample stream was split and sent to the four chemistry manifolds where simultaneous analysis was carried out. The FRP and nitrate/nitrite manifolds are shown in Figures 2.2 and 2.3 respectively. Automatic background correction was achieved for each determinand using a reference photometer (6250 Matrix). Refractive index effects were eliminated by subtracting the absorbance at a reference wavelength (FRP – 1100 nm, nitrate/nitrite – 620 nm) from the absorbance at the analyte wavelength (FRP – 880 nm, nitrate/nitrite – 540 nm). Analog data from the detector were then transferred to a computer, via a digital interface (Skalar SAN^{++} 8502), where it could be displayed and recorded using Flow Access software.

2.2.3.2 Phosphorus interference studies

The effect of arsenate (0 - 50 μg L^{-1} As), silicate (0 – 10 mg L^{-1} Si), nitrate (0 – 1000 mg L^{-1} N), nitrite (0 – 1000 mg L^{-1} N), Cu(II) (0 - 50 μg L^{-1} Cu) and possible hydrolysis products of complex P molecules (adenosine triphosphate (ATP), sodium tripolyphosphate (STP), and phytic acid (PTA), each made up to 95 μg L^{-1} P) on the determination of FRP (40 μg L^{-1} P and 100 μg L^{-1} P) in aqueous solution was investigated. For each potential interference investigated, a blank solution containing the interference in the absence of FRP was also analysed.

2.2.3.3 Nitrogen interference studies

The effect of phosphate (0 - 100 mg L^{-1} P), Cu(II) and salinity (0-35) on the determination of nitrate/nitrite (2.5 mg L^{-1} N) in aqueous solution was investigated. For each potential interference investigated, a blank solution containing the interference in the absence of

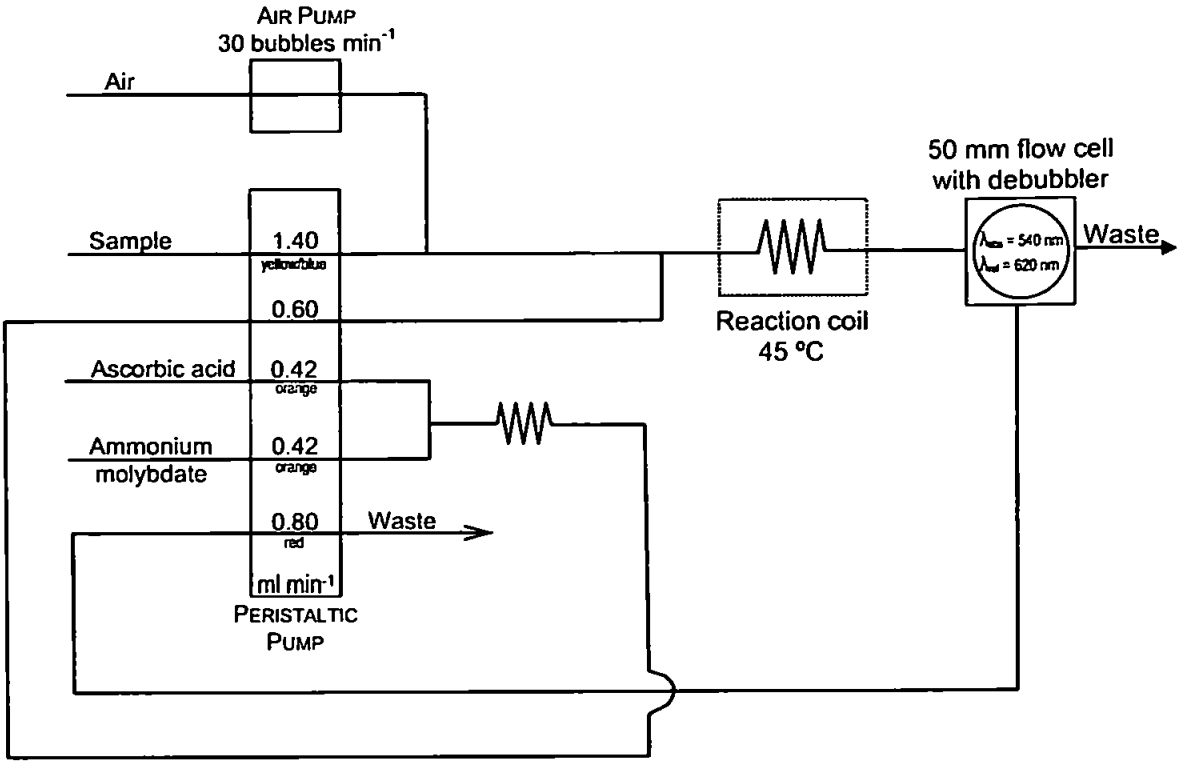


Figure 2.2: Flow diagram for FRP determination using segmented flow analysis

nitrate/nitrite was also analysed.

2.2.3.4 Quality assurance

Participation in the 'NOAA/NRC 2nd annual intercomparison study for nutrients' (Clancy and Wille, 2003), to analyse two seawater samples for FRP and nitrate/nitrite using the Skalar SAN Plus flow analyser was undertaken in 2002 to assess analytical capability for quantifying nutrients in seawater. Two seawater samples, a proposed certified reference material (MOOS-1) and a control sample (Nutrient 2002 that was collected from Pensacola Sound, Florida, USA) were distributed to participants. MOOS-1 was collected on June 24 1996, from a depth of approximately 200 m at Latitude 47.062833 °N, Longitude 59.982333 °W, off the northern tip of Cape Breton, Nova Scotia, Canada. A bulk sample was collected using a rosette containing 22 x 10 L Niskins. The contents of each Niskin was transferred, using a peristaltic pump, through a 0.05 µm filter cartridge to 50 L

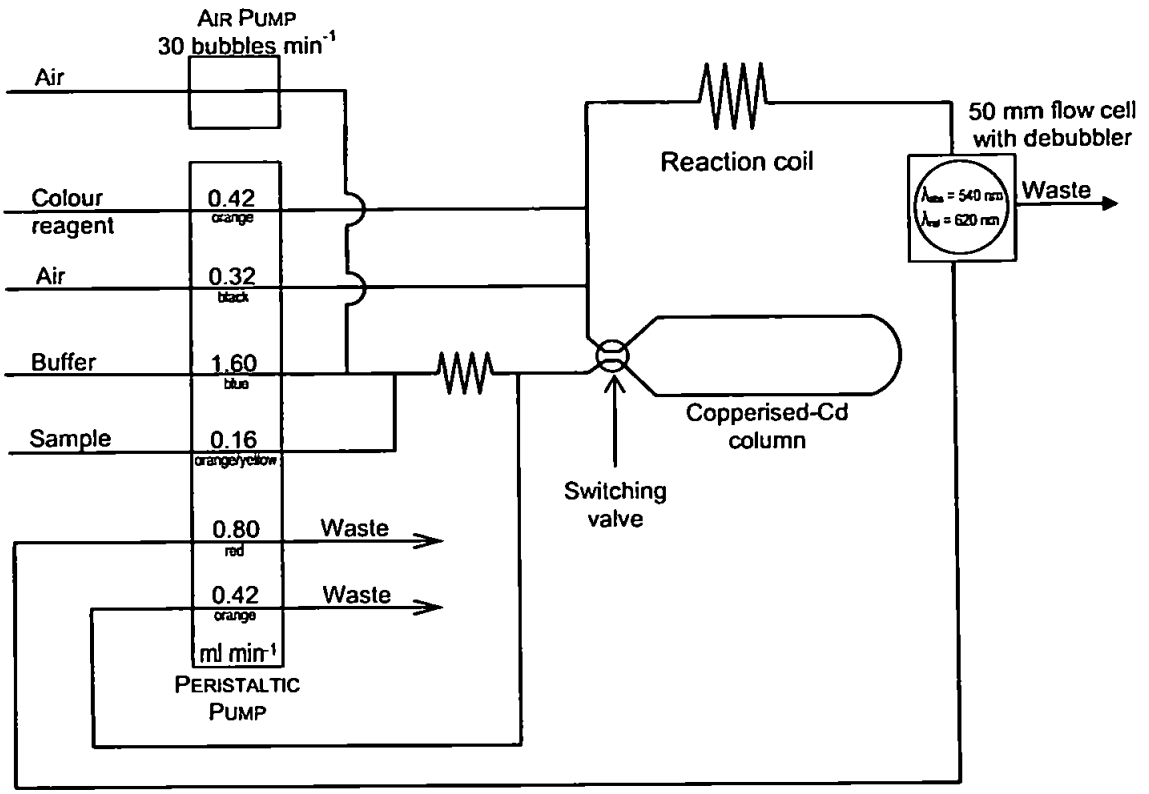


Figure 2.3: Flow diagram for nitrate/nitrite determination using segmented flow analysis. The determination of nitrite only was carried out by by-passing the copperised-Cd column.

carboys. The water was homogenised in a 400 L tank bottled in 50 mL aliquots (in 60 mL bottles) on 11 and 12 July 1996, then sealed and irradiated. The bottles were irradiated on 16 July 1996. It was considered insufficiently homogeneous and was reblended and bottled on 3 April 2001.

2.2.4 Autoclave digestion of phosphorus and nitrogen species in natural waters

2.2.4.1 Efficiency of digestion of phosphorus compounds

A working solution of 95 μg L^{-1} P of each selected P model compounds (ATP, sodium tripolyphosphate, phytic acid) and KH_2PO_4 was prepared from appropriate stock solutions. 5 mL alkaline persulphate oxidising reagent was added to 50 mL of each P model

compound solution in an autoclave bottle and autoclaved at 121 °C for 30 min. Phosphate standard solutions (0 - 125 μg L^{-1} P) were also autoclaved. Samples were allowed to cool, then analysed for FRP using the Skalar SAN Plus flow analyser.

2.2.4.2 Efficiency of digestion of nitrogen compounds

A 2.5 mg L^{-1} N solution of each selected N model compounds (EDTA, L-cysteine, nicotinic acid, urea) and sodium nitrate was prepared from appropriate stock solutions. 5 mL alkaline persulphate oxidising reagent was added to 50 mL of each N model compound solution in an autoclaved bottle and autoclaved at 121 °C for 30 min. Nitrate standard solutions (0 – 5 mg L^{-1} N) were also autoclaved. Samples were allowed to cool, then analysed for nitrate using the Skalar SAN Plus flow analyser.

2.3 Results and Discussion

2.3.1 Analytical performance of the Skalar SAN Plus Segmented Flow Auto Analyser

The accurate determination of phosphorus and nitrate/nitrite concentrations in natural waters is essential when providing data for assessing the health of aquatic ecosystems (Hanrahan et al., 2002; Zhang et al., 1999; Aston, 1980), investigating the biogeochemical cycling of N and P and monitoring compliance with legislation (CEC, 1975). Analytical procedures must be characterised with respect to analytical performance and the factors which influence the analytical performance. This is especially important given the complex nature of environmental matrices and the role of environmental data in informing and influencing monitoring programmes and environmental management strategies. Therefore, quantitative charcterisation of the linear range, the limit of detection of the method (the lowest analyte concentration that can be detected with a particular degree of confidence), repeatability, reproducibility and other analytical figures of merit that typify

an analytical method is necessary to describe the conditions for which accurate and precise results can be obtained.

All environmental samples should be filtered prior to analysis of nitrate and phosphate in the dissolved phase. The common operational definition of dissolved nutrients refers to the nutrients contained in the fraction of a water sample which passes through a 0.45 μm filter (Auflitsch et al, 1997; Lyddy-Meaney et al., 2002; Hanrahan et al., 2001 a; Gardolinski et al., 2001). In this study, all water samples were filtered using 0.45 μm cellulose acetate membrane filters within 24 h of collection.

The Skalar SAN Plus analyser is a commercially available air-segmented continuous flow system configured for wet chemical analysis, including the analysis of nitrate/nitrite and FRP in aqueous solution. FRP detection is based on the reaction of FRP with molybdate under acidic conditions (pH < 2) to produce phosphomolybdic acid (Baldwin, 1998; Drummond and Maher, 1995); this heteropolyacid is then reduced by ascorbic acid to form a blue complex (Drummond and Maher, 1995) that can be detected spectrophotometrically at 880 nm:

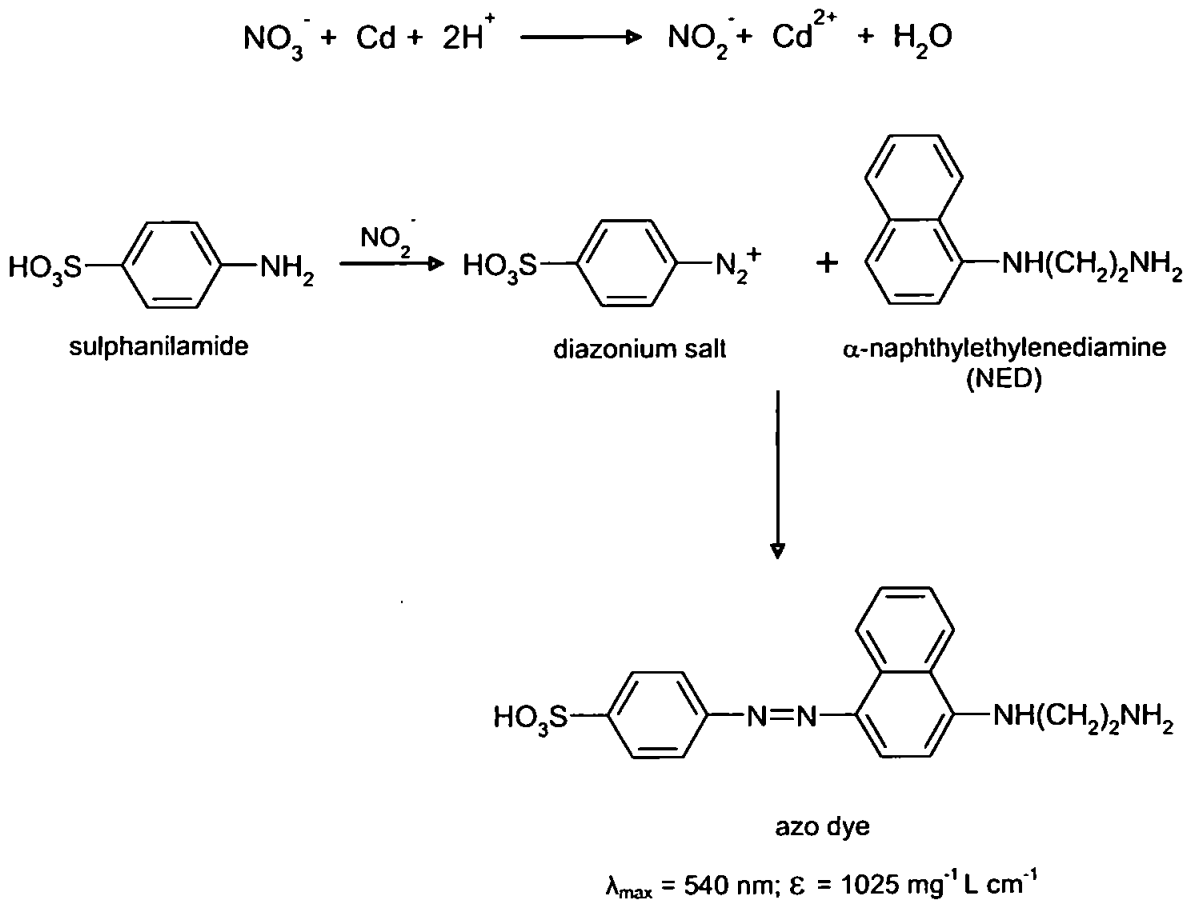
$$12\ MoO_4^{2-} + PO_4^{3-} + 27\ H^+ \longrightarrow H_3PO_4(MoO_3)_{12} + 12\ H_2O$$

$$H_3PO_4(MoO_3)_{12} \xrightarrow{\text{Ascorbic acid}} \text{Phosphomolybdenum blue complex [Mo(V)]}$$

The molybdate reagent used in the Skalar FRP manifold contains potassium antimony tartrate, which acts as a catalyst for the reduction step (Broberg and Pettersson, 1988). Ascorbic acid on its own reduces the heteropolyacid to form the blue colour very slowly; the addition of antimony increases the rate of reduction of the complex (Murphy and Riley, 1962). Drummond and Maher (1995), in an investigation of the effect of the $[H^+]/[MoO_4]$ ratio on colour development, found that ratios ranging from 45 – 80 gave a constant response at all pH investigated; a ratio of 70 ± 2 was recommended. The $[H^+]/[MoO_4]$ ratio for the Skalar in this work was 73, with a pH of 0.42; these conditions are typical of

methods using reagent concentrations described by Murphy and Riley (Murphy and Riley,1962; Drummond and Maher, 1995).

Many existing FI methods for the determination of nitrate and nitrite in water are based on the simultaneous determination of both species (Mikuška and Večeřa, 2003; Cerdà et al., 1998) and most are based on the conversion of nitrate to nitrite with subsequent diazotisation-coupling of an aromatic amine under acid conditions to form the highly absorbing azo-dye from which the nitrite concentration can be determined (Mikuška and Večeřa, 2003; Moorcroft et al., 2001). The Skalar nitrate/ nitrite manifold used in this work is based on this described chemistry. The simultaneous determination of nitrate and nitrite is based on complete reduction of nitrate to nitrite using a copperised-Cd column; the nitrite then forms a diazo compound with sulphanilamide under acidic conditions and the diazo compound couples with NED to form the pink-coloured azo dye which is detected spectrophtometrically at 540 nm (Moorcroft et al., 2001). The overall reaction, also known as the Greiss reaction, is shown below:



The Skalar nitrate/nitrite manifold was configured such that flow of the buffered sample stream can be directed through the copperised-Cd column for the simultaneous determination of nitrate and nitrite species, or bypasses the column for the determination of nitrite only (Figure 2.3).

Typical analytical figures of merit for the performance of the Skalar San Plus flow analyser for the determination of FRP, nitrate/nitrite and nitrite in aqueous solution are outlined in the Table 2.1. The limits of detection (LODs) were calculated using 3 standard deviations of the slope, and represent the smallest analyte concentration that can be detected with a 99 % degree of confidence (Miller and Miller, 1992). The limits of detection described for the FRP and nitrate/nitrite manifolds in Table 2.1 are higher than the LODs of 2 $\mu g\ L^{-1}$ P and 0.1 $mg\ L^{-1}$ N, respectively, described by the manufacturer. A gradual decline in instrument performance can be distinguished only if analytical performance is recorded regularly. The linear range of the FRP and nitrate/nitrite manifold have been noted over a 2-year period, and analytical performance with respect to detection limit and linear range was not significantly different ($p<0.05$) from the figures reported in Table 2.1. The limit of quantitation (LOQ), determined from 10 standard deviations of the slope, is regarded as the lower limit for precise quantitative measurements (Miller and Miller, 1992).

The linear ranges reported for FRP and nitrate/nitrite are wide enough for the measurement to be useful for determining concentrations in most surface waters, including P and N concentrations in oligotrophic, mesotrophic and eutrophic stream waters as defined by Smith et al. (1999) and the UK Environmental Agency (EA, 1998 a) in Table 1.2 (Section 1.1.3). However, nitrite concentrations in natural waters and nitrate/nitrite concentrations in seawater are often lower than the detection limit reported in Table 2.1. Nitrite concentrations for the Tamar catchment at Gunnislake on the Tamar River ranged from less than 10 $\mu g\ L^{-1}$ to 110 $\mu g\ L^{-1}$ N over a 27 year period from 1974 to 2002 (Section 3.3).

Table 2.1: Typical analytical performance of the FRP and nitrate/nitrite manifolds of the Skalar SAN Plus flow analyser

	Determinand		
	FRP	**nitrate/nitrite**	**nitrite**
Sample throughput (h^{-1})	20	20	20
LOD	3.0 µg L^{-1} P	0.45 mg L^{-1} N	0.34 mg L^{-1} N
LOQ	10.0 µg L^{-1} P	1.51 mg L^{-1} N	1.14 mg L^{-1} N
Linear range	3 – 150 µg L^{-1} P	0.45 – 5.0 mg L^{-1} N	0.34 – 5.0 mg L^{-1} N
Linear relationship	y = 14.34x - 16.47 (y = absorbance in arbitrary digital units ; x = concentration (µg L^{-1} P))	y = 1024.9x + 170.46 (y = absorbance in arbitrary digital units ; x = concentration (mg L^{-1} N))	y = 1006.1x + 99.7 (y = absorbance in arbitrary digital units ; x = concentration (mg L^{-1} N))
Regression coefficient (r^2)	0.9998	0.9932	0.9961
SD (n=3)	1.66 µg L^{-1} (40 µg L^{-1} P)	0.003 mg L^{-1} (1 mg L^{-1} N)	0.004 mg L^{-1} (1 mg L^{-1} N)
	1.48 µg L^{-1} (100 µg L^{-1} P)	0.004 mg L^{-1} (4 mg L^{-1} N)	0.006 mg L^{-1} (4 mg L^{-1} N)
% RSD (n=3)	3.2 (40 µg L^{-1} P)	0.4 (1 mg L^{-1} N)	0.4 (1 mg L^{-1} N)
	1.2 (100 µg L^{-1} P)	0.1 (4 mg L^{-1} N)	0.2 (4 mg L^{-1} N)

LOD calculated as the analyte concentration giving a signal equal to the blank signal plus three standard deviations of the slope (Miller and Miller, 1992).

LOQ calculated as the analyte concentration giving a signal equal to the blank signal plus ten standard deviations of the slope (Miller and Miller, 1992).

The Skalar nitrate/nitrite manifold can be reconfigured to measure 2 – 100 µg L^{-1} N by increasing the sample flowrate (1.4 ml min^{-1}), reducing the buffer flowrate (0.8 ml min^{-1}) and increasing the buffer concentration (50 mg L^{-1} ammonium chloride). According to the EA (1998 a), FRP concentrations in oligotrophic, mesotrophic and meso-eutrophic surface waters range from 20 – 200 µg L^{-1} P, with eutrophic waters being defined as greater than 200 µg L^{-1} P. FRP concentration in the Tamar River at Gunnislake ranged from 10 µg L^{-1} to 500 µg L^{-1} P from 1974 to 2002, with a mean concentration of 72 µg L^{-1} P (Section 3.3). The Skalar FRP manifold can be used to determine such concentrations in freshwater, with dilution of samples containing greater than 150 µg L^{-1} P. The Skalar has

also been successfully used to quantify FRP and nitrate/nitrite in seawater (Willie and Scott, 2003) and is discussed in Section 2.3.2.

The Skalar showed good reproducibility in compliance accordance EU legislation, with within-day precision (n=3) of less than 5 % for the FRP manifold and less than 0.5 % for the nitrate/nitrite and nitrite manifold (Table 2.1). The instrument also exhibits good repeatability.

In order to assess the accuracy of analysis and allow comparison of data between laboratories, analytical procedures must include sufficient QA/QC procedures (Asmund et al., 2004). The Skalar SAN Plus flow analyser has several QA/QC procedures in built into the software programme such as regular maintenance of the flow injection manifold according to the manufacturer's guide, the inclusion of 'drift' samples into the sample table and monitoring the efficiency of the copperised-Cd reduction column. Regular maintenance of the Skalar SAN Plus flow analyser (Table 2.2) includes maintenance guidelines for the autoinjector, the chemistry unit, including pumps and pump tubing and adjusting the settings on the photometer after changing pump tubings.

Each sample table must be set up with a 'tracer', which refers to the highest standard concentration (> 2000 arbitrary digital absorbance units from baseline) to trigger a timing cycle within the software, followed by a QA/QC 'drift' sample at the start of a calibration, or the software will not interpolate sample data using the calibration standards. Manual manipulation of large data sets would be very tedious and is not recommended. A drift sample should be injected after every 12 sample injections and again at the end of the sample run. The software programme statistically determines whether there has been any baseline drift or change in analytical response over the analytical run period. The Skalar is a robust instrument used for high sample throughput. The QA/QC 'drift' samples are essential to the process of characterising instrument performance and response over prolonged periods of analysis, to identifying change in performance and can

Table 2.2: List of procedures included in the general maintenance of the Skalar SAN Plus flow analyser.

Skalar sub-unit	Maintenance	Schedule
SA 1050 auto injector	Clean standard solution containers with cleaning reagent, e.g. 5 % hypochlorite	weekly
	Inspect pump tubing for capacity, elasticity, elasticity, algal growth and contamination. Tubing replaced if necessary.	weekly
	Inspect sample transmission tubing (needle to analysis system) for algal growth and contamination. Tubing replaced if necessary. Needle cleaned with 'pinners'	weekly
	Replace pump tubing	monthly
	Replace rinsing liquid supply tube	every 3-months
	Replace rinsing liquid discharge tubes, rinsing vessel to pump, and pump to waste container	every 3-months
	Replace springs that guide the 'Z' cable to the injector needle	annually or every 20,000 sampling movements
Chemistry analysis unit	Grease peristaltic pumps if necessary	daily
	Replace overstretched pump tubing on the peristaltic pumps, or use the extra bridge on each tubing (3-bridge tubing) to renew tension.	recommended every 2 – 3 months
		tubing inspected monthly and replaced as necessary
6250 Photometer	Adjust the settings on the photometer after changing pump tubings	As necessary

indicate the need for troubleshooting the system.

The efficiency of the copperised-Cd column was assessed by comparing the digital response of the Skalar to 5 mg L^{-1} NO_3-N and 5 mg L^{-1} NO_2-N. The efficiency was considered acceptable if the NO_3-N response was within ± 2 % of the response obtained for the NO_2-N standard. Efficiency was noted after every 20 - 25 h of operation. Figure 2.4 shows the change in sensitivity of the nitrate/nitrite determination over a 72 h period after using the manifold 24 h per day for 1 month, with new reagents made up every 24 hours. Therefore, the change in sensitivity can be attributed to the change in the efficiency of reduction by the copperised-Cd column. According to the reduction chemistry, cadmium is dissolved by the oxidation processes and this ultimately leads to loss of reductor material over time. Also, small cadmium particles dissolve first such that the overall specific

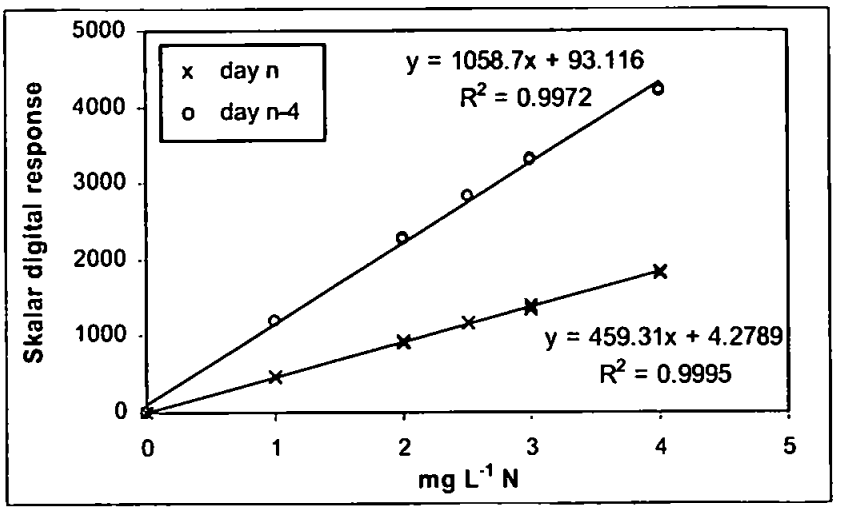


Figure 2.4: Calibration graphs for nitrate (as mg L^{-1} N) obtained on Day n-4 and Day n, with 99 % reduction efficiency on Day n-4 and 43 % reduction efficiency on Day n after approximately 72 hours later.

surface area available for reduction decreases rapidly (Gal et al., 2004) with an adverse effect on reduction efficiency (Gabriel et al., 1998). Gal et al. (2004) suggested regenerating the copperised-Cd column by successive injection of the acid and copper sulphate solutions initially used in the coating of the cadmium particles. However, in this research (regeneration using 4 M HCl and 2 % m/v copper sulphate as used in the initial coating of the cadmium material), this approach was not successful and a visible layer of copper was deposited on the column. Gal et al. (2004) also suggested that regeneration is only a temporary measure and repeated regeneration results in a shorter column life. A loss of reduction efficiency over time has been reported by several researchers and in discussions with manufacturers of FI equipment (Gal et al., 2004). The average lifetime of the copperised-Cd column for the Skalar varied from 1 to 8 months and was dependant on frequency of use and type of samples analysed. Gal et al. (2004) also noted similar findings.

The stability of the sulphanilamide/NED reagent is prolonged by storing the reagent in a dark bottle at 4 °C when not in use. Formation of the azo dye in the colour reagent prior to

introduction in the nitrate/nitrite manifold would result in decreased sensitivity of analysis. The use of "low-nitrite" o-phosphoric acid in the preparation of the colour reagent reduced the formation of the azo dye in the colour reagent. Therefore, as a precautionary measure, the absorbance of the colour reagent was monitored before use.

2.3.2 Quality assurance

Environmental monitoring and research programmes provide valuable data for assessing environmental quality and decision making. Therefore, accurate and reliable analytical results are essential to this process. Programmes involving multi-national participation and international databanks are especially concerned with quality control of data (Aminot and Kérouel, 1996) and require adequate QA/QC schemes to ensure the data integrity necessary in the comparison of data from various sources. Adherence to QA guidelines, participation in interlaboratory studies, use of reference materials (RMs) and certified reference materials (CRMs) are all means of achieving QA/QC of data quality (Quevauviller, 1998).

Certified Reference Materials (CRMs): The use of CRMs in analytical methodology is the most efficient way to measure and control accuracy (Benoliel and Quevauviller, 1998) and can help produce reliable calibration and validation of measurement procedures; quality control; verification of standardised methods; and the development and validation of new methods (Hanrahan et al., 2002). A CRM is a reference material for which component values have been certified by a technically valid procedure and is accompanied by or traceable to a certificate or other documentation issued by a certifying body (ISO Guide, 1981; Taylor 1990). CRMs can be either calibration CRMs, which are high purity substances or synthetically prepared mixtures, or matrix-matched CRMs which may be natural samples or artificial samples simulating the composition of natural samples (Benoliel and Quevauviller, 1998).

The suitability of CRMs for verifying accuracy depends on, among other factors, the degree of similarity of their matrix concentration and analyte concentrations to those expected for real samples (Quevauviller, 1996). However, few CRMs are available on the international market for the determination of P species in environmental matrices, despite the need for such QA/QC materials in routine environmental analysis and quality control (Quevauviller, 1998). In this thesis, a catalogue of CRMs currently available on the international commercial market for the analysis of P in environmental matrices has been compliled and is presented in Table 2.3, and for the analysis of N in environmental matrices in Table 2.4. CRMs are not currently available for all environmental matrices routinely analysed for P and N species, such as estuarine waters, nor do they adequately span the range of P and N concentrations characteristic of environmental matrices.

Prior to the development of MOOS-1, which became commercially available in 2004, no seawater CRM was available for N and P species. The National Research Council (NRC) of Canada recognised and urgent need for CRMs for nutrients, including FRP and nitrate/nitrite, for use in the marine sciences (Clancy and Willie, 2004). MOOS-1, a natural seawater CRM available for the determination of nutrients in seawater, was developed in direct response to this need and is described by Clancy and Willie (2004).

Interlaboratory comparison studies: Interlaboratory comparison studies are essential features of method development and validation (Hanrahan et al., 2002). These studies can also play an important role in the certification of reference materials, which depend on collaborative certification (Emons et al., 2004), such as described by Clancy and Willie (2004) for MOOS-1. Participation can also identify best practice with respect to method, sample preparation and sample storage and can serve to identify training needs. Such exercises involve several laboratories analysing one or more homogeneous and stable materials under specified conditions, the results of which are compiled, compared and put into a single report. *Z*-scores (Thompson and Wood, 1993) have been widely used as an

Table 2.3: Commercially available CRMs for the determination of phosphorus species in environmental matrices

CRM	Matrix	Phosphorus species	Concentration	Comments	Supplier	Reference
MOOS-1	Seawater	Orthophosphate	1.56 ± 0.07 μmol L^{-1}	Natural seawater sample, of Cape Breton Island, NS, Canada at a depth of 200 m	NRC	Clancy and Willie, 2004 and 2003
QC RW1	Freshwater	Orthophosphate	100 μg L^{-1}	Artificial sample, distributed as an ampoule to be 100 times with pure water	VKI	VKI Chemicals; Merry 1985
QC RW2	Freshwater	Total phosphorus	200 μg L^{-1}	Artificial sample, distributed as an ampoule to be 100 times with pure water	VKI	VKI Chemicals; Merry 1985
Australian natural water CRM	Natural water/ freshwater	Orthophosphate	27 ± 0.8 μg L^{-1}	Natural water sample obtained from Christmas Creek in the Lamington National Park, QLD, Australia	Queensland Health Scientific Services	
		Total dissolved phosphorus	37 ± 1.2 μg L^{-1}			
BCR-616	Groundwater (high carbonate content)	Orthophosphate	3.36 ± 0.13 mg kg^{-1}	Artificial groundwater sample, prepared from ultrapure water, to which required salts were added; stabilised by autoclaving	BCR	www.irmm.jrc.be
SRM®-2702	Marine sediment	Total phosphorus	0.1552 ± 0.0.0066 %	Material for SRM® was collected from Chesapeake Bay,USA, freeze-dried, seived at 70 μm (100 % passing) and cone blended, then radiation sterilised and bottled	NIST	www.nist.gov
SRM®-1646a	Estuarine sediment	Total phosphorus	0.027 ± 0.0.001 %	Material for SRM® was dredged from Chesapeake Bay, USA, freeze-dried, lightly deagglomerated and < 1 mm fraction ball milled and the < 75 μm blended and bottled	NIST	www.nist.gov
BCR-684	River sediment	NaOH-extractable P	500 ± 21 mg kg^{-1}	Material for the CRM was collected from the lower reaches of the River Po, Italy, then sieved and the < 2mm fraction was dried, lightly deagglomerated, crushed and hammer-milled and < 90 μm blended and bottled	BCR	www.irmm.jrc.be
		HCl-extractable	536 ± 28 mg kg^{-1}			
		Inorganic P	1113 ± 24 mg kg^{-1}			
		Organic P	209 ± 9 mg kg^{-1}			
		Conc. HCL-extract. P	1373 ± 35 mg kg^{-1}			

Table 2.4: Commercially available CRMs for the determination of nitrogen species in environmental matrices

CRM	Matrix	Nitrogen species	Concentration	Comments	Supplier	Reference
MOOS-1	Seawater	Nitrate/nitrite	23.7 ± 0.9 μmol L^{-1}	Natural seawater sample, of Cape Breton Island, NS, Canada at a depth of 200 m	NRC	Clancy and Willie, 2004 and 2003
BCR-479	Freshwater	Nitrate	214 ± 4 μmol kg^{-1}	artificial freshwater with a relatively low level of nitrate	BCR	www.irmm.jrc.be
BCR-480	Freshwater	Nitrate	885 ± 13 μmol kg^{-1}	artificial freshwater with a relatively high level of nitrate	BCR	www.irmm.jrc.be
TROIS-94	Freshwater	Ammonia	0.03 ± mg L^{-1}	a bulk sample, collected from Trois Rivieres, Quebec in 1994, was centrifuged and filtered. Nitrite is not known to be present in this sample.	Environment Canada	www.nwri.ca
	(natural river water)	Nitrate	0.07 ± mg L^{-1}			
		Nitrate + nitrite	0.07 ± mg L^{-1}			
		Total Kjeldahl Nitrogen (TKN)	0.24 ± mg L^{-1}			
BATT-01	River water (hard)	Nitrate + nitrite	0.046 ± mg L^{-1}	sample collected from Battle River, Saskatchewan.	Environment Canada	www.nwri.ca
BCR-616	Groundwater (high carbonate content)	Nitrate	50.4 ± 0.9 mg kg^{-1}	artificial groundwater sample prepared from ultrapure water, to which required salts were added; stabilised by autoclaving.	BCR	www.irmm.jrc.be
BCR-617	Groundwater (low carbonate content)	Nitrate	25.8 ± 0.5 mg kg^{-1}	artificial groundwater sample prepared from ultrapure water, to which required salts were added; stabilised by autoclaving.	BCR	www.irmm.jrc.be
ION-20	Fortified lake water (hard)	Nitrate + nitrite	0.043 ± mg L^{-1}	sample collected from Lake Ontario, clarified, diluted then fortified with various salts. ION-20 is diluted 5 times before it is used as a CRM	Environment Canada	www.nwri.ca
DORSET-94	Lake water (soft)	Nitrate	0.005 ± mg L^{-1}	formerly named PLASTIC-94. Sample collected from Plastic Lake, near Dorset, Ontario, then filtered in the lab.	Environment Canada	www.nwri.ca
ION-915	Lake water (soft)	Nitrate + nitrite	0.343 ± mg L^{-1}	bulk sample collected from Lake Superior, in northern Ontario.	Environment Canada	www.nwri.ca
BMOOS-1	Lake water (soft)	Nitrate + nitrite	0.41 ± mg L^{-1}	bulk sample collected from Big Moose Lake, New York.	Environment Canada	www.nwri.ca

analytical tool for the statistical assessment of data to give a comparative indication of performance in intercomparison exercises, with $|z|<2$ indicating satisfactory performance (Wells, 1994; Aminot and Kirkwood, 1994; Kirkwood et al., 1996; Clancy and Willie, 2003; Mc Govern, 2002).

Laboratory performance in the intercomparison studies conducted by NOAA/NRC in 2000 and 2002 (Clancy and Willie, 2003; Gardolinski et al., 2002) was used to assess capabilities of international laboratories to quantify nutrients in seawater for the development of MOOS-1 (Clancy and Willie, 2004). Analysis of MOOS-1 in 2002 was carried out by the NRC as well as 24 expert laboratories participating in the 'NOAA/NRC 2nd annual intercomparison study for nutrients' (Clancy and Wille, 2003). Labs were selected on the basis of their previous satisfactory performance in the NOAA 2000 intercomparison study. Participating laboratories, including the University of Plymouth, were sent one bottle of MOOS-1 and one bottle of a nutrient seawater sample Nutrient 2002, which was a control sample. The assigned analyte concentrations for MOOS-1 were known while the concentrations for the Nutrient 2002 were unknown to the participants.

Analytical methods were not specified and the methods used included both flow and manual methods. However, all methods for the determination of FRP and for nitrate/nitrite were based on the spectrophotometric procedures of Strickland and Parsons (1972). FRP and nitrate/nitrite data received from each participating laboratory was used to calculate z-scores for each determinand. The z-score is a comparison of the bias estimate for each analyte, calculated from the difference between the mean analyte concentration obtained by the participating laboratory (x_i) and the assigned mean ($\bar{x}$) which was calculated from laboratory analyses prior to the intercomparison study, with a target value for the standard deviation, σ_{target}:

$$z = \frac{x_i - \bar{x}}{\sigma_{target}}$$

In the NOAA 2002 intercomparison study, a target standard deviation (σ_{target}) of 5 % was selected. The assigned mean for FRP was 1.61 ± 0.21 µM and for nitrate/nitrite was 24.1 ± 1.7 µM.

Each participating laboratory was sequentially assigned a number on receipt of data. The University of Plymouth was assigned number 24, and the NRC laboratory number 25. Of twenty-five laboratories participating, eighteen (including Plymouth) achieved satisfactory *z*-scores for the determination of phosphate and twenty-two of twenty-four laboratories (including Plymouth) achieved satisfactory performance *z*-scores for the determination of nitrate/nitrite in the MOOS-1 seawater sample as shown in Figures 2.5 a and b, respectively (Clancy and Wille, 2003). The MOOS-1 sample was analysed at the University of Plymouth using the Skalar SAN Plus segmented flow analyser and had *z*-scores of 0.22 and -0.21 for FRP and nitrate/nitrite analyses, respectively. These z-scores were well within the limits of satisfactory performance and confirmed that the Skalar SAN Plus analyser is a reliable method for determining FRP and nitrate concentration in seawater samples. It also confirms that the Skalar provides an accurate, reliable and precise method for the determination of FRP and nitrate/nitrite in natural waters and reinforces the quality of data collected in freshwater and estuarine surveys analysed using the Skalar.

National and regional laboratory intercomparison schemes: The QUASIMEME project (Quality Assurance of Information for Marine Environmental Monitoring in Europe), now QUASIMEME Laboratory Performance Studies, was established to assist EU labs to develop their quality assurance/quality control (QA/QC) procedures in order to satisfy the data quality requirements of monitoring programmes in which they participate such as the International Marine Monitoring Programmes of the Oslo and Paris Commissions (OSPARCOM), the Helsinki Commission (HELCOM) and the Mediterranean Research and Pollution Monitoring Plan (MEDPOL) of the Barcelona Convention (Wells and

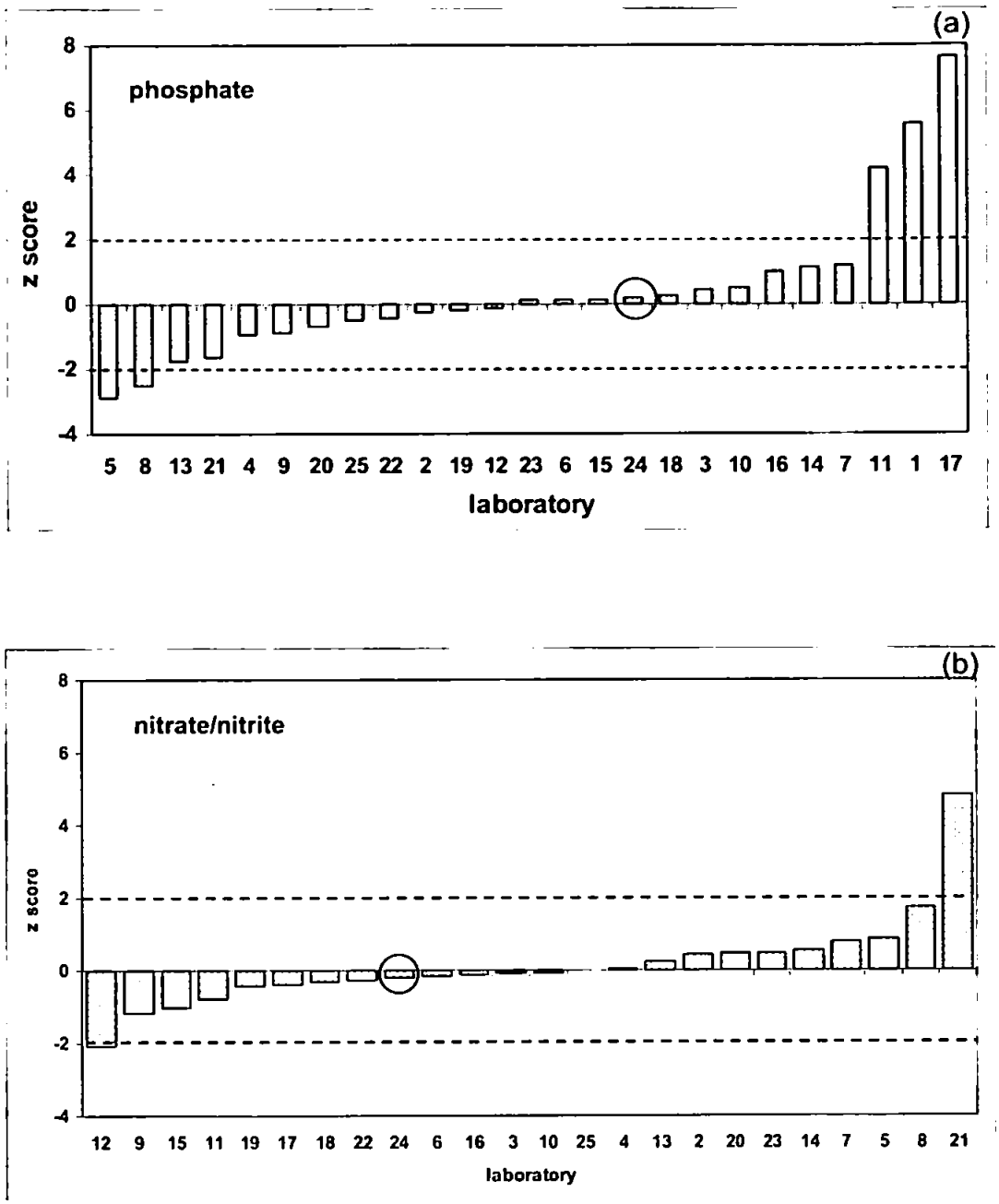


Figure 2.5: Plot of *z*-scores obtained by laboratories participating in the NOAA 2002 intercomparison study for the analysis of (a) orthophosphate, and (b) nitrate/nitrite in MOOS-1. *Z*-scores were calculated from the mean orthophosphate concentration (assigned value set at 1.61 ± 0.21 μM) and the mean nitrate/nitrite concentration (assigned value set at 24.1 ± 1.7 μM). $|z| \leq 2$ represent the satisfactory *z* score value for MOOS-1 (Clancy and Willie, 2004; Clancy and Willie, 2003). The *z*-score for the University of Plymouth data is highlighted by ◯.

Cofino, 1997; Aminot et al., 1997). Initially funded by the EU (1992-1996), the programme continues by subscription of participating institutes and all institutes, worldwide, involved in chemical measurements in seawater can participate. The laboratory programmes for proficiency testing of most determinands are conducted twice per year and routinely include aqueous test materials containing orthophosphate and total phosphorus at concentrations similar to those found in estuaries, coastal and open water environments (QUASIMEME Laboratory Performance Studies, 2003). Regular testing is necessary to assure the quality of environmental data submitted since the performance of many laboratories does not remain constant (Wells, 1994; Wells and Cofino, 1997). The assessment of the quality of data must be made at the time that the environmental samples are analysed. Such exercises provide vital information for improving the quality and performance of laboratories and provide a structure for strong analytical technique. To this end, QUASIMEME Laboratory Performance Study was designed to follow the IUPAC/ISO/AOAC international protocol for international testing (Thompson and Wood, 1993; ISO/IEC, 1996). All laboratories that submit data to the UK National Marine Monitoring Programme (NMMP) routinely participate in the QUASIMEME Laboratory Performance Studies as a means of external QA/QC of the data collected, including orthophosphate (National Marine Monitoring Programme Working Group, 2003).

Databases: Environmental monitoring and research programmes generate large amounts of data and can provide a valuable database for analytical information if QA/QC measures are used to preserve data quality. Databases have been generated from of the NMMP monitoring programme and the 'Winter Monitoring of the Western Irish Sea' (McGovern, 2002); both programmes incorporated QA/QC schemes to ensure data integrity. Legislation such as the European Commission Water Framework Directive (EC WFD) outlines an approach for managing water quality for the member states of the European Union and will require monitoring and environmental quality data collected by member states to be reported at the EU level. There is vast potential here to add to the repository of

data already held by the European Environmental Agency. Adherence to QA/QC practices such as intercomparison studies, in conjunction with routine in-house use of RMs and CRMs, can provide a robust dataset for nutrients in environmental matrices on a multinational level.

2.3.3 Interferences in the determination of P

Many components of natural waters may interfere with phosphate analysis (Blomquist and Westin, 1998). Interferences such as arsenate (As(V)) and silicate can also form molybdate complexes and can cause an overestimation of FRP in natural waters. Other interferences such as Cu(II), Fe(III), Al(III) and Ca(II) combine with orthophosphate ion and reduce the amount of FRP available to react with the molybdate, causing an underestimation of FRP (Peat et al., 1997). Other interferences in the analysis of FRP in natural waters include chromium, nitrate, nitrite, and sulphide (Worsfold et al., 2005; Neal et al., 2000).

Silicate: A high ratio of silicate to phosphate is often encountered in rivers, lakes and groundwaters resulting from the dissolution of ubiquitous amorphous silica minerals (Zhang et al., 1999; Garrels and Christ, 1965). The interference from high silicate can overwhelm the phosphate signal (Zhang et al., 1999) in FRP analysis. Zhang et al. (1999), in an exhaustive study of the effects of temperature and pH on the interference of silicate in FRP determination, showed that as temperature increases above 40 °C silicate interference increases. Drummond and Maher (1995) also reported that at room temperature (20 – 25 °C) up to 10 mg L^{-1} Si can be present without causing over-estimation of the FRP concentration. However, 100 mg L^{-1} Si resulted in a 25 % over-estimation of the FRP. Silicate in streams and rivers are generally less than 5 mg L^{-1} Si but may be as high as 15 mg L^{-1} (Robards et al., 1994). Campbell and Thomas (1970) showed that at the elevated temperature and pressure necessary for autoclaving samples in the acidic persulphate

digestion of P, silicate is polymerised and becomes non-reactive to the molybdate reagent in the subsequent FRP determination.

Most methods used for FRP determination in natural waters are based on Murphy and Riley (1962), with reagent composition differing between methods. Therefore, it is necessary to determine whether a particular method is susceptible to silicate interference, and it has also been recommended that individual laboratories assess methods to determine if there is silicate interference in their FRP method (Neal et al, 2000).

In the present study, the potential interference of silicate (0-10 mg L^{-1} Si) on the determination of FRP in natural waters using the Skalar SAN Plus autoanalyser was quantified by measuring apparent phosphate concentration in samples containing only silicate and in samples containing silicate and phosphate. Silicate concentrations of 0-15 mg L^{-1} Si are typical of natural waters (Zhang et al., 1999) and span the range of silicate concentrations recorded for the Tamar River; the highest silicate concentration recorded for the Tamar River from 1974 to 2004 was 3.9 mg L^{-1} Si. Investigations were carried out at fixed temperature (45 °C) and pH (final concentration pH 0.42). These investigations showed that silicate concentrations up to 10 mg L^{-1} Si did not affect the determination of P (0-100 µg L^{-1} P) (Table 2.5). Studies by Zhang et al. (1999) showed that for pH< 0.78, silicate interference increased with temperatures above 40 °C, however, such a trend was using the Skalar FRP method (45 °C). Therefore, silicates in freshwater samples from the Tamar River are not expected to cause any measurable interference in the analysis of FRP in these samples.

Early studies by Koroleff (1983) found that absorbance due to silicate increased with time since the development of the silicomolybdenum blue is slower than that of the phosphomolybdenum blue complex. In manual analysis the time interval between adding reagents and measuring the absorbance is not always constant from one sample to the next, resulting in different degrees of interference. However, automated flow analysis

Table 2.5: Effect of silicate on the calculated phosphate concentration

[Si] added (mg L^{-1} Si)	[P] added (ug L^{-1} P)	Mean P measured (ug L^{-1} P)	% detected
0	0	1.3	-
0.5	0	1.0	-
1	0	0.8	-
1.5	0	0.6	-
2	0	0.5	-
3	0	0.7	-
5	0	0.5	-
10	0	0.6	-
0	80	80.1	100.0 ± 0.2
1	80	80.9	101.0 ± 0.3
2	80	82.1	102.5 ± 0.2
3	80	81.6	101.9 ± 0.1
5	80	81.5	101.8 ± 0.0
10	80	81.8	102.2 ± 0.1

instrumentation, such as the Skalar, provide precise timing with respect to addition of sample to reagents, reagent mixing and time of detection of the blue complex. In addition, the presence of antimony in the molybdate reagent increases the rate of formation of the phosphomolydenum blue complex, thus increasing the time difference between the formation of the slower-forming silicomolybdenum blue and the antimonylphosphomolybdenum blue complex (Zhang et al., 1999).

Arsenate: Arsenate forms similar complexes with molybdate as phosphate and, therefore, can positively interfere in the measurement of phosphate (Linge and Oldham, 2001). Arsenate interference may be of concern in areas affected by arsenic contamination (Linge and Oldham, 2001). The Tamar River at Gunnislake was the area selected in this project for studying the techniques used for quantifying and understanding the biogeochemistry of nutrients in the Tamar River catchment. The dissolved arsenic concentrations reported for the Tamar River at Gunnislake in 2001 by the EA were higher than the expected

background concentrations for surface waters (Langston et al., 2003). Concentrations of dissolved arsenic at Gunnislake ranged from 1.5 – 5.5 μg L^{-1} As, with a median value of 4 μg L^{-1} As in 2001 (Langston et al., 2003). Tamar freshwater streams and adits are known to have elevated concentrations of dissolved arsenic, up to 115 μg L^{-1} As, which increases the dissolved As concentration in the waters of the Tamar at Gunnislake.

Arsenate interference in FRP measurement using the Skalar SAN Plus flow analyser was investigated by analysing a series of phosphate solutions with varying concentrations of arsenate and the apparent P concentration for each solution was calculated (Table 2.6). Sulphuric acid (2 % v/v) was added to ensure that As was present as arsenate. Arsenate concentration up to 10 μg L^{-1} As did not cause any measurable interference in the determination of P (0-125 μg L^{-1} P). However, 25 and 50 μg L^{-1} As showed a small negative interference in the measurement of P (Table 2.6). Arsenate concentrations in the Tamar catchment do not exceed 10 μg L^{-1} As (Langston et al., 2003) and therefore this method is suitable for the determination of FRP in the present study. Drummond and Maher (1995) also reported that arsenate concentrations less than 10 μg L^{-1} As did not cause any error in their measurements of FRP.

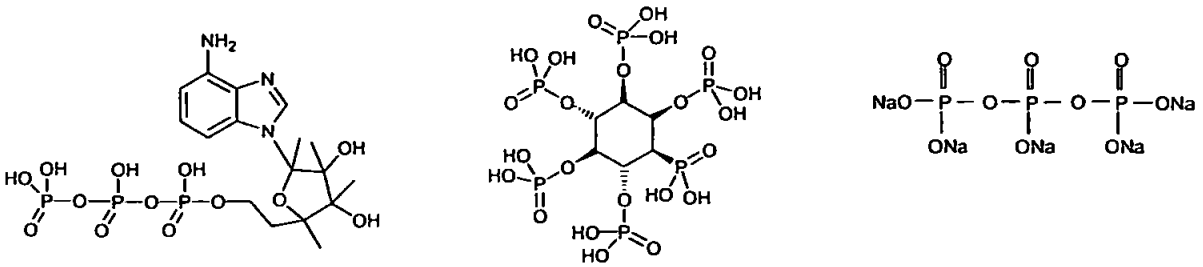
Hydrolysable P compounds: The analysis of FRP in natural waters is carried out in acidic media which can contribute to the hydrolysis of organic P compounds. Baldwin (1998) investigated the possibility of analysis-induced hydrolysis/release of dissolved organic P during the determination of FRP in environmental waters, using native P species isolated from a eutrophic wetland. Hydrolysis/release of organic P to FRP would elevate the overall amount of P detected in water samples, resulting in an overestimation of the FRP concentration in the natural waters being analysed. Such errors would be undesirable both from a QA/QC perspective and monitoring/legislative point of view. Investigations carried out by Baldwin (1998) showed that DNA, RNA, tripolyphosphate, and phytic acid (also called insositol hexaphosphate) were resistant to hydrolysis under the conditions used to determine FRP. However, phytic acid and adenosine monophosphate (AMP) were

Table 2.6: Effect of arsenate on the observed phosphate concentration

[As] added (ug L^{-1} As)	[P] added (ug L^{-1} P)	Mean P measured (ug L^{-1} P)	% P detected
0	0	0.0	-
1	0	-1.1	-
2	0	-1.2	-
5	0	-1.4	-
10	0	-1.0	-
25	0	-0.6	-
50	0	-0.5	-
0	40	40.4	100.0 ± 0.9
1	40	40.9	101.2 ± 0.6
2	40	41.1	101.7 ± 1.0
5	40	41.1	101.6 ± 0.7
10	40	40.4	100.0 ± 0.3
25	40	38.8	96.1 ± 1.7
50	40	38.3	94.6 ± 0.6

susceptible to 10 % and almost 100 % hydrolysis, respectively. These results indicate that not all environmentally relevant organic P compounds are refractory and some can be readily hydrolysed during FRP analysis. As a result, the possibility of analysis-induced hydrolysis/realease of organic P during the determination of FRP in aqueous solution using the Skalar SAN Plus flow analyser was investigated in this study. Solutions of adenosine triphosphate (ATP), phytic acid and tripolyphosphate (Figure 2.6), each containing 95 μL^{-1} P were analysed; potassium dihydrogen orthophosphate was used as the control. The final pH of the FRP channel on the Skalar was 0.42. These investigations showed that ATP, phytic acid and tripolyphosphate were resistant to hydrolysis under the conditions of analysis used in the Skalar method to determine FRP. Therefore, overestimation of FRP due to hydrolysis/release of organic P is unlikely during the determination of FRP using the Skalar method.

Other P interferences: Research has suggested that Cu(II) (Peat et al., 1997) and nitrate



ATP

(a)

Phytic acid

(b)

Sodium tripolyphosphate

(c)

Figure 2.6: Model P compounds (a) adenosine-5′-triphosphate (ATP); (b) Phytic acid (PTA), and (c) Sodium tripolyphosphate (STP)

and nitrite ions could interfere with the determination of FRP in natural waters (Worsfold et al., 2005; Neal et al., 2000). Since the analysis of FRP is often performed simultaneously with the nitrate/nitrite determination using the Skalar flow analyser, possible interference from environmental concentrations of nitrate/nitrite present in the Tamar were relevant to this study. Nitrate concentrations up to 1000 mg L^{-1} N and nitrite concentration up to 1000 mg L^{-1} N did not cause any measurable interference in the determination of P (0-100 µg L^{-1} P). The effect of high environmental concentrations of Cu(II) (0-50 mg L^{-1} Cu) on the determination of FRP (0-100 µg L^{-1} P) was also investigated and there was no measurable interference on the FRP determination using the Skalar method.

2.3.4 Interferences in the determination of N

Cerdá et al. (1998), in a study of interferences on the determination of nitrate/nitrite using sequential injection, observed loss of activity of the reduction column at high phosphate concentrations (≥ 50 mg L^{-1} P). This trend was not observed in other studies. Daniel et al. (1995 b) reported that the development of the azo-dye is considerably faster in seawater than in freshwater, and the reaction kinetics are such that the slope of the calibration is

independent of salinity between 17 and 34, but is 15 % lower at salinity 0. However, Galhardo and Massini reported negligible (< 0.15 %) interference from Na^+ and Cl^- ions.

The Skalar manual suggests that metals such as copper may have a negative interference on nitrate determination. Moorcroft et al. (2001) suggested that transition metal ions can reduce nitrite to nitric oxide. Such an occurrence within the reaction stream would cause less nitrite to be present to react with the chromogenic reagents and therefore the intensity of the signal be lower than the original nitrate/nitrite concentration present in the sample. The Skalar method suggests that such an interference can be eliminated by the addition of 1 g L^{-1} EDTA to the ammonia buffer when performing the nitrate analysis.

In the present study, the effect of salinity, phosphate and Cu(II) on the determination of nitrate/nitrite using the Skalar air-segmented flow analyser, was investigated (Table 2.7). Salinity did not affect the sensitivity of the analysis, of nitrate/nitrite as indicated by the limit of detection and linear range of the analysis which remained unchanged from salinity 0-35 (Table 2.7). Historical data indicate that dissolved Cu concentrations in the Tamar do not exceed 14 ug L^{-1} Cu (Langston et al., 2003). Experiments with the Skalar indicated that copper concentrations in the Tamar River would not adversely affect the nitrate/nitrite determination in these waters (Table 2.7).

Phosphate concentrations up to 10 mg L^{-1} P did not affect the determination of nitrate/nitrite but phosphate concentrations of 50 and 100 mg L^{-1} P caused a small negative interference (3 and 6 % respectively). However, there was no loss of activity of the reduction column after five injections and subsequent injections of 'drift' sample indicated no significant difference ($p<0.05$) in reduction efficiency of the column. Nevertheless, it is unlikely that such excessively high P concentrations would be encountered in freshwater systems. Long-term records (30 years) of FRP concentrations in the Tamar catchment indicate that this parameter does not exceed 500 µg L^{-1} P. Therefore, the small negative P interference on the determination of nitrate/nitrite was not expected to affect the analytical

Table 2.7: Effect of various ionic species on the nitrate/nitrite determination

Species	(mg/L)	added as	Mean measured N (mg L^{-1} N)	% N detected
phosphate	0	KH_2PO_4	2.46 ± 0.01	100.00 ± 0.170
phosphate	0.1	KH_2PO_4	2.49 ± 0.02	101.11 ± 0.304
phosphate	0.2	KH_2PO_4	2.48 ± 0.02	100.91 ± 0.293
phosphate	0.5	KH_2PO_4	2.48 ± 0.05	100.83 ± 0.738
phosphate	1	KH_2PO_4	2.47 ± 0.02	100.51 ± 0.243
phosphate	10	KH_2PO_4	2.47 ± 0.04	100.61 ± 0.569
phosphate	50	KH_2PO_4	2.40 ± 0.04	97.62 ± 0.576
phosphate	100	KH_2PO_4	2.33 ± 0.04	94.87 ± 0.642
Cu(II)	0.005	$Cu(NO_3)_2 \cdot 3H_2O$	2.42 ± 0.09	96.10 ± 3.74
Cu(II)	0.02	$Cu(NO_3)_2 \cdot 3H_2O$	2.63 ± 0.00	104.61 ± 0.18

Salinity	Matrix	Linear relationship§	Linear range
0	ultrapure water	y = 1024.9x + 170.46	0.5 – 5 mg L^{-1} N
17.5	ultrapure water/low nutrient seawater	y = 1030.2x + 152.16	0.5 – 5 mg L^{-1} N
35	low nutrient seawater	y = 1022.2x + 179.44	0.5 – 5 mg L^{-1} N

§ (y = absorbance in arbitrary digital units ; x = concentration (mg L^{-1} N)

performance of the method for analysing freshwater samples from the Tamar catchment.

Temperature is reported to influence the Greiss reaction above 30 °C (Gal et al., 2004), but the reaction is reported to be independent of temperature between 10 – 20 °C. The reactor on the Skalar nitrate/nitrite channel is not thermostated, however, the room is kept at a constant temperature of 22 °C. Therefore, the investigation of temperature on the rate of reaction and on the intensity of the signal produced was not considered relevant to this work. In addition, the method was successfully validated through participation in an interlaboratory comparison study (Section 2.3.2).

2.3.5 Determination of Total Nitrogen and Total Phosphorus

Total phosphorus (TP) and total nitrogen (TN) are often used as indicators of water quality

with respect to eutrophication and trophic status (Benson et al., 1994; Maher et al., 2002;Johnes and Heathwaite, 1997). TP data is used in the prediction of algal biomass (Jones and Lee, 1986; Vollenweider, 1968; Maher et al., 2002) and the TN:TP ratio has been used to predict algal species type (Maher et al., 2002; Seitzinger, 1988). TN and TP data are also frequently used to determine the nutrient loads from a water body or discharge system (Benson et al., 1994; Johnes and Hodgkinson, 1998; Johnes, 1996; Beaulac and Recknow, 1982; Johnes, 1990; Johnes and Heathwaite, 1997). Therefore, as with dissolved inorganic N and P species, it is important to obtain accurate TN and TP data. Nutrient load calculations can be used to determine nutrient export from river catchments or in nutrient discharge from sewage treatment works (Hanrahan et al., 2002). Consequently, these calculations and predictions can have environmental as well as economic implications regarding environmental management decisions, eg. the installation (or not) of costly phosphorus removal technology, or the impact of increased agriculture on water quality. There are also fewer interferences in the determination of TP in natural waters than for FRP as silicate is polymerised in the acidic persulphate digestion of P and becomes non-reactive to the molybdate reagent in FRP analysis (Campbell and Thomas, 1970).

Maher and Woo (1998) cited two important considerations to obtain accurate measurements: sample storage, and sample digestion. Stability of the various species in the water column may alter composition of the particulate/dissolved fractions. Water samples for FRP and TFP need to be filtered immediately on collection as adsorption/desorption onto particles may occur in the sample container (Maher and Woo, 1998) and FRP may also undergo biological conversion.

The determination of TN and TP in natural waters requires the use of digestive or oxidative processes to convert particulate, organic and condensed species to nitrate and phosphate, respectively (Maher and Woo, 1998). Many of the nitrogen and phosphorus species

present in natural waters contains bonds that need to be broken to release easily measurable N and P species: P—O—P, C—O—P and C—P bonds need to be broken down to release phosphorus as phosphate, which can then be determined using molybdenum blue chemistry (Murphy and Riley, 1962); N=N, C—N and C=N need to be oxidised to nitrate (Johnes and Heathwaite, 1998). The digestion step must also be able to release phosphorus and nitrogen from biological material such as algal cells and plant detritus and from species adsorbed or occluded onto particulate matter (Maher and Woo, 1998). Digestion techniques including microwave heating (Columbini et al., 1998; Johnes and Heathwaite, 1992; Maher et al., 2002), autoclaving (Valderrama, 1981; Ebina et al., 1983; Hansen and Koroleff, 1999; Maher et al., 2002), UV photo-oxidation (Roig et al., 1988), conventional heating (using hot-plate, sand bath or aluminium blocks) (Maher at al., 2002), dry ashing (OrnazaGonzalez and Statham, 1996), and fusion (APHA/AWWA, 1976). These have all been used in several studies in combination with a range of oxidative techniques such as perchloric acid (Harwood et al., 1969), sulphuric acid-nitric acid (APHA/AWWA, 1992), acid persulphate (Worsfold et al., 2005) and alkaline persulphate (Aminot and Kérouel, 1996 b; Hansen and Koroleff, 1999; Maher et al., 2002) (Worsfold et al., 2005; Maher and Woo, 1998). Recent literature suggests that alkaline persulphate oxidation is still the only suitable technique for the simultaneous determination of TN and TP in water and sediments (Columbini et al., 1998).

2.3.5.1 Alkaline persulphate digestion

Koroleff (1969/1970) introduced alkaline persulphate oxidation for determining TN in water. This method was later modified by D'Elia (1977), who introduced a buffer which kept the system at pH 9.7 at the beginning and 4-5 at the end, then further modified by Nydahl (1978). The digestion of TN and of TP both involved the use of persulphate, however, since these procedures were carried out at different pH (TN digestion in alkaline media; TP digestion in acidic media), they were performed as separate procedures

(Koroleff, 1977; Valderrama, 1981). In 1981, Valderama successfully carried out the simultaneous determination of TP and TN in natural waters using alkaline persulphate oxidation (Table 2.8) and obtained similar recoveries to Nydahl in a study of TN digestion efficiency (Nydahl, 1978): greater than 92 % recovery for nitrate, nitrite, ammonia, urea and some aliphatic acids on digestion, but poor yields for compounds with an N—N bond, and the oxidation of N=N to nitrate appeared to be entirely inhibited (Valderrama, 1981).

The alkaline persulphate oxidising agent breaks down organic compounds, releases phosphorus as phosphate and oxidises nitrogen to nitrate (Hansen and Korleff, 1999). $K_2S_2O_8$ decomposes in aqueous solution to produce oxygen and acid (Maher and Woo, 1998; Ebina et al., 1983):

$$K_2S_2O_8 + H_2O \rightarrow 2\,KHSO_4 + O_2$$

such that 2 mol of H^+ is formed per mol of $K_2S_2O_8$. The decomposition of persulphate increases with temperature and pH (Johnes and Heathwaite, 1992). Hence, reaction time is limited by the decomposition rate. For this reason these digestions are often carried out under increased pressure and temperature, eg. using an autoclave. Valderrama (1981) suggested that since the digestion time required is a function of the autodecomposition of persulphate, it need not be greater than 30 min if a temperature of 110-115 °C is used under high pressure.

In the presence of 1 mol sodium hydroxide per mol of $K_2S_2O_8$, the oxidising solution becomes acidic after oxidation due to the build up of H^+. This shift in pH allows simultaneous digestion of TN and TP using the alkaline persulphate digestion. Therefore the concentrations and relative proportions of sodium hydroxide and potassium persulphate in the oxidising reagent are critical in determining the efficiency of the digestion technique. Sufficient NaOH should be present to ensure an alkaline environment is maintained long enough for complete digestion of all N compounds in the sample, and sufficient persulphate should remain after the digestion of N compounds in the alkaline phase to ensure digestion of all organically bound phosphorus in the liquid sample during the acid

Table 2.8: Alkaline persulphate digestion methods for the simultaneous digestion of TN and TP

Matrix	pH	Digestion reactant	Digestion time	Digestion temperature	Model compounds	Comments	Reference
Natural waters	Initial: 9.7 Final: 5-6	oxidising rgt.: 50 g $K_2S_2O_8$ and 30 g M H_3BO_3 were dissolved in 350 mL NaOH and made up to 1 L deionised water 4 mL oxidising rgt. added to 30 mL sample	30 min	110-115 °C	N: ammonium chloride, urea, EDTA	Simultaneous detn of TN and TP: N recovery – greater than 92 % recovery; no significant difference from Nydahl (1978) Model compounds for testing efficiency of P digestion were not used	Valderrama, 1981
River water	Initial: 12.6 Final: 2.0	oxidising rgt.: 20 g $K_2S_2O_8$ and 3 g NaOH and were dissolved in made up to 1 L deionised water 5 mL oxidising rgt. added to 5 mL sample	30	120 °C	N: ammonium chloride, potassium nitrate; sodium nitrite; urea, EDTA; glycine, guanidinium carbonate; 4-AAP; creatinine; caffeine P: ATP, ADP, G-6-P-K_2; TSPP, STP, G-1-Na_2; β-GLY	N recovery – 97 - 100 % for all compounds except guanidinium carbonate (46.3-73.6%); 4-AAP (46.3 %); creatinine (89.0 %); caffeine (90.0 %) P recovery – 96 - 102 %	Ebina et al., 1983
River water and wastewater	Initial: 9.7 Final: 5-6	oxidising rgt.: 0.185 M $K_2S_2O_8$ and 0.485 M H_3BO_3 and 0.35 M NaOH (as in Valderrama, 1981) 7 mL oxidising rgt. added to 50 mL sample	30 min	120 °C (95 % power using a 1000 W microwave)	None reported Simulated freshwater samples analysed to determine accuracy of TN	Microwave assisted digestion for simultaneous determination of TN and TP	Colombini et al., 1998
Freshwater	Initial: 12.8 Final: 2-2.1	oxidising rgt.: 40 g $K_2S_2O_8$ and 9 g NaOH in 1 L dist. water 5 mL oxidising rgt. added to 10 mL sample	1 h	120 °C	N: ammonium chloride, sulfanilamide, NED, urea, pyridine, acetonilamide P: ATP, ADP, TSPP, STP, G-6-P-K_2	Simultaneous detn of TN and TP: N recovery – 92 - 102 % P recovery – 94 - 102 % CRMs also used: National Bureau of Standards reference material 1571; NIES chlorella; NIES orchard leaves; NIES pepperbush Suggests smaller NaOH:S_2O_8 gives better recoveries of both N and P	Hosomi and Sudo, 1986
Freshwater and sediment	Final pH: 2	oxidising rgt.: 50 g $K_2S_2O_8$, 15 mL 3.75 M NaOH made up to 1 L in double-distilled deionised water. 4 mL oxidising rgt. added to 8 ml sample	45 min	Microwave digestion at 80 % power (480 W)	N: ammonium chloride, sulfanilamide, NED, urea, 4-aminoantipyrine, pyridoxine hydrochloride (vitamin B6) P: ATP-Na_2, ADP-Na_2, TSPP, SPP, KHP, G-1-Na_2, G-6-P-K_2	Simultaneous detn of TN and TP: N recovery – 95 - 102 %, excludes 4-aminoantipyrine (60 - 73 % recovery) which contains a HN=C which is resistant to persulphate digestion P recovery – 96 - 102 % National Bureau of Standards reference material 1571 was also analysed	Johnes and Heathwaite, 1992

Matrix	pH	Digestion reactant	Digestion time	Digestion temperature	Model compounds	Comments	Reference
Pond water	Not reported	5 mL of 0.075 M NaOH and 0.1 mg $K_2S_2O_8$ added to 10 mL sample After digestion, 1 mL borate buffer (61.8 g H_3BO_3 and 8 g NaOH in 1 L distilled water) was added	30 min	110 °C	Water samples spiked with KHP	N recovery – similar to Eaton et al., 1995 P recovery – 85-112 %	Gross and Boyd, 1998
Orchard leaves and aufwuchs:	Orchard leaf: Initial - 12.0 Final - 2.5 Aufwuchs: Initial - 12.8 Final - 3.7	oxidising rgt.: 13.4 g $K_2S_2O_8$ and 6 g NaOH in 1 L water to give 200 mg persulphate per 15 mL allquot. Other concentrations of persulphate used: 300, 400 and 500mg 15 mL oxidising rgt. added to 0.5 mL live aufwuchs 15 mL oxidising rgt. added to 15 mg orchard leaves suspended in 15 mL deionised water	1 h	100-110 °C	National Bureau of Standards reference material 1571	Orchard leaf: N recovery –% P recovery – 86.9-88.7% (using 500 mg persulphate) Aufwuchs: N recovery –% P recovery – 93.6 % (300 mg persulphate) ; 101.4 % (400 mg persulphate)	Langor and Hendrix, 1982
Turbid waters	Final: 2	oxidising rgt.: 0.27 M $K_2S_2O_8$ and 0.24 M NaOH 2 mL oxidising rgt. added to 10 mL sample	1 h (autoclave) 40 min (microwave) 1 h (hot water bath)	120 °C (autoclave) 95 °C (microwave) 100 °C (hot water bath)	N: EDTA; glutamic acid; hexamine; nicotinic acid; urea, P: KHP; G-6-P; PTA; α-GLY; 2-AEP; PFA; STP, o-phosphonyl ethanol; SHMP; PEP; aluminium phosphate, calcium phosphate	Comparison of autoclave digestion, microwave digestion and hot water bath and digestion by Kjeldahl method Autoclave digestion recoveries: N (96-103 %); P (103-114 %, except aluminium phosphate (23 %)) Microwave digestion recoveries: N (96-100 %); P (94-108 %, except SHMP (34 %) and aluminium phosphate (17 %)) Hot water bath recoveries: N (94-112 %); P (96-107 %, except aluminium phosphate (8 %) Oxidising reagent suitable for sample with SS concentration up to 150 mg L^{-1} Best recoveries obtained via microwave digestion TN and TP recoveries from pond sediment suspensions lower with alkaline persulphate oxidation than with Kjeldahl method at high SS concentrations	Maher et al., 2002

phase (Johnes and Heathwaite, 1992). Maher and Woo (1998) suggested that the alkali concentration must not be too high when using alkaline persulphate digestion as this could result in a final pH that is not acidic and therefore hydrolysis of P compounds such as polyphosphate compounds would not occur. The sodium hydroxide to persulphate ratio should be such that the final pH of 2 is achieved (Maher and Woo, 1998).

Digestions in studies carried out by Hosomi and Sudo (1986) and Ebina et al. (1983) attained a final pH of approximately 2 (Table 2.8), and both studies achieved greater than 90 % recovery of N compounds and greater than 95 % recovery of P compounds. Johnes and Heathwaite (1992) stated that pH change could be used as an indicator to identify the degree of completion of the digestion since literature inferred that digestion was complete when the initial pH of the sample-oxidising reagent mixture was reduced from pH 12 to pH 2. They used a final pH of 2 as an endpoint for digestion and achieved greater than 95 % recovery for N and P compounds with the exception of refractory compounds containing N=C (4-aminoantipyrine). Initial alkaline conditions may be more effective in hydrolysing some alkyl phosphates than acidic conditions (Corbridge, 1985). In some procedures, boric acid is used as a buffer to maintain an initial pH of 9 (Korleff, 1983; Hansen and Koroleff, 1999; Valderrama; 1981; Aminot and Kérouel, 1996 b). The final pH in these procedure tends to be between 4 and 6 (Valderrama; 1981). Although a final pH of 2 was not achieved in these procedures, good recoveries have been reported by Roig at al. (1999) and by Valderrama (1981) for all model compounds analysed and by Gross and Boyd (1998) for water samples spiked with KHP. The Koroleff method, reported in 'Standard Methods for Sewater Analysis', proposed a 1:5 ratio of oxidising agent (3 g persulphate, 2 g boric acid in 100 mL 0.375 M sodium hydroxide) to sample. The boric acid buffers the initial pH at 9.7, which becomes acidic (pH 4 - 5) after 30 min autoclave digestion at 121 °C. This method has been reported in the literature for the determination of TN and TP in natural waters (Aminot and Kérouel, 1996 b; Hansen and Koroleff, 1999; Liu et al., 2003).

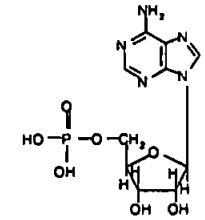
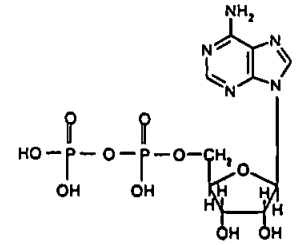
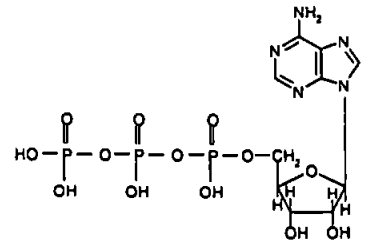
Studies carried out by these researchers confirmed that the alkaline persulphate oxidation has become the preferred method to release TN and TP in a range of natural waters including freshwater, seawater and wastewater and also in sediment and in organic material such as orchard leaves and aufwuchs (Table 2.8). Maher et al. (2002) reiterated this finding in a comparison of digestion techniques used in conjunction with simultaneous alkaline persulphate oxidation of TN and TP.

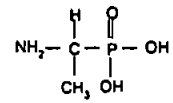
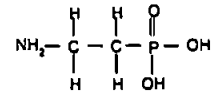
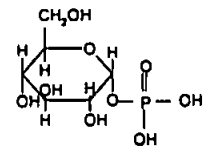
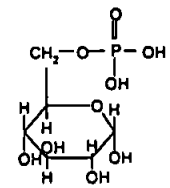
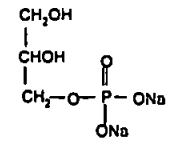
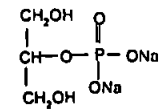
2.3.5.2 Model compounds

The efficiency of a digestion or oxidation technique needs to be established using a range of compounds that are representative of the different chemical bonds and stabilities of naturally occurring compounds. Model N and P compounds that have been used to test the efficiency of methods for the simultaneous determination of TN and TP are listed in Tables 2.9 and 2.10 respectively and the rationale for selecting such compounds are discussed below.

P model compounds: P compounds occurring in natural waters are classified as orthophosphates, condensed phosphates (pyro-, meta, and orthophosphates), organically bound phosphates and phosphonates. These compounds may contain PO_4^{3-}, P—O—P, C—O—P and C—P bonds (Maher and Woo, 1998; Kérouel and Aminot, 1996). Therefore, the synthetic model compounds used to test the efficiency of a digestion procedure should mimic these bonds. Most naturally occurring compounds contain C—O—P or P—O—P bonds or both (Worsfold et al., 2005). Maher and Woo (1998) generalised that C—O—P bonds are readily decomposed using oxidants such as persulphate although this oxidation may be more difficult in a seawater matrix than in aqueous solution or freshwater and refractory C—O—P compounds may be difficult to oxidise. However, OrmazaGonzalez et al. (1996) reported good recoveries (ca. 100 %) for the refractory C—O—P containing compound 4-nitrophenyl phosphate using the alkaline persulphate

Table 2.9: Model P compounds used in autoclave digestion methods (Adapted from Worsfold et al., 2005)

Model Compound	Synonyms	Abbreviations used in text	Chemical Formula	Bond type	stability	Structural Formula
Adenosine-5'-monophosphate	adenosine-5'-monophosphoric acid; 5-adenylic acid; adenosine phosphate; tert-adenylic acid; ergadenylic acid	AMP	$C_{10}H_{14}N_5O_7P$	C–O–P	refractory	
adenosine-diphosphate		ADP	$C_{10}H_{15}N_5O_{10}P_2$	P–O–P and C–O–P	refractory	
adenosine-5'-diphosphate (sodium salt)		5'-ADP-Na_2	$C_{10}H_{13}N_5O_{10}P_2Na_2$	P–O–P and C–O–P	refractory	Similar to ADP
adenosine-5'-triphosphate		ATP	$C_{10}H_{16}N_5O_{13}P_3$	P–O–P	refractory	
adenosine triphosphate disodium	adenosine 5'-(tetrahydrogen triphosphate) disodium salt; adenosine 5'-triphosphate, disodium salt; adenosine 5'-triphosphate, disodium salt hydrate	5'-ATP-Na_2	$C_{10}H_{14}N_5O_{13}P_3Na_2$	P–O–P and C–O–P	refractory	Similar to ATP

Model Compound	Synonyms	Abbreviations used in text	Chemical Formula	Bond type	stability	Structural Formula
1-aminoethylphosphonate	1-aminoethylphosphonic acid	1-AEP	$C_2H_8NO_3P$	C–P	refractory	
2-aminoethylphosphonate	2-aminoethylphosphonic acid	2-AEP	$C_2H_8NO_3P$	C–P	refractory	
glucose-1-phosphate	glucose-1-phosphoric acid	G-1-P	$C_6H_{13}O_9P$	C–O–P	labile	
glucose-6-phosphoric acid (dipotassium salt)	α-D-glucose-6-phosphoric acid dipotassium salt	G-6-P-K_2	$C_6H_{11}O_9PK_2$	C–O–P	labile	Similar to G-6-P
glucose-6-phosphate sodium salt		G-6-P-Na	$C_6H_{12}O_9PNa$	C–O–P	labile	Similar to G-6-P
glucose-6-phosphate	glucose-6-phosphoric acid	G-6-P	$C_6H_{13}O_9P$	C–O–P	labile	
DL-α-glycerophosphate disodium salt	rac-glycerol 1-phosphate disodium salt; DL-α-glycerophosphate	α-GLY	$C_3H_7O_6PNa_2$	C–O–P	labile	
β –glycerophosphate disodium salt hydrate	glycerol 2-phosphate disodium salt hydrate; sodium β-glycerophosphate	β-GLY	$C_3H_7O_6PNa_2$	C–O–P	labile	

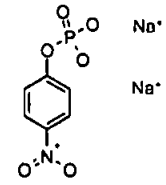
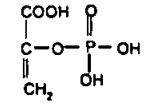
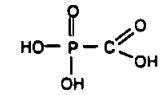
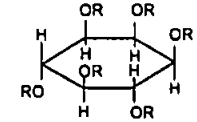
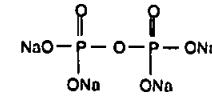
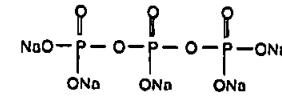
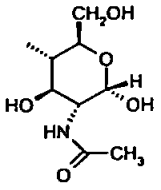
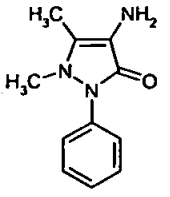
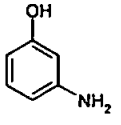
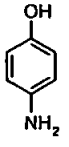
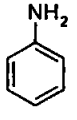
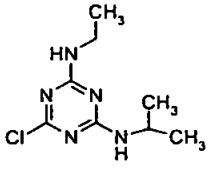
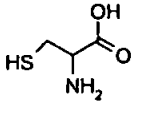
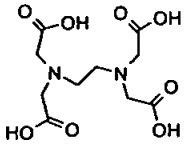
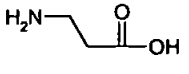
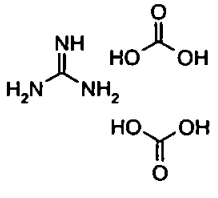
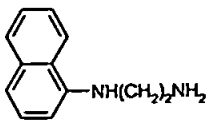
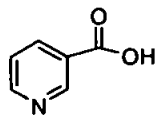
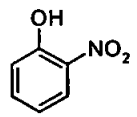
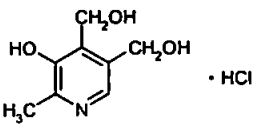
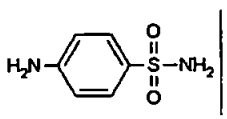
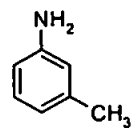
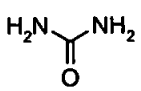
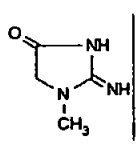
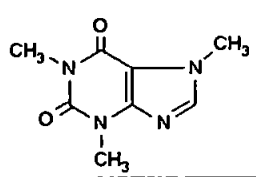
Model Compound	Synonyms	Abbreviations used in text	Chemical Formula	Bond type	stability	Structural Formula
4-nitrophenyl phosphate	4-nitrophenyl phosphate disodium salt Disodium 4-nitrophenyl phosphate		$C_6H_4NNa_2O_6P$	C–O–P	refractory	
phospho(enol) pyruvate		PEP	$C_3H_5O_6P$	C–O–P	labile	
phosphonoformate	phosphonoformic acid	PFA	CH_3O_5P	C– P	refractory	
phytic acid	myo-inositol hexakis (dihydrogen phosphate); inositol hexaphosphoric acid	PTA	$C_6H_{18}O_{24}P_6$	C–O–P	refractory	 where R=PO_3H_2 also see Figure 2.6 b
tetrasodium pyrophosphate	sodium pyrophosphate; pyrophosphoric acid tetrasodium salt; diphosphoric acid, tetrasodium salt; pyrophosphoric acid, tetrasodium salt	TSPP	$Na_4O_7P_2$	P–O–P	labile	
sodium tripolyphosphate	pentasodium tripolyphosphate dihydrate; sodium triphosphate; sodium polyphosphate; triphosphoric acid pentasodium anhydrous	STP	$Na_5P_3O_{10}$	P–O–P	labile	
sodium hexametaphosphate	sodium metaphosphate; metaphosphoric acid, hexasodium salt; sodium polymetaphosphate	SHMP	$(NaPO_3)_n$	P–O–P	labile	$(NaPO_3)_n$; a mixture of condensed sodium phosphates including cyclohexaphosphates and highly polymerised linear polyphosphates

Table 2.10: Model N compounds used in autoclave digestion methods

Model Compound	Synonyms	Bond type	Chemical Formula	Structural Formula
N-acetyl-glucosamine	2-Acetamido-2-deoxy-D-glucose; D-GlcNAc	C–N	$C_8H_{15}NO_6$	
4-aminoantipyrine	4-Amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one; Ampyrone	C–N and N–N	$C_{11}H_{13}N_3O$	
3-aminophenol		C–N	C_6H_7NO	
4-aminophenol		C–N	C_6H_7NO	
aniline	benzenamine phenylamine aminobenzene aminophen	C–N	C_6H_6N	
atrazine	2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine	C–N and N=N refractory	$C_8H_{14}ClN_5$	
L-cysteine	(*R*)-2-Amino-3-mercaptopropionic acid; (*R*)-(+)-Cysteine	C–N	$C_3H_7NO_2S$	
EDTA	Ethylenediaminetetraacetic acid	C–N	$C_{10}H_{16}N_2O_8$	
glycine	Glycocoll Aminoacetic acid Aminoethanoic acid	C–N	$C_2H_5NO_2$	
guanadinium carbonate		C=N	$[(NH_2)_2C=NH]_2H_2CO_3$	

Model Compound	Synonyms	Bond type	Chemical Formula	Structural Formula
NED	α-naphthylethylenediamine; α-naphthylethylenediamine dihydrochloride	C–N	$C_{12}H_{15}N$	
nicotinic acid	Pellagra preventive factor 3-Picolinic acid Pyridine-3-carboxylic acid Niacin Vitamin B3 Vitamin B	C=N	$C_6H_5NO_2$	
2-nitrophenol		C–N	$C_6H_5NO_3$	
pyridoxine hydrochloride (vitamin B6)	Vitamin B6 hydrochloride; Pyridoxol hydrochloride; Adermine hydrochloride; PN HCl	C=N	$C_8H_{11}NO_3 \cdot HCl$	
sulfanilamide	4-Aminobenzenesulfonamide	C–N and S–N	$C_6H_8N_2O_2S$	
3-toluidine	1-Amino-3-methylbenzene 1-Aminophenylmethane 3-Amino-1-methylbenzene 3-Aminophenylmethane 3-Methylbenzenamine Aniline, 3-methyl- m-Toluidin	C–N	$CH_3C_6H_4NH_2$	
urea	Carbamide Carbonyldiamide	C–N	CH_4N_2O	
creatinine	2-Imino-1-methylimidazolidin-4-one 2-Imino-*N*-methylhydantoin 2-Amino-1-methyl-2-imidazolin-4-one	C–N and C=N	$C_4H_7N_3O$	
caffeine	1,3,7-Trimethylxanthine	C–N and C=N	$C_8H_{10}N_4O_2$	

oxidation/autoclave digestion procedure described by Valderrama (1981). Compounds containing P—O—P bonds can only be hydrolysed in the presence of an acid (Maher and Woo, 1998; Halliwell, 1996; Zaiyou and Limin, 1986), but also need to be heated to temperatures of 90 – 120 °C (Maher and Woo, 1998; Armstrong et al., 1966; Hinkamp and Schwedt, 1990; McKelvie et al., 1989). Reported autoclave and microwave digestion

techniques (Table 2.8), all achieved a temperature high enough to promote release of orthophosphate from P—O—P bonds. Alkaline persulphate has been successfully used to break these bond types in several P model compounds, eg. STP, TSPP, SHMP (Table 2.8). It is advisable to include both inorganic and organic polyphosphates in recovery studies. Compounds such as ATP contain both C—O—P and P—O—P bonds. ATP is an organic condensed P compound and is widely found in nature and it has been used as a model compound by several researchers (Maher and Woo, 1998); ATP is recommended by Worsfold et al. (2005) for testing P digestion efficiency. Some researchers use both ATP and its derivative ADP (Johnes and Heathwaite, 1992; Roig, 1998).

Phytic acid contains C—O—P bonds and is one of the most refractory organic P compounds. It is an inositol phosphate, an important class of naturally occurring organic compounds, and is found in natural waters. Baldwin (1998) extracted phytic acid from waters samples collected in an eutrophic wetland. The carbon atoms of C—O—P bonds are bound within a hexane ring, thus increasing the stability of the bond, making it one of the one of the more resistant compounds to hydrolysis (Worsfold et al., 2005). Therefore, phytic acid has been recommended as a refractory organic P model compound (Worsfold et al., 2005) and has been used in studies by Maher et al. (2002), Aminot and Kerouel (1996 b), Denison et al. (1998), and Espinosa et al. (1999) for assessing digestion efficiency in various environmental matrices.

Few compounds reported in the literature contain C-P bonds, which are very resistant to oxidation and hydrolysis (Worsfold et al., 2005; Corbridge, 1985). Phosphonates contain strong C—P bonds and are refractory organic P compounds. This type of compound may be released from biological sources in marine systems (Maher and Woo, 1998; Kérouel and Aminot, 1996), and has been detected in soils and soil leachate (Worsfold et al., 2005). Therefore these compounds are useful indicators in testing digestion efficiency when working with seawater and sediment matrices.

Sugars such as 6-glucose-1-phosphate and D-glucose-6-phosphate are labile organic P compounds containing C—O—P bonds. D-glucose-6-phosphate has also been extracted from waters samples collected in an eutrophic wetland by Baldwin et al. (1998). Such compounds are also found in soil leachates and can make their way into freshwater systems via diffuse runoff from soils. These compounds are often used as model compounds for labile organic P compounds containing C—O—P bonds (Maher et al., 2002; Roig et al., 1999; Johnes and Heathwaite, 1992).

Worsfold et al. (2005) recommended that model compounds selected to determine P digestion efficiency should include compounds representative of all three bond types, and should also include refractory and labile examples of each bond type. Johnes suggests selection of straight chain organic compounds such as G-6-P-K_2, G-1-P, ATP and ADP and inorganic heterogeneous compounds such as KHP, and a pyrophosphate. Model compounds should also reflect the forms that exist in the environmental matrix under study. Soils and sediments contain P compounds bound to calcium and aluminium and compounds such as calcium phosphate and aluminium phosphate have been recommended as model compounds for digestion methods applied to these matrices (Maher and Woo, 1998; Maher et al., 2002). Orthophoshates such as KHP should be included as a method control (Worsfold et al., 2005; Kérouel and Aminot, 1996). Studies which involved digestion of turbid water samples have also included solid reference materials such as leaves and aufwuchs (Table 2.8).

N model compounds: Although a wide a range N compounds (Table 2.10) has been used in studies to determine the efficiency of the simultaneous digestion of TN and TP, the criteria for choosing these compounds has rarely been discussed with respect to bond stability and chemical structure. Johnes and Heathwaite (1992) suggested selection of straight chain organic compounds such as urea and pyroxidin, and inorganic heterogeneous compounds such as ammonium chloride as well as a refractory N compound containing

C=N such as 4-aminoantipyrine. Nydahl (1978) used forty-nine N model compounds in a study of alkaline persulphate oxidation efficiency in TN determination and found that the C=N, N=N and N—N bonds were particularly difficult to break. Similar findings were reported by Ebina et al. (1983) and Johnes and Heathwaite (1992).

2.3.5.3 Recovery Studies

In the current work, simultaneous digestion of TN and TP was carried out on water samples collected bimonthly from the Tamar River at Gunnislake. Samples were digested using alkaline persulphate oxidation with autoclave digestion as described by Hansen and Koroleff (1999). This method, using a 1:5 ratio of oxidising agent (3 g persulphate, 2 g boric acid in 100 mL 0.375 M sodium hydroxide) to sample has been extensively used by researchers for simultaneous digestion of TN and TP in a variety of natural waters (Aminot and Kérouel, 1996 b; Hansen and Koroleff, 1999).

The efficiency of the digestion procedure was tested with three P model compounds and four N model compounds: ATP (both C—O—P and P—O—P bonds); sodium tripolyphosphate (P—O—P bonds); phytic acid (refractory C—O—P compound); L-cysteine (C-N bonds); EDTA (C-N bonds); urea (C-N bonds); and nicotinic acid (C=N bonds). KHP was used as the P method control and sodium nitrate was used as the N method control. Model compounds were selected on the basis of bond type and on the frequency of occurrence in freshwater systems.

Recoveries for P model compounds were greater than 89 ± 13 % (n=6) (Figure 2.7) for phytic acid (PTA), 100 ± 13 % (n=6) for sodium tripolyphosphate (STP) and 85 ± 4 % (n=6) for ATP. Therefore, this method is shown to efficiently break P—O—P bonds and C—O—P bonds, including highly resistant C—O—P bonds in refractory compounds such as ATP and phytic acid. Compounds containing C-P bonds were not used in this study as these compounds are primarily found in marine systems.

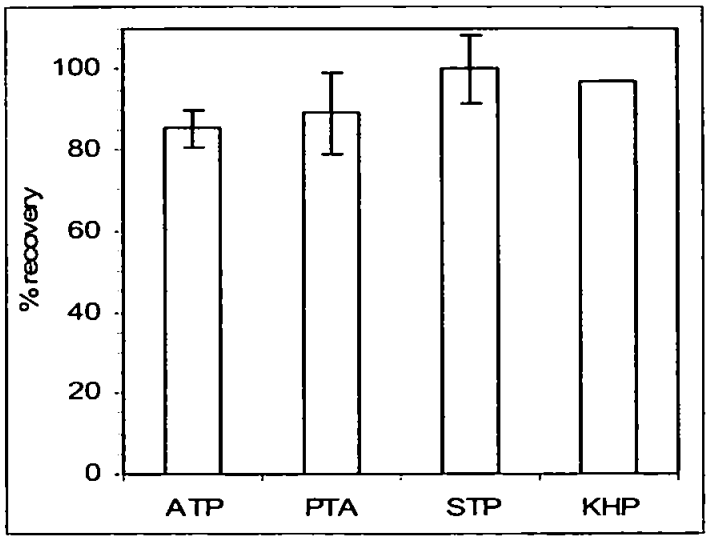


Figure 2.7: Comparison of recoveries for selected P model compounds using alkaline persulphate autoclave digestions. ATP – adenosine triphosphate; PTA – phytic acid ; STP –sodium tripolyphosphate ; KHP – potassium dihydrogen orthophosphate. Error bars show ± 3 standard deviations (n=6).

Quantitative recoveries were obtained for the alkaline persulphate digestion of all N compounds used in this study: L-cysteine (92.8 ± 0.9 %); EDTA (96.7 ± 0.5 %); nicotinic acid (97.7 ± 0.3 %), and urea (103.1 ± 1.2 %). Therefore the alkaline persulphate/autoclave digestion procedure effectively oxidised the C=N of the nicotinic acid (Vitamin B_3). Johnes and Heathwaite obtained poor recoveries for Vitamin B_6, and concluded that the C=N was resistant to their alkaline persulphate/microwave digestion procedure which did not use a borate buffer to maintain alkaline pH at the start of the digestion procedure.

The method of digestion used in this study achieved satisfactory recoveries of both TN and TP using model compounds. This, in combination with reliable methods of measuring the products of digestion (nitrate/nitrite and FRP), suggests that the simultaneous determination of TN and TP in natural waters can be considered reliable in this study.

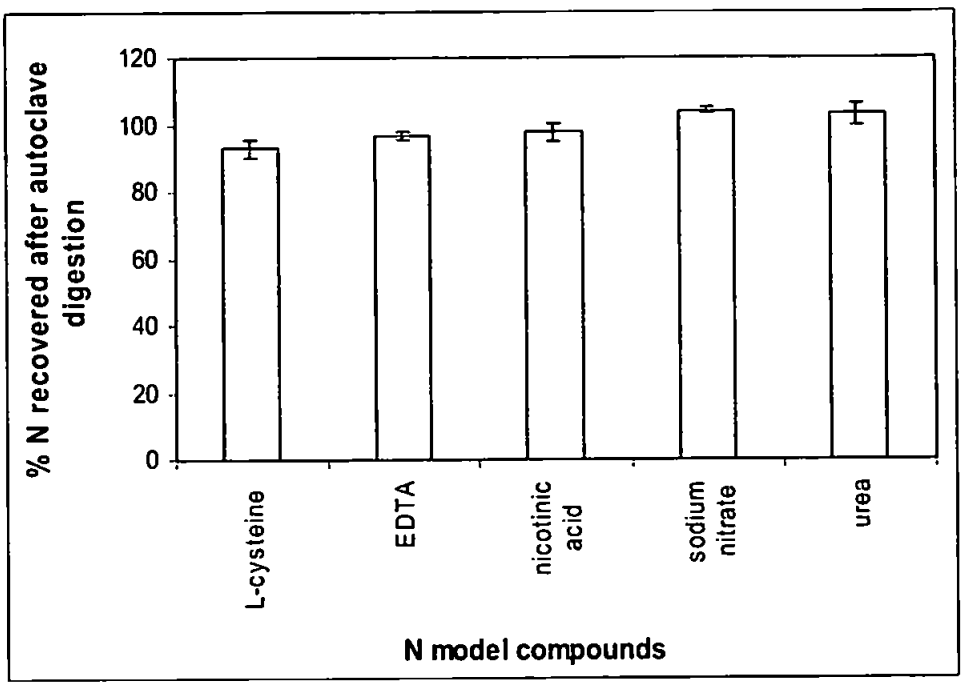


Figure 2.8: Comparison of recoveries for selected N model compounds using alkaline persulphate autoclave digestions. EDTA- ethylenediamine-tetraacetic acid. Error bars show ± 3 standard deviations (n=6).

2.4 CONCLUSIONS

Several conclusions have been derived from the methods discussed in this chapter:

1. No significant interference from silicate, arsenate, hydrolysis products of P compounds soluble in aqueous solution, nitrate or nitrite in the analysis of FRP using the Skalar air-segmented flow analyser was observed.

2. No significant interference from salinity, phosphate or copper in the analysis of nitrate/nitrite using the Skalar air-segmented flow analyser was observed.

3. The Skalar air-segmented flow analyser can be used to determine FRP (3 – 150 μg L^{-1} P) and nitrate/nitrite (0.5 – 5.0 mg L^{-1} N) in aqueous solution and in natural waters.

4. Successful participation in the 'NOAA/NRC 2nd annual intercomparison study for nutrients' indicated that FRP and nitrate/nitrite in natural waters can be accurately analysed using the Skalar San Plus flow analyser.

5. Quantitative recoveries for N and P model compounds were achieved using alkaline persulphate/autoclave digestion and can be considered to achieve quantitative conversion of N and P compounds in natural waters to nitrate and phosphate respectively. Therefore, this alkaline persulphate/autoclave digestion followed by analysis of phosphate and nitrate using the Skalar San Plus flow analyser can be considered an accurate and reliable method for the simultaneous determination of TN and TP in the freshwaters leaving the Tamar catchment in this study.

CHAPTER 3

Biogeochemistry of nutrients in the Tamar catchment

3.1 INTRODUCTION

Nutrient water quality and the processes driving nutrient mobility within river catchments play a major role in defining environmental quality objectives (Harris and Heathwaite, 2005), which are an essential part of environmental policy, legislation and management strategy. However, water quality data can be highly variable and the large range of temporal and spatial variations encountered is partly driven by fluctuations in climate and hydrodynamics and partly by fluctuations in internal biological and physical processes (Harris and Heathwaite, 2005). Therefore, the analysis of long-term time series data is necessary to identify trends and baseline conditions within the catchment.

This chapter sets out to describe the biogeochemistry of nutrients in a freshwater system. The Tamar River in South West England (Figure 3.1) was selected as the study area based on geographical proximity and ease of access to collect samples and also because a large repository of physico-chemical data for the selected sampling site is available. Historical physico-chemical data for this catchment will be used to describe environmental trends with respect to nutrient water quality and nutrient biogeochemistry of the waters leaving the catchment and entering the Tamar Estuary. Environmental interpretation of recent physico-chemical data collected between May 2003 and May 2004 will be used to identify short-term trends over this study period.

3.2 METHODS

3.2.1 Description of Study Area

The Tamar freshwater catchment, located in South West England, is approximately 915 km^2 (EA, 1999) and is made up of ten sub-catchments (Figure 3.1). The catchment, as defined in this study, is lowest in elevation along the middle and lower Tamar River valley (0-50 m) and slopes upwards in excess of 200 m at the eastern and western borders of the catchment (Findlay et al., 1984). The geology of the area is largely of Devonian siltstones and mudstones and Carboniferous shales and sandstones with upland areas of

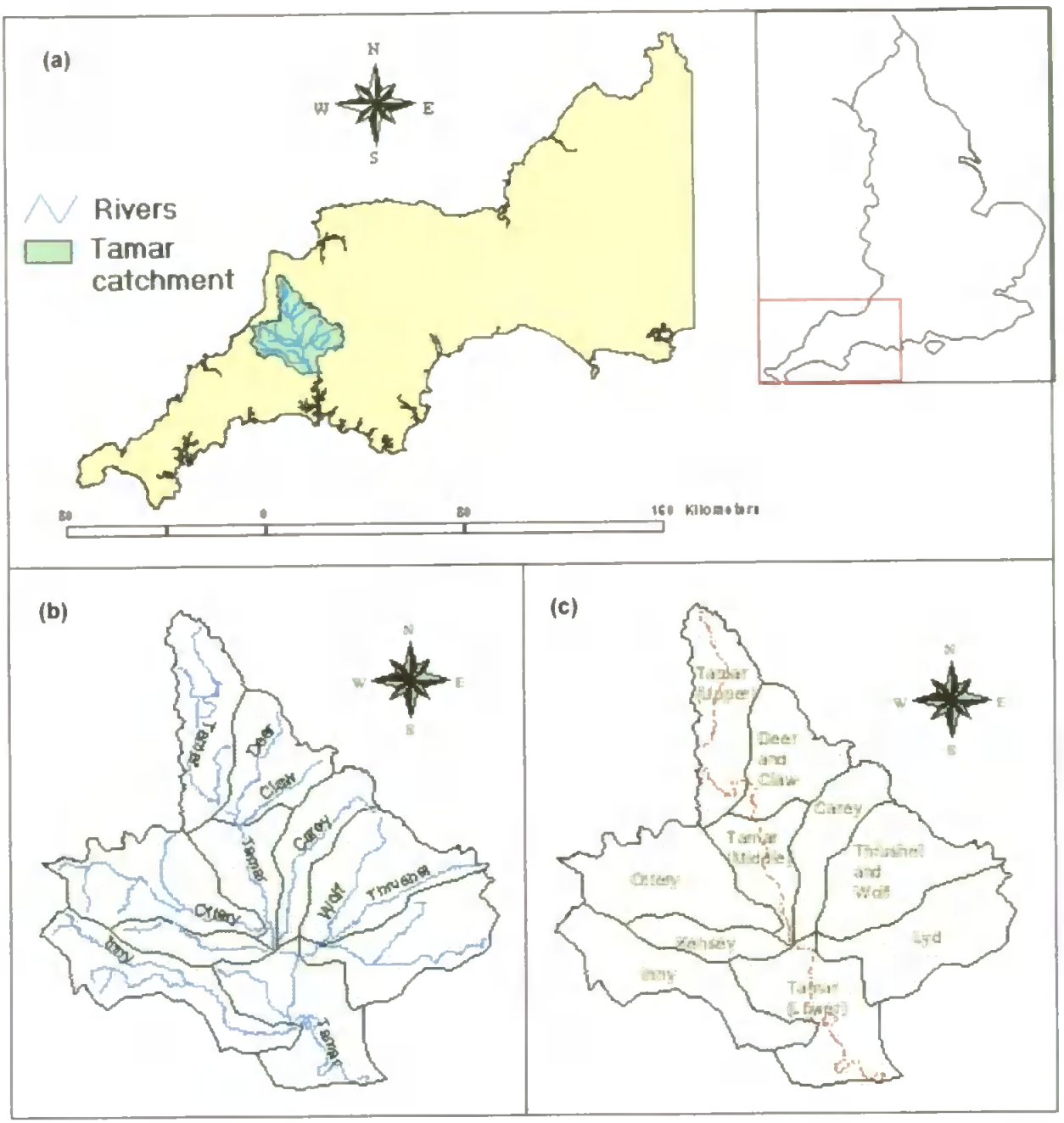


Figure 3.1: Map Showing (a) The Location Of The Tamar Catchment, SW England; (b) Rivers (~) within the Tamar Freshwater catchment; (c) Subcatchments within the Tamar catchment and the Devon-Cornwall county boundary (– · – ·)

granite (Findlay et al., 1984) with thin deposits of aeolian loamy and silty drifts overlying the area. Water movement through the clay soils of the Tamar River valley, between Launceston to Holworthy, is generally slow and as a result this area has historically provided high quality grassland for grazing. Approximately 75 % of the land cover is

designated to agriculture, chiefly managed grasslands dedicated to dairy, beef cattle and sheep (NRA, 1996). There is extensive land drainage to the north of the catchment, with approximately 75 % of the land being drained (NRA, 1996). This primarily rural catchment has two small towns, Launceston and Holworthy, although the majority of the 32,900 residents of the catchment are spread thinly over the area (NRA, 1996); less than 1.3 % of the catchment is used for human settlements (0.35 persons ha^{-1}). The catchment has no large industry, but from the extensive historical mining activity in the area, drainage from these workings still affects rivers and sediments (EA, 2000).

The Tamar River drains into the Tamar Estuary; the river is physically separated from the estuary by a weir at Gunnislake (National Grid Reference SX4332722). The long-term mean river discharge volume at Gunnislake is 707,699 million litres per annum (NRA, 1996). Annual mean discharge rate of the Tamar River is 30 m^3s^{-1} whilst instantaneous flow can exceed 100 m^3s^{-1} (Miller, 1999). The Upper Tamar Lake Reservoir and Roadford Reservoir provide water to large parts of Devon and Cornwall (NRA, 1996; EA, 1999 a). Incidents of non-compliance of the Tamar Lakes with nutrient water quality objectives in 1997 have been linked with algal blooms in the Tamar River (EA, 1999). Similar incidents led to failure to comply with the EC freshwater Fish Directive in previous years (EA, 1999 b). Agriculture was a significant contributing factor to these incidents (EA, 1999 b).

3.2.2 Historical physico-chemical data for the Tamar catchment at Gunnislake and the meterological data for Pentillie Castle

Physico-chemical data: Nitrogen and phosphorus concentration data for the period 1974 to 2004 were obtained from a Microsoft Access database provided by the Environment Agency for three sampling stations, all located at Gunnislake (National Grid Reference SX4332722, SX43377235, SX42657250). Temperature, suspended solids concentrations and pH data for 1974 to 2004 were also obtained from this database.

Dissolved oxygen, silicate and chlorophyll a data were obtained from a dataset provided by the EA for the period 1974 to 2000 for the three sampling stations located at Gunnislake (National Grid Reference SX4332722, SX43377235, SX42657250).

River discharge: Daily mean river discharge rates from 1974 to 2004 at Gunnislake (National Grid Reference SX4332722) were obtained from the Environment Agency, Bodmin.

Rainfall: Rainfall from 1961 to 2005 at Pentillie Castle (National Grid Reference SX41206460) were obtained from the National Rivers Archives website (www.nerc-wallingford.ac.uk/ih/nrfa/index.htm).

Data analysis: Summary statistics such as the calculation of means and medians were performed using Statgraphics Plus 5.1 (Statgraphics Corporation). This software was used to represent the long-term annual data discussed in Section 3.3 as box and whisker plots (Figure 3.2). Box and whisker plots are particularly useful for non-parametric data. The data is ranked in order of magnitude, from smallest to largest. It divides the data into four equal areas of frequency. Each one of the horizontal lines represents an important number related to the data set: minimum and maximum data values are represented by the top and bottom lines, respectively and the three lines that form the box are drawn at the 25th percentile, 50th percentile (median), and 75th percentile of the data. Therefore the 'box' encloses the middle 50 percent of the data, where the median is represented as a vertical line inside the box. The mean, symbolised by '+', may be plotted as a point on the box.

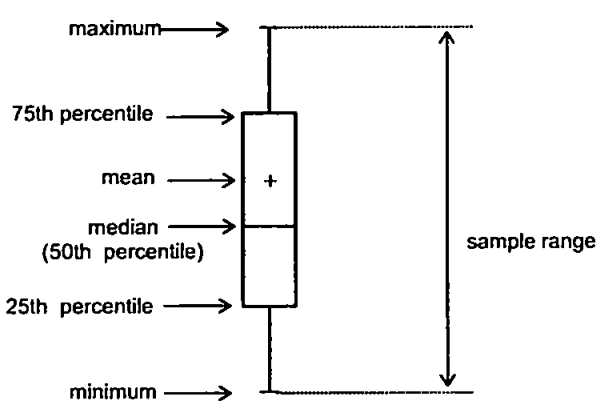


Figure 3.2: Basic representation of a box and whisker plot

3.2.3 Nutrient and physico-chemical data for the Tamar catchment for 2003 – 2004

Discrete water samples were collected bi-monthly at Gunnislake (SX43377235) from May 2003 to May 2004. Dissolved oxygen, temperature, conductivity and pH measurements were recorded on site using *in situ* field instruments. Dissolved oxygen (DO) concentrations and temperature were determined using a YSI 55 meter (YSI (UK) Ltd, Hampshire). The DO meter was calibrated in the field, immediately prior to use. Conductivity was measured using a HI 9635 conductivity meter (Hanna Instruments Ltd, Hertfordshire). pH measurements were performed using a HI 9025 pH meter (Hanna Instruments Ltd) fitted with a Gelplas probe (BDH). The pH meter was calibrated immediately prior to use with buffer solutions of pH 7.0 and 4.0 (NIST), and calibrated for temperature. Suspended solids concentrations were determined spectrophotometrically using a Hach DR/800 series colorimeter (Hach Company, Colorado).

A portion of each sample was filtered on site using pre-washed (10 % v/v HCl) 0.45µm cellulose acetate membrane filters. Samples were then transported to the laboratory where analyses to determine TP, TDP, FRP, TN, TDN and nitrate/nitrite concentrations were performed within 24 h of collection as described in Section 2.2. Prior to sampling, all laboratory glassware, bottles and containers used for experiments, sampling and sample storage were soaked in nutrient-free detergent (Decon®-Neutracon) for 24 h, then rinsed 3 times with ultra pure water. They were then soaked in 10 % v/v HCl for a minimum of 24 h and subsequently rinsed 3 times with ultra pure water, dried at room temperature and stored in resealable plastic bags until required for use.

3.3 Results and Discussion

3.3.1 Historical nutrient and physico-chemical data for the Tamar catchment

The biogeochemistry of nutrients in natural waters can be studied through the determination of the chemical speciation of nutrients in the water column, thereby building

up a database which allows the elucidation of long-term environmental trends. High frequency, long-term time series data are necessary to elucidate such trends and as such historical physico-chemical datasets can be invaluable resources in the interpretation of environmental trends.

An analysis of time-series data for various physico-chemical parameters collected for the Tamar River at Gunnislake to identify environmental trends are discussed in this section. The periodicity of the data available from the EA for the Tamar River at Gunnislake varied widely. This was because data collection was managed by a succession of regulatory bodies over this period, and was accompanied by changes in regulatory and budgetary priorities. Littlewood et al. (1998) suggests that the most comprehensive and best organised national database for UK river water quality is the Harmonised Monitoring Scheme (HMS) database which covered the period 1974 to 1996. Subsequently, the Environment Agency for England and Wales has been responsible for the collection of the water quality data. Historical water quality data available for Gunnislake indicate that dissolved inorganic nutrients species are more extensively monitored than other nutrient species; nitrate, nitrite, ammonia data were available from 1974 to 2004; FRP data from 1976 to 2004, and silicate data from 1974 to 1998. The importance of monitoring total nitrogen and total phosphorus loads has been recognised and the monitoring of TP at Gunnislake began in 1988 but there are no TN data for this site.

3.3.1.1 Phosphorus

TP concentrations in the Tamar ranged from 20 to 500 μg L^{-1} P from 1989 to 2004. However, throughout this period, TP was usually less than 160 μg L^{-1} P. The long-term mean TP concentration (97 μg L^{-1} P) was 20 % higher than the long-term median TP concentration (80 μg L^{-1} P) and the annual means were often higher than the annual median TP concentrations (Figure 3.3). Therefore, annual export of TP from the Tamar catchment to the estuary will be strongly influenced by these short periods of high nutrient

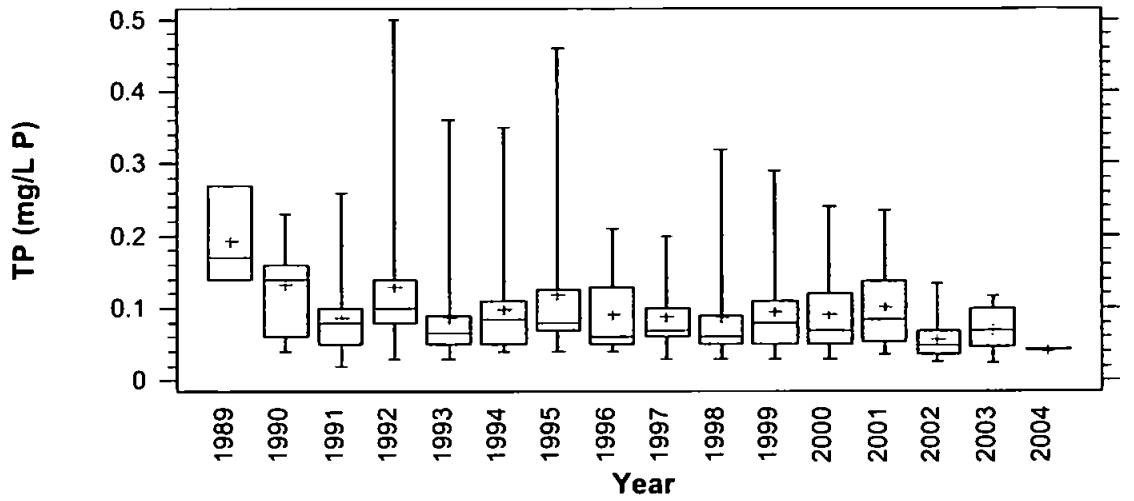


Figure 3.3: Time series TP data for the Tamar River at Gunnislake from 1989 to 2004

concentrations. This observation reinforces the need for high frequency, high resolution data that are representative of all environmental conditions.

Seasonal trends based on the mean TP concentration recorded for each month of the year over a 15-yr period (1989 to 2004) indicate that TP is highest from July to October and lowest in February (Figure 3.4 a). Over the period, mean monthly TP concentration increased as the mean daily discharge per month decreased from February to August (Figure 3.4 b). However, from September to January, mean monthly TP concentrations were less influenced by river discharge rates but were generally higher than from February to August (Figure 3.4 c).

FRP concentrations are generally less than 130 μg L^{-1} P although concentrations of 470 - 500 μg L^{-1} P were reported in 1976 and 1983 (Figure 3.5a), both years with extremely dry summers. These incidences of excessively high FRP concentrations, although infrequent, are reflected in the long-term mean FRP concentration of 72 μg L^{-1} P which is 20 % higher than the long-term median FRP concentration of 60 μg L^{-1} P. Nutrient classification systems proposed for P in flowing waters are generally based on mean annual FRP concentrations, for example, the Environment Agency have published interim standards for phosphorus in standing and running freshwaters based on mean annual FRP concentrations

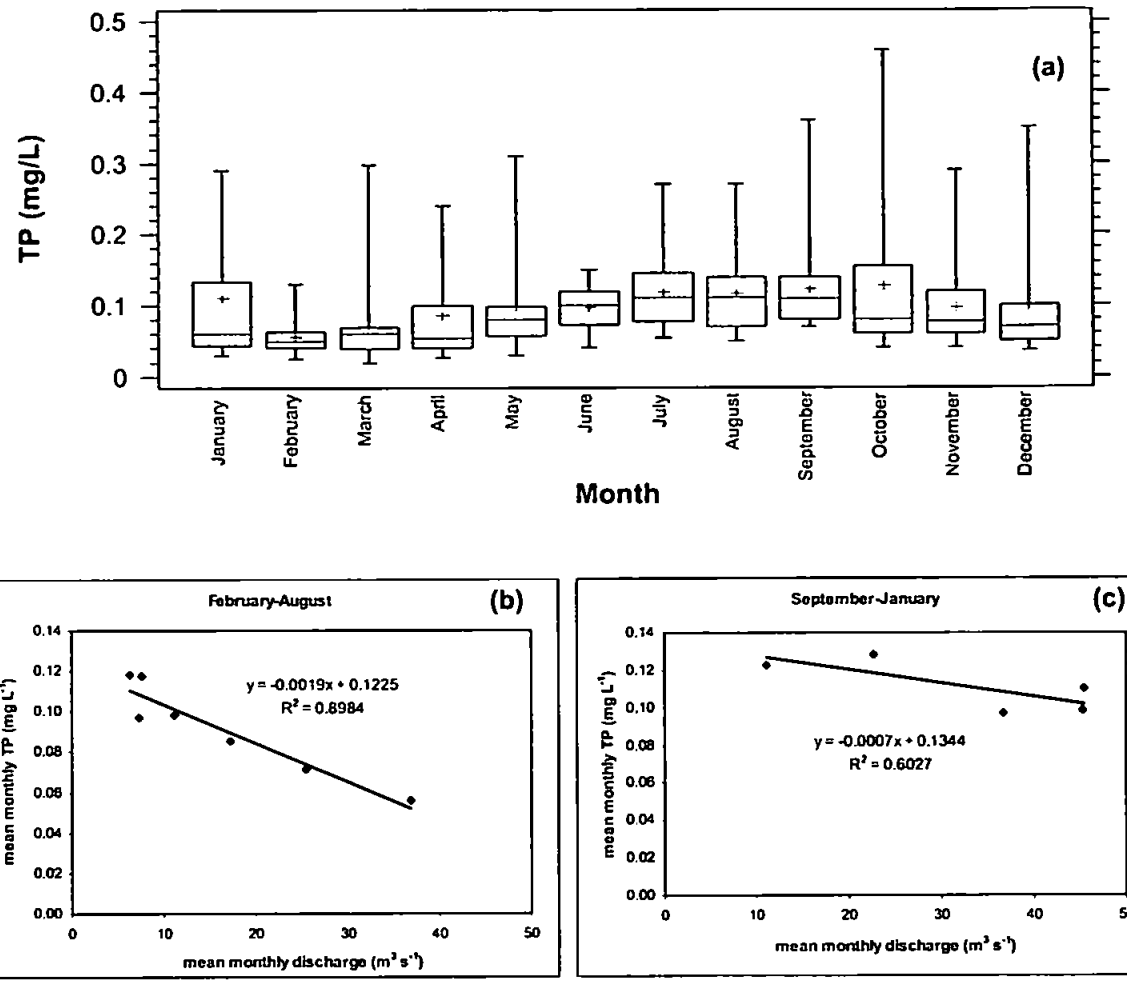


Figure 3.4: Time series TP data for the Tamar River at Gunnislake: (a) box and whisker plots of mean monthly TP (1989 to 2004); and the relationship of mean monthly TP (1989 to 2004) to mean monthly discharge; (b) February to August and, (c) September to January.

(Section 1.4.), whilst mean annual FRP concentrations <47 μg L^{-1} P have been suggested by McGarrigle (1993) as necessary to prevent nuisance growth of algae and to preserve water quality suitable for salmonid fish in Irish rivers (Smith, 2003). In 1976 and 1990, mean annual FRP concentrations exceeded 100 μg L^{-1} P, and the Tamar was eutrophic using interim standards set by the EA (Figures 3.5 a and b). However, mean annual FRP concentrations have decreased since 1990 (Figure 3.5 b). The average FRP concentration from 1976 to 1990 was 84 μg L^{-1} P (mesoeutrophic), while from 1991 to 1999 it was 59.2 μg L^{-1} P (mesotrophic) and 49.5 μg L^{-1} P (mesotrophic) from 2000 to 2004, indicating a decrease in P inputs to the Tamar River over this period. Improved

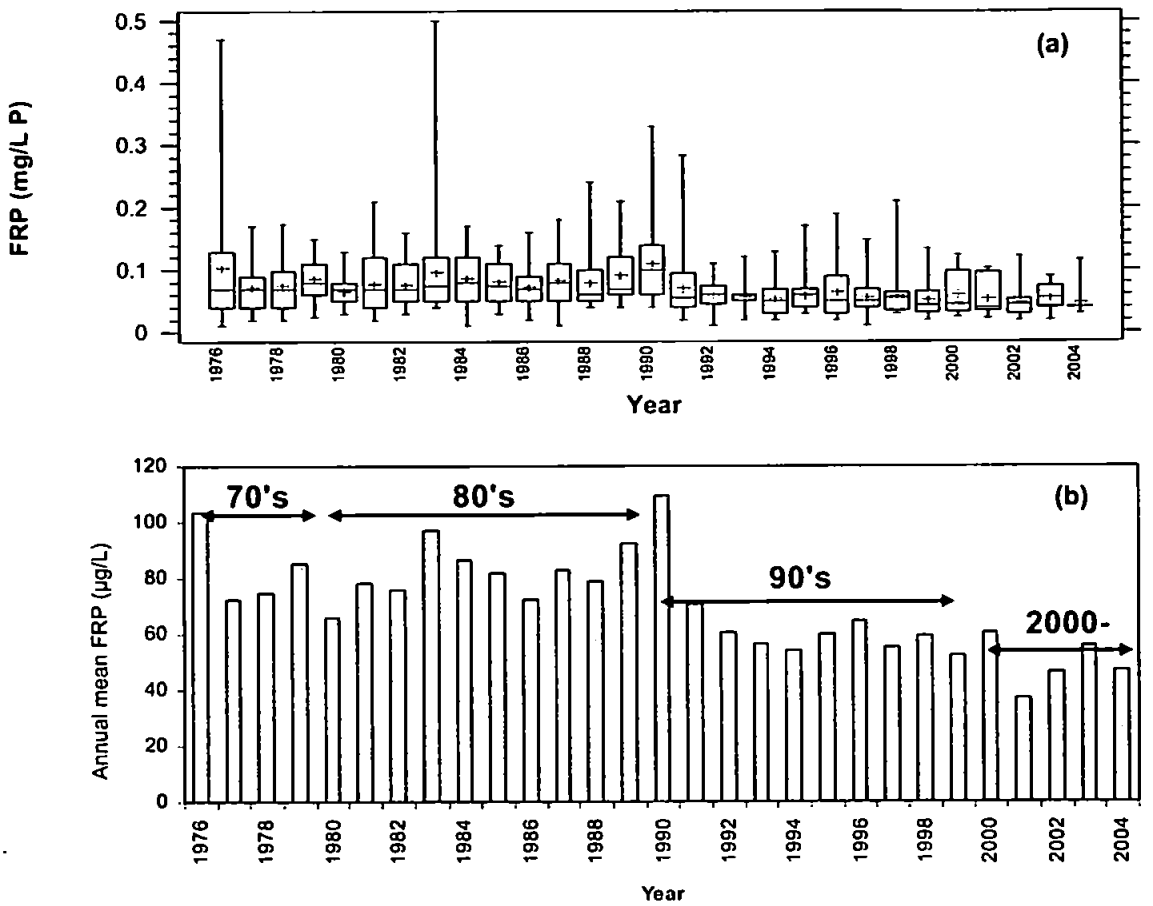


Figure 3.5: FRP in the Tamar River at Gunnislake: (a) box and whisker plots of FRP concentrations from 1976 to 2004; (b) histograms of annual mean FRP concentrations (1976 to 2004).

treatment of wastewater, such as outlined by the Urban Wastewater Directive (UWWD), would contribute to such a decrease. A similar trend of decreasing P in EU rivers has been attributed to changes implemented in accordance with the UWWD (European Environmental Agency, 1999).

FRP:TP ratios indicate the proportion of TP that is readily bioavailable. Hanrahan et al. (2001 b) suggested that trends in FRP concentrations may be estimated from trends in TP concentrations, thereby reducing the number of water quality parameters that need to be frequently monitored. However, this is not the case for the Tamar catchment: the FRP:TP ratios from 1989 to 2004 ranged from 0.1 to 1.0 with a long-term average of 0.65 (Figure 3.6); the dataset was non-parametric and the annual median FRP:TP ratios were

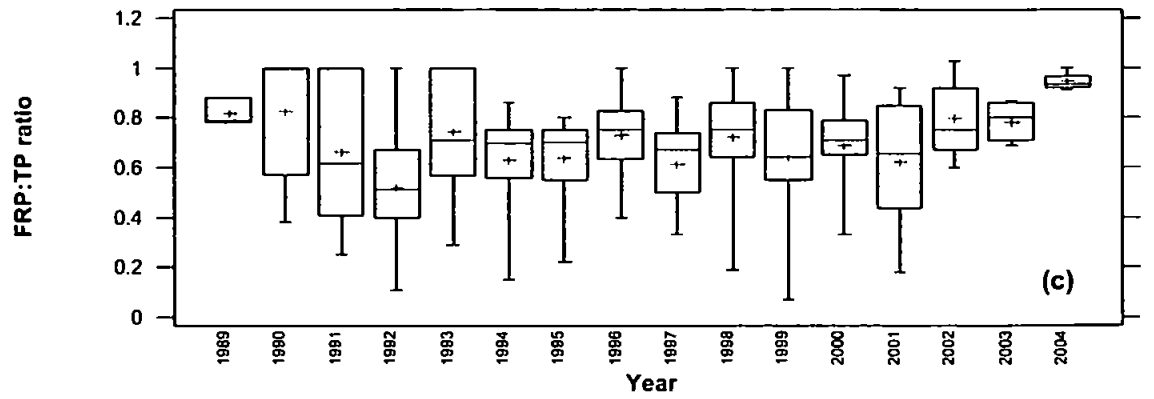


Figure 3.6: FRP:TP ratios in the Tamar River at Gunnislake (1989 to 2004).

significantly different over this period ($p<0.05$).

Sampling events where FRP:TP ratios greater than 1 were reported in the EA dataset provided, but were not considered in the statistical analysis of the data. FRP:TP ratios of 1 were accepted in the statistical analysis although such a ratio implies that 100 % of TP in a riverine water sample is FRP component, suggesting that biotic or mineral components of the water column were absent. This FRP:TP dataset highlighted the need for QA/QC in environmental monitoring programmes and reinforced the need to establish analytical figures of merit for methods of analysis and establish competence to perform these analyses as discussed in Sections 2.3.1 and 2.3.2.

3.3.1.2 Dissolved inorganic nitrogen

Nitrate/nitrite data collected at Gunnislake from 1974 to 2004 (Figure 3.7 a) showed that the guide level set by the Nitrates Directive for polluted waters (5.65 mg L^{-1} NO_3-N) had been exceeded on only one occasion in 1976 although this guide level was approached on several occasions between 1996 to 1997. Nitrite concentrations in the Tamar River range from 0 to 110 µg L^{-1} N from 1974-2004 (Figure 3.7 b), with a long-term mean nitrite concentration over this period, 20 µg L^{-1} N. Although these concentrations are typical of natural waters (Chapman, 1998), 92 % of all nitrite data recorded at Gunnislake exceeded

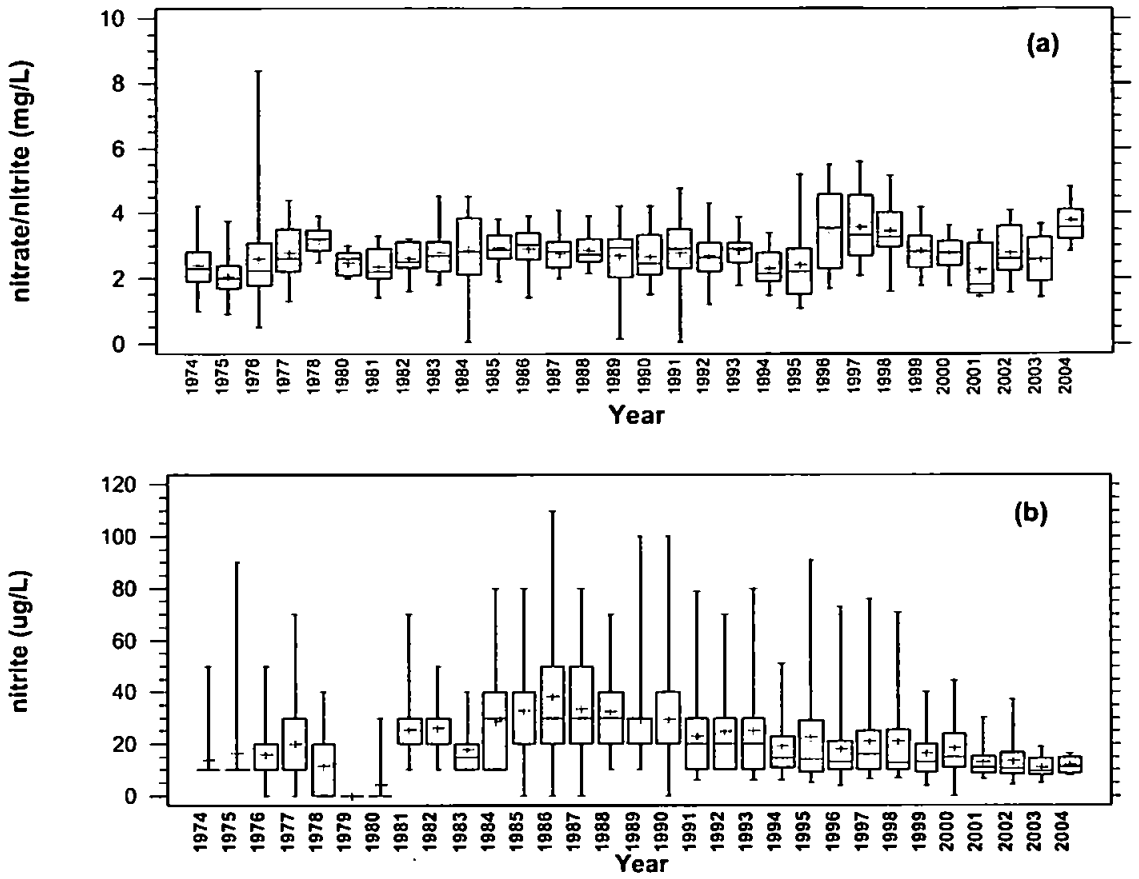


Figure 3.7: Box and whisker plots of (a) nitrate/nitrite concentration data collected at Gunnislake from 1974 to 2004 and, (b) nitrite concentration data collected at Gunnislake from 1974 to 2004

the EU guideline of 9 μg L^{-1} N for the protection of course freshwater fish.

Annual mean nitrate/nitrite concentrations ranged from 2.1 to 3.8 mg L^{-1} N (Figure 3.7). Criteria proposed by Dodds et al. (1997) suggest that TN levels exceeding 1.5 mg L^{-1} N (Table 1.1, Section 1.1.3) are indicative of eutrophic waters. Therefore, in the absence of time series data for TN at Gunnislake, using the criteria proposed by Dodds et al. (1997) the nitrate/nitrite data indicate that the waters leaving the Tamar catchment have been eutrophic with respect to N for the past 30 years.

Gardolinski (2002) plotted the weekly distribution of water quality data at Gunnislake (Figure 3.8) to establish statistical correlations within the dataset. These plots indicate that nitrate concentrations are likely to be lowest from July through September (weeks 23-40) and higher over the winter months (Figure 3.8). Studies of the Slapton Ley and Windrush

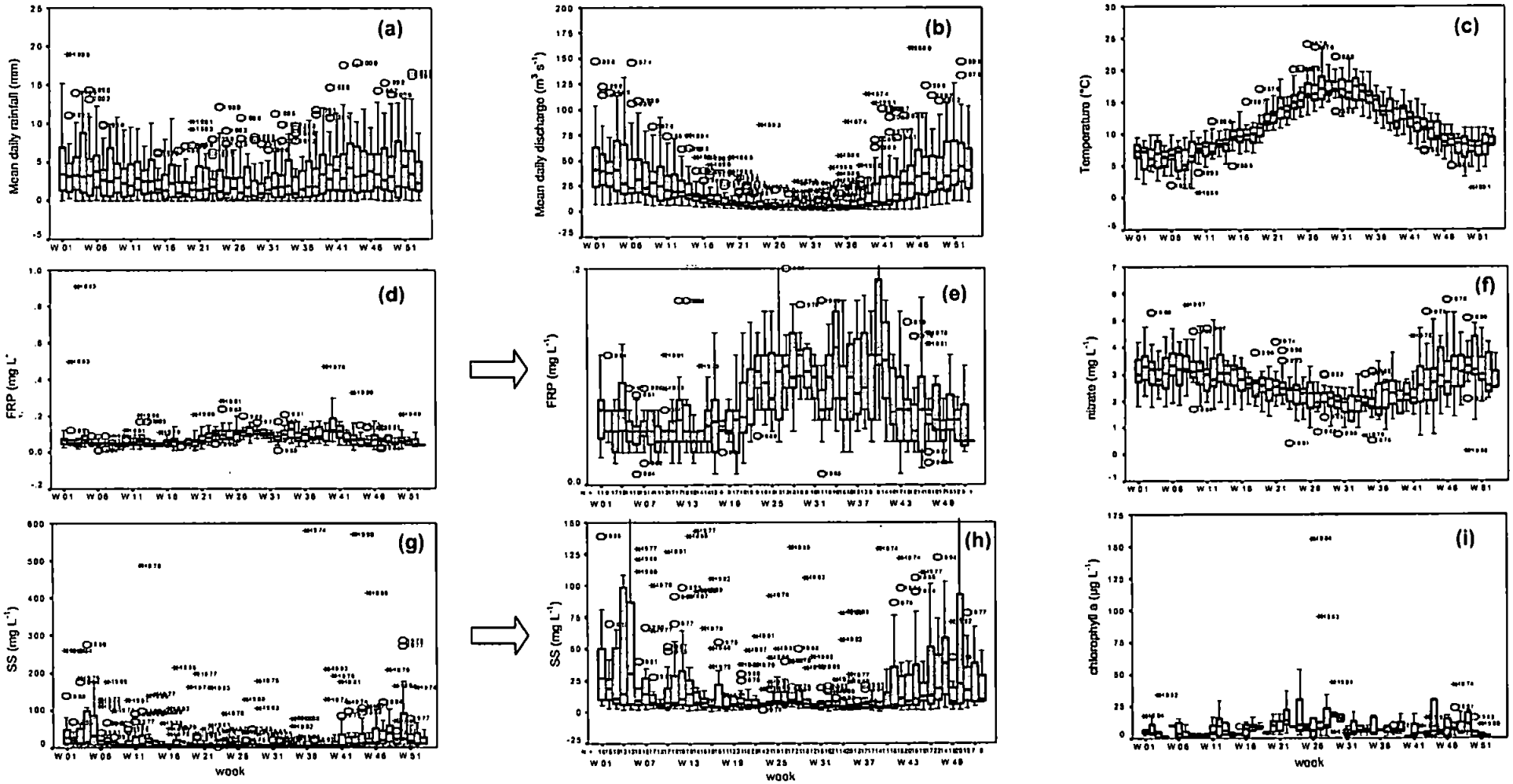


Figure 3.8: Box and whisker plots showing seasonal weekly distribution of (a) rainfall, (b) discharge, (c) temperature, (d) FRP (e) FRP expanded, (f) nitrate, (g) suspended solids (h) suspended solids expanded and (i) chlorophyll a recorded for the Tamar at Gunnislake from 1974 to 2002. Reproduced from Gardolinski, 2002

catchments have shown similar trends to the Tamar catchment, where nitrate concentrations tended to be highest during the winter months, usually coincident with highest river discharges (Heathwaite and Johnes, 1996).

Ammonia can be toxic to aquatic life and concentrations <0.031 and <0.155 mg L^{-1} N have been quoted as guide levels for the protection of salmonid and cyprinid fish, respectively. 90 % of all ammonia concentrations recorded at Gunnislake were less than 0.155 mg L^{-1} N, but concentrations exceeding the mandatory levels for the protection of all fish (0.78 mg L^{-1} N) were recorded in 1986, 1995, 1998 (Figure 3.9). However, ammonia concentrations could be considered suitable for salmonid fish on only 45 % of the sampling occasions from 1974 to 2004 according to the criteria stated above. Mean annual concentrations of ammonia at Gunnislake range from 0.033 to 0.18 mg L^{-1} N.

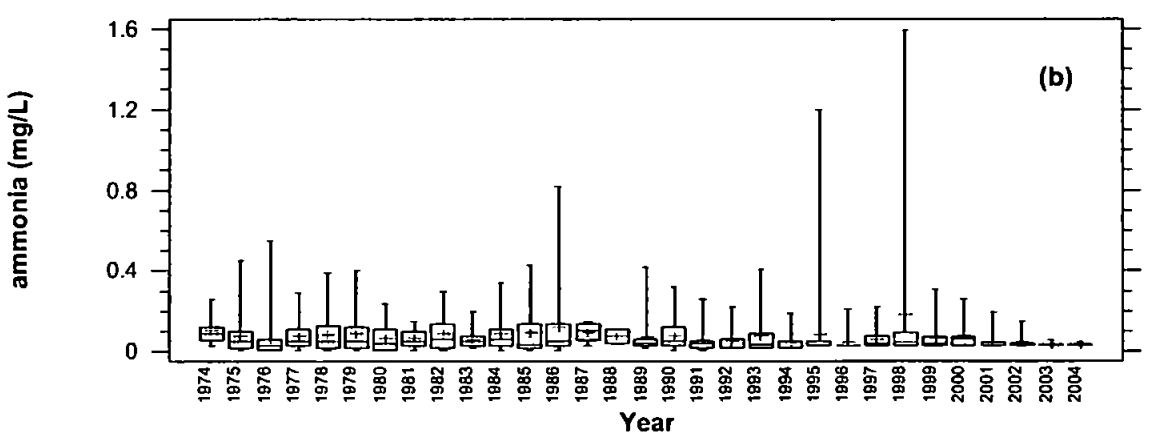


Figure 3.9: Ammonia water quality data collected at Gunnislake (1974 to 2004).

Nitrate is generally reported to be the largest contributor to the annual TN load within riverine systems (Heathwaite and Johnes, 1996) and is considered to be the predominantly bioavailable form of nitrogen. Therefore, from a practical environmental perspective, nitrates are more frequently included in most routine environmental monitoring programmes than other N species (Johnes and Burt, 1991; 1993; Heathwaite, 1993). However, it is important to characterise the nitrogen biogeochemistry before taking a decision on which N species are to be monitored. Research has shown that in some riverine systems, DON dominates the dissolved nitrogen phase (Berman, 2001; Berman

and Bronk, 2003; Antia et al., 1991) and some components of the DON pool can play an active role in supplying N directly or indirectly to phytoplankton and bacteria (Berman, 2001). However, time series TN, TDN and DON data were not available for the Tamar. This historical dataset does not provide information as to which N species dominates the dissolved phase or the TN in the Tamar River.

3.3.1.3 Silicon

Silicates in natural waters are primarily derived from weathering of rocks and are essential nutrients for diatoms (Reynolds et al, 1984; Neal et al., 2005). Silicate data at Gunnislake, available from 1974 to 1998, indicate that 28 % of silicate concentrations are less than 2 mg L^{-1} Si, while 65 % of silicate concentrations ranged from 2 to 3 mg L^{-1} Si. Mean annual silicate ranged from 1.7 to 3.0 mg L^{-1} Si with a 25 year average of 2.2 mg L^{-1} Si (Figure 3.10 a). Silicate concentrations in the Tamar did not exceed 3.9 mg L^{-1}. In a summary of silicate in eastern UK rivers, Neal et al. (2005) found that concentrations ranged from 0 to 16 mg L^{-1} Si with mean concentrations for each river varying from 1.2 – 7.5 mg L^{-1} Si, with the lowest concentrations recorded in rural catchments. Jarvie et al. (2002) suggests that the R. Tweed is typical of concentrations of silicate in a rural catchment (mean 1.3 mg L^{-1} Si; range, 0.3 - 1.9 mg L^{-1} Si). Silicate concentrations in the Tamar appear to be typical of UK rivers draining rural catchments.

3.3.1.4 Chlorophyll a

Chlorophyll a is primary photosynthetic pigment found in all plants that release oxygen and chlorophyll a concentrations in natural waters are used as indicators of photosynthetic productivity in aquatic systems. Chlorophyll a data for the Tamar River at Gunnislake were available from 1978 to 1998 (Figure 3.10 b). Analysis of these data did not indicate a correlation between TP, FRP or nitrate with chlorophyll a concentrations. It should be noted that chlorophyll a data was not available for 1976 when the Tamar River would have

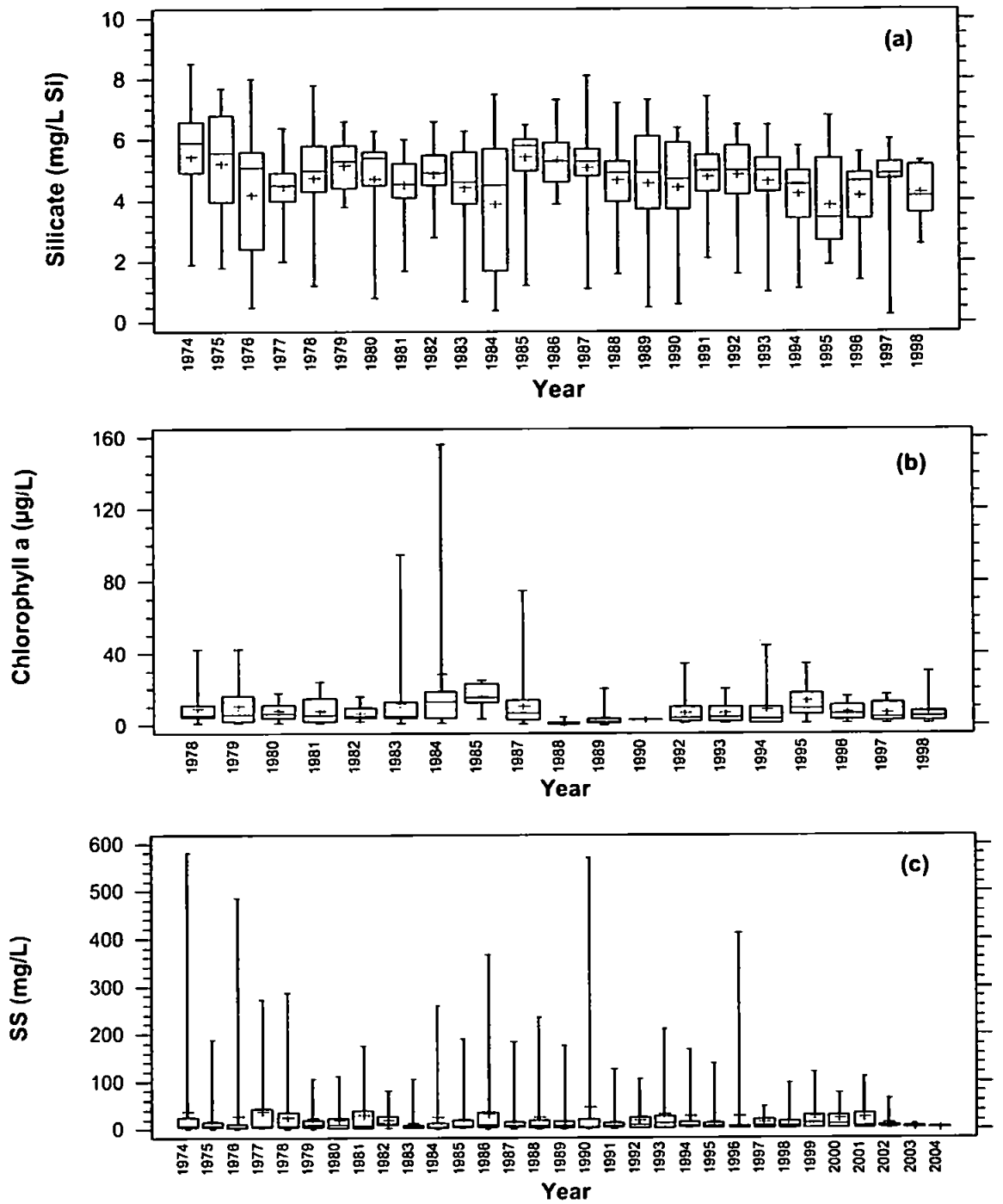


Figure 3.10: Box and whisker plots of (a) silicate and (b) chlorophyll a and (c) suspended solids (SS) data collected at Gunnislake from 1974 to 1998.

been categorised as eutrophic with respect to P, and also that chlorophyll a concentrations were not excessively high in 1990 when the Tamar River was also classed as eutrophic. Chlorophyll a concentrations exceeded 25 µg L^{-1}, the level recommended as the maximum summer concentration (OECD, 1982) in 1979, 1984, 1987, 1994 and 1995. An indicator

value of 10 μg L^{-1} chlorophyll a (mean annual concentration) for suspected eutrophic conditions in UK waters (Langston et al., 2003) was also exceeded in these years as well as in 1983 and 1985. Plots by Gardolinski (2002) show that chlorophyll a concentrations are typically highest during the summer months (Figure 3.8).

3.3.1.5 Suspended solids (SS)

Within stream ecosystems, primary producers and invertebrate communities can be sensitive to increasing suspended solid concentrations (Dodds and Whiles, 2004), and benthic communities can be adversely affected indirectly through excess sedimentation. Water with SS concentrations in excess of 400 mg L^{-1} provide poor fish habitat and in excess of 80 mg L^{-1} (Dodds and Whiles, 2004) fisheries may be harmed. Mean suspended solids concentration at Gunnislake over the 30 year period (1974 to 2004) was 23.5 mg L^{-1} (Figure 3.10 c). However, 80 % of the data at Gunnislake were <23.5 mg L^{-1}, as reflected by the annual median values which ranged from 3.2 mg L^{-1} in 2004 to 13.9 mg L^{-1} in1999 (Figure 3.10 c). SS concentrations in excess of 80 mg L^{-1} were not uncommon (7.5 %) and levels as high as 580 mg L^{-1} have been recorded at this site in 1974 and in 1990. These occurrences arise from spate events rather than sustained periods of high SS. Suspended solids concentrations are lowest in summer (weeks 19 to 40) and highest over the late autumn to early spring months (Figure 3.8). Time series data indicate that spate events are not limited to any particular time of the year.

3.3.1.6 Hydrological trends

Hydrological data are collected on a daily basis. Rainfall data is reported as the total precipitation collected for each day of the year. River discharge rates are measured every 15 min and the mean discharge for each day is reported each day of the year as the daily discharge rate. The mean annual discharge rate refers to the mean daily discharge rate calculated for each year, and the mean annual rainfall refers to the mean daily rainfall

calculated for each year.

Statistical analysis of the rainfall data collected at Gunnislake from 1961 to 2004 suggests that mean daily rainfall over this period is strongly influenced by periods of heavy rainfall: the mean daily rainfall calculated for each year (25 to 44 mm; 43 yr mean - 34 mm) far exceeded the median daily rainfall for each year (0 to 22 mm; 43 yr mean - 4 mm) (Figure 3.11 a). Annual rainfall from 1965 to 2004 ranged from 923 mm (1973) to 1622 (2000) with a mean annual rainfall of 1255 mm ± 173 mm over this period.

Median discharge rates of the Tamar River at Gunnislake from 1974 to 2004 was 11.4 $m^3 s^{-1}$ and ranged from 6.3 to 19.3 $m^3 s^{-1}$ over this period (Figure 3.11 b). Biggs (2000) suggests that discharge rates greater than 3 times the median discharge signals flood or spate events. Using this definition, river discharge rates greater than 34.3 $m^3 s^{-1}$ are indicative of spate events in the Tamar. Approximately 20 % of flows in the Tamar at Gunnislake exceeded this value and flows as high as 484 $m^3 s^{-1}$ have been recorded during spate events (Figure 3.11 b). Spate events increase the mean discharge rates (30 yr mean - 22.4 $m^3 s^{-1}$) to twice that of the annual median discharge rates. It is highly likely that nutrient export from the Tamar will be strongly influenced by these spate events.

River discharge in the Tamar catchment is highly variable but this is typical of small riverine catchments (< 1000 km^2) (Ryding and Rast, 1989) and long-term mean discharge recorded for the Tamar at Gunnislake was comparable with mean discharge rates of 18.2-22.5 $m^3 s^{-1}$ reported by Neal and Davies (2003) for eastern UK river catchments of a similar size (900 ± 100 km^2).

The mean annual rainfall and mean annual discharge rate show a strong correlation (r^2= 0.808) but daily rainfall and discharge in the catchment were not strongly correlated (r^2= 0.212). This suggests that although river discharge is influenced by rainfall, the discharge at Gunnislake is influenced by short term changes due to other factors such as abstraction (from the Tamar upstream of Gunnislake drinking water supply) and also by percolation of rainfall in to the soil, evaporation rates and river baseflow.

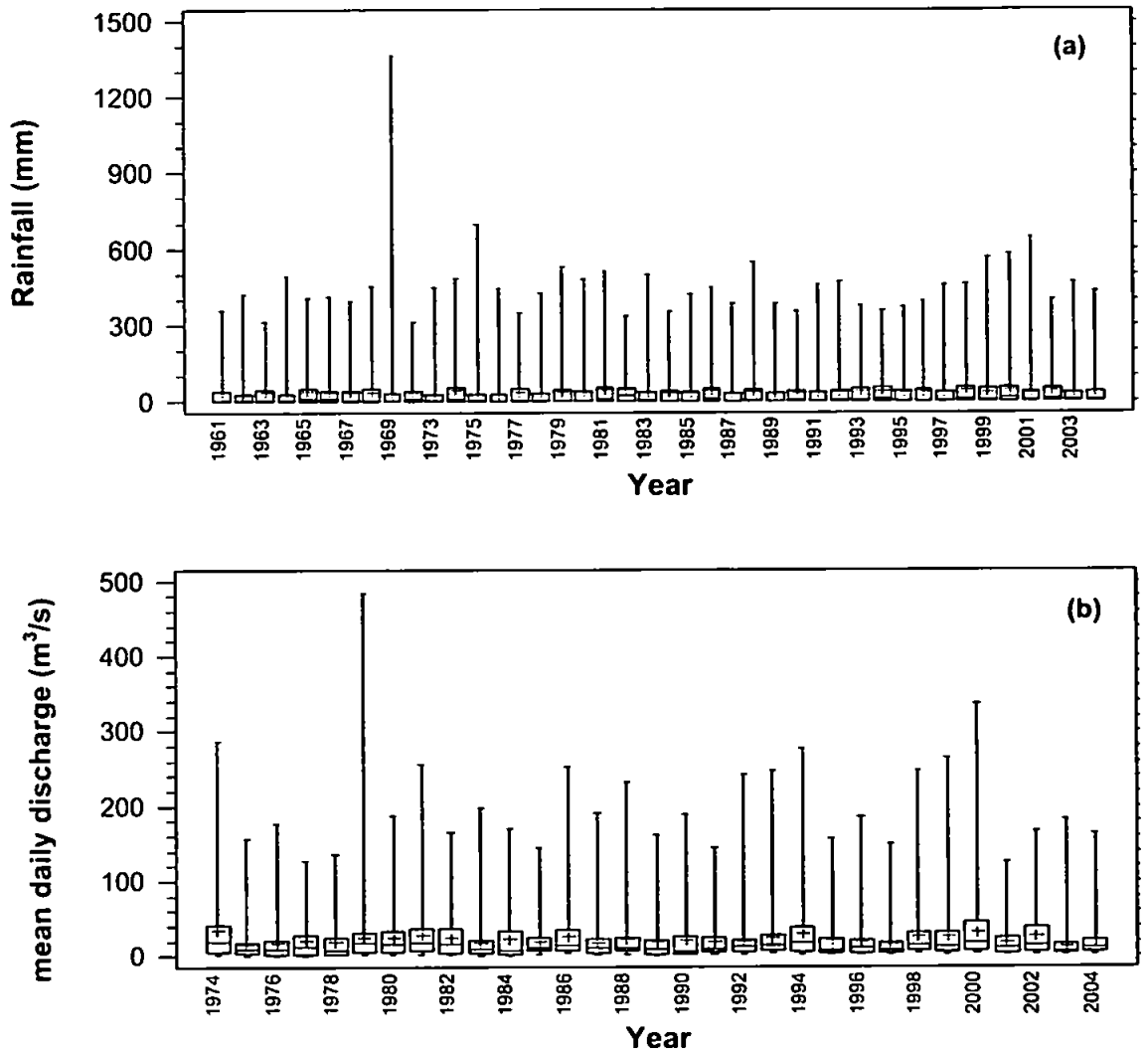


Figure 3.11: Box and whisker plots of (a) rainfall at Pentillie Castle from 1961 to 2004 and (b) mean daily discharge at Gunnislake from 1974 to 2004.

Seasonal hydrological trends: Long-term (1956-2002) mean monthly statistics for river discharge for the River Tamar at Gunnislake and catchment rainfall and catchment runoff data (gauging station 47001) (Figure 3.12) were available from the National River Flow Archive (www.nerc-wallingford.ac.uk/ih/nrfa/index.htm). Long-term trends show that daily discharge rates are highest during December and January and rainfall and runoff are lowest during June and July and highest from September to January.

Mean daily rainfall was strongly correlated to catchment runoff ($r^2 = 0.913$; Table 3.1) and river discharge ($r^2 = 0.941$; Table 3.1) when a delay-time of 1month was factored in to the

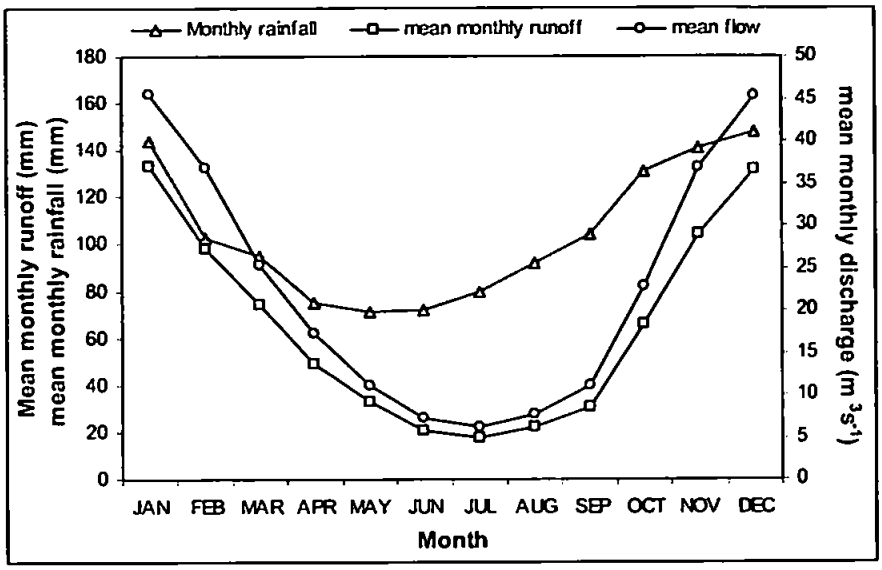


Figure 3.12: Mean monthly discharge statistics and rainfall for the period 1956-2002. Data available from the National Rivers Flow Archives (www.nerc-wallingford.ac.uk/ih/nrfa/index.htm).

rainfall data suggesting that catchment runoff and river discharge rates at Gunnislake are influenced by rainfall within the catchment from 1 month earlier. This relationship can be used to predict periods of low river discharge, which are often associated with increased TP concentrations, from periods of low rainfall up to a month in advance. Gardolinski (2002), in analysis of weekly water quality data in the Tamar, also found improved correlation between rainfall and discharge when a 4-5 week delay time between rainfall and discharge was considered.

Approximately 59 % of rainfall over the Tamar catchment is lost to runoff (EA website, 2001). The Slapton Ley catchment is of similar geology and topography as the Tamar catchment and 56 % of rainfall from that catchment was also lost to runoff (Johnes, 1996). P export from a catchment is not directly influenced by rainfall but it is affected by discharge which in turn is affected by rainfall. Therefore, annual rainfall as well as availability of nutrient water quality data should be taken into consideration when constructing empirical P export models.

Table 3.1: Correlation between various hydrological variables and seasonal TP based on regression coefficient (r^2).

	mean daily flow per month ($m^3 s^{-1}$)	mean daily runoff per month (mm)	mean daily rainfall per month (mm)	mean daily rainfall per month with 1 month lag-time (mm)	mean monthly TP (Feb-Aug)	mean monthly TP (Sept-Jan)
mean daily flow per month ($m^3 s^{-1}$)	1.000					
mean daily runoff per month (mm)	0.996	1.000				
mean daily rainfall per month (mm)	0.688	0.740	1.000			
mean daily rainfall per month with 1 month lag-time (mm)	0.941	0.913	-	1.000		
mean monthly TP (Feb-Aug)	0.898	0.907	0.267	0.838	1.000	
mean monthly TP (Sept-Jan)	0.603	0.676	0.482	0.816	-	1.000

3.3.1.7 Other physico-chemical characteristics of the Tamar catchment

Dissolved oxygen (DO) concentrations in the Tamar at Gunnislake ranged from 6.5 to 16.2 mg L^{-1} (Figure 3.13 a). The lowest DO concentrations were recorded in 1984. The temperature of the waters at Gunnislake ranged from 1-27°C from 1974-2004 (Figure 3.13 b). The solubility of gases, such as oxygen, in water decreases as water temperature increases; DO concentrations at Gunnislake from 1974 to 1998 reflected this trend, exhibiting a linear decrease with increasing temperature (r^2 = 0.742) , with lowest DO concentration recorded during the summer months. Seasonal trends show that water temperatures were lowest in January and February (Weeks 1-8, Figure 3.8), then increased from mid April with highest temperatures in mid-summer, July and August (weeks 25-35, Figure 3.8), and decreased again to below 10° C by mid-October. EA data indicates that pH in the Tamar at Gunnislake ranged from 6.1-9.3 over the past 30 years (Figure 3.13 c) with little variation of the mean annual pH (pH 7.0 to 7.7) over this period (Figure 3.13 c). Long-term mean pH over this period was 7.5.

3.3.2 Nutrient and physico-chemical data for the Tamar River for 2003 – 2004

The variable periodicity of the historical nutrient water quality dataset provided by the EA has highlighted the need for regular monitoring, both in periodicity and in the number of

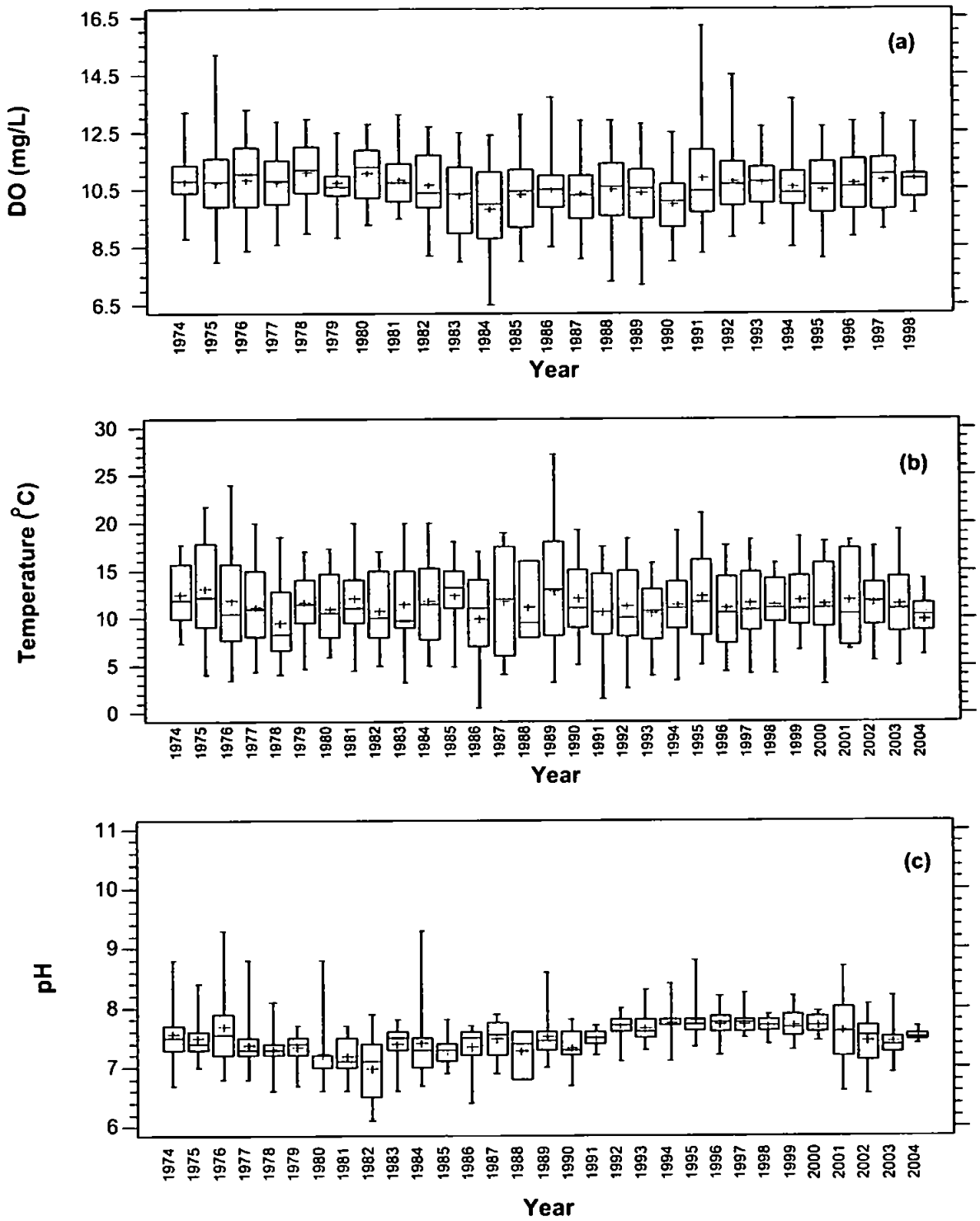


Figure 3.13: Box and whisker plots of physico-chemical data collected at Gunnislake from 1974 to 2004; (a) dissolved oxygen (DO); (b) temperature, and (c) pH.

sampling events per year. In addition, the historical dataset provides very limited information as to P and N speciation within the water column. In this study, bi-monthly

sampling of the Tamar River at Gunnislake was undertaken from May 2003 to May 2004 (Table 3.2) to monitor TN and TP concentrations present in the waters leaving the Tamar catchment and to identify short-term trends with respect to the biogeochemistry of N and P in the Tamar.

3.3.2.1 Phosphorus

TP concentrations at Gunnislake ranged from 37 – 160 μg L^{-1} P from May 2003 to May 2004, with a mean concentration of 92 μg L^{-1} P (Figure 3.14). This was in good agreement with TP concentrations recorded from 1989 to 2004 by the EA. TP in the Tamar as dominated by dissolved P; mean TDP and mean PP were 66 % and 34 % of TP, respectively. Approximately 50 % of TP on average in the waters at Gunnislake was FRP (FRP:TP ratio of 0.51) from May 2003 to May 2004. The FRP:TP ratio in this study was highly variable (0.21 to 0.91) reflecting a similar trend to that observed with historical FRP:TP data recorded for the Tamar (0.1-1.0).

The total dissolved P fraction was generally dominated by FRP (mean, 77 %; range, 41-99 %) and a significant positive correlation between FRP and TDP was observed over the period May 2003 to May 2004 (Figure 3.15) (Table 3.3). These trends are characteristic of rural, agricultural catchments, such as the Tamar catchment, which tend to be dominated by soluble inorganic P, such as orthophosphate from diffuse sources (Jarvie et al., 1998). Jarvie et al. (1998) suggested that this relationship could be used to differentiate between rural/agricultural and urban/industrial rivers since P concentrations in urban/industrial catchments tend to be dominated by dissolved organic P (associated with sewage and domestic waste).

Mean annual FRP from May 2003 to May 2004 was 47 μg L^{-1} P. Therefore, according to interim standards published by the EA (1999), the waters leaving the Tamar River catchment and entering the Tamar Estuary were mesotrophic over the study period. This assessment of the trophic status of the Tamar at Gunnislake with respect to P is in

Table 3.2: Date and location of sampling events

Sampling event	Date	Sample Location
1	28 May 2003	Gunnislake
2	20 June 2003	Weir Head
3	30 June 2003	Gunnislake
4	14 July 2003	Gunnislake
5	01 August 2003	Gunnislake
6	15 August 2003	Gunnislake
7	19 August 2003	Weir Head
8	29 September 2003	Gunnislake
9	30 September 2003	Gunnislake
10	20 October 2003	Weir Head
11	30 October 2003	Gunnislake
12	19 November 2003	Weir Head
13	27 November 2003	Gunnislake
14	03 December 2003	Weir Head
15	19 December 2003	Gunnislake
16	09 January 2004	Gunnislake
17	27 January 2004	Gunnislake
18	25 February 2004	Gunnislake
19	26 February 2004	Gunnislake
20	11 March 2004	Gunnislake
21	15 April 2004	Gunnislake
22	27 April 2004	Gunnislake
23	27 April 2004	Weir Head
24	13 May 2004	Gunnislake
25	27 May 2004	Gunnislake

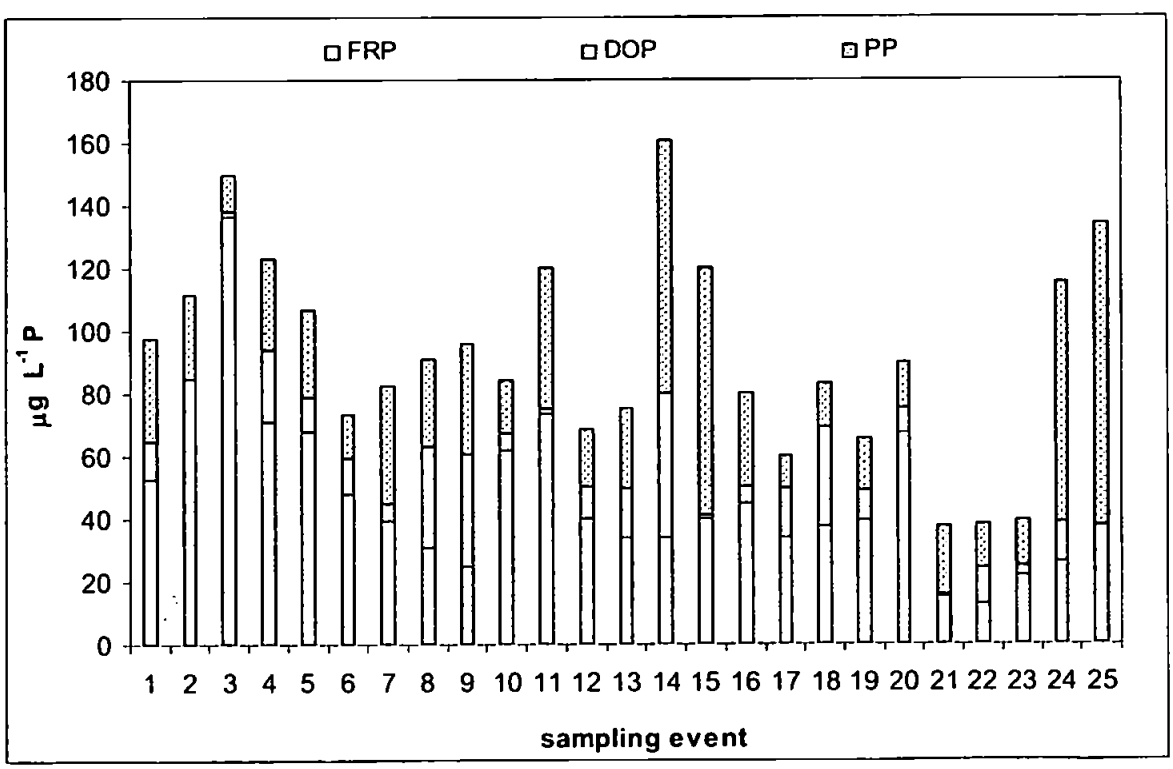


Figure 3.14: Monitoring of phosphorus species (FRP, DOP and PP) in the Tamar River at Gunnislake from May 2003 to May 2004.

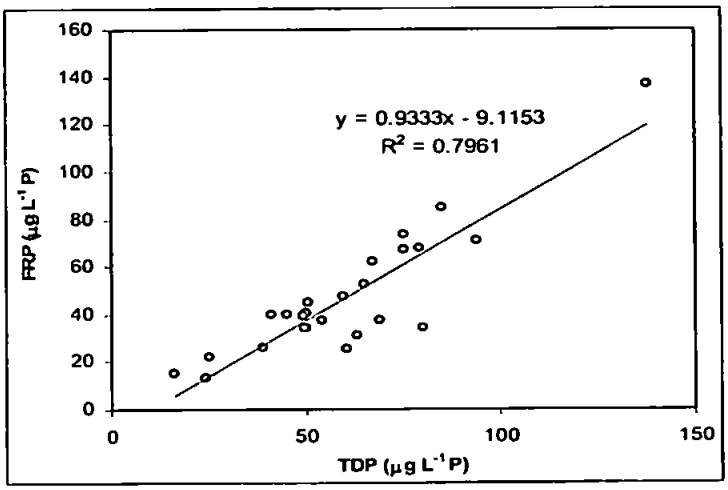


Figure 3.15: Relationship between FRP and TDP in the Tamar River at Gunnislake from May 2003 to May 2004

Table 3.3: Correlation table (2003-2004)

	Rainfall (daily)	Mean discharge (daily)	Temperature
Mean discharge (daily)	0.217[a]	1.000	-
TP	0.107	0.004	-
FRP	0.250	0.015	-
TDP	0.219	0.023	-
FRP:TP	0.107	0.004	-
FRP:TDP	0.055	0.003	-
TN	0.041	0.096	-
TDN	0.038	0.040	-
nitrate/nitrite	0.022	0.219	-
Suspended solids	0.000	0.703	-
DO	-	0.364[b]	0.842

[a] based on mean daily rainfall vs mean daily discharge from May 2003 to May 2004

[b] curvilinear logarithmic relationship

agreement with the historical data provided by the EA, which also indicated mesotrophic status from 2000 to 2004.

3.3.2.2 Nitrogen

TN concentrations ranged from 1.51 to 6.13 µg L^{-1} N during the sampling period, with an average concentration of 4.07 µg L^{-1} N (Figure 3.16). According to the criteria outlined by Dodds et al. (1997), the waters of the Tamar at Gunnislake would be considered eutrophic

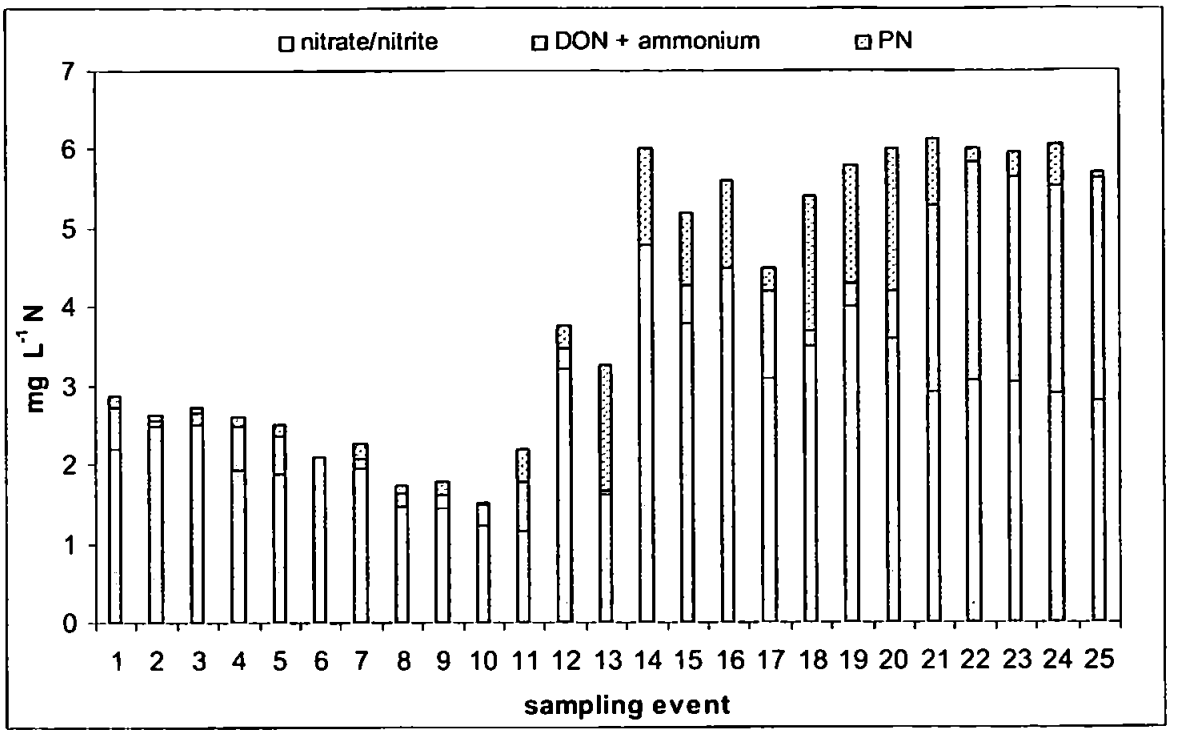


Figure 3.16: Nitrogen species in the Tamar River at Gunnislake from May 2003 to May 2004 showing nitrate/nitrite, DON + ammonia and PN.

(>1.5 mg L^{-1} N) with respect to N; in contrast, these waters would not be considered polluted according to criteria for the identification of polluted surface waters under the Nitrates Directive (<11.3 µg L^{-1} NO_3-N). However, nitrate concentrations approached the guide levels of 5.65 µg L^{-1} NO_3-N for surface waters intended for the abstraction of drinking water on one occasion (3 December 2003). Both TN and nitrate/nitrite concentrations are highest over the winter months (December to February) when biological uptake is lowest.

Nitrogen in the Tamar River was predominantly in the dissolved phase; TDN made up 87 % of TN (mean concentration) from May 2003 to May 2004. Also, nitrate/nitrite made up a large proportion of the dissolved nitrogen fraction (50 – 100 %; mean, 82 %) (Figure 3.16). This agrees with EA data (EA, 1999 b) which suggests that nitrates account for approximately 84 % of TN in surface waters in SW England. Nitrate/nitrite concentrations are linearly correlated to the TDN (Figure 3.17) from May 2003 to March 2004 (r^2= 0.938) but not from April to May 2004 at the start of the growing season when increasing

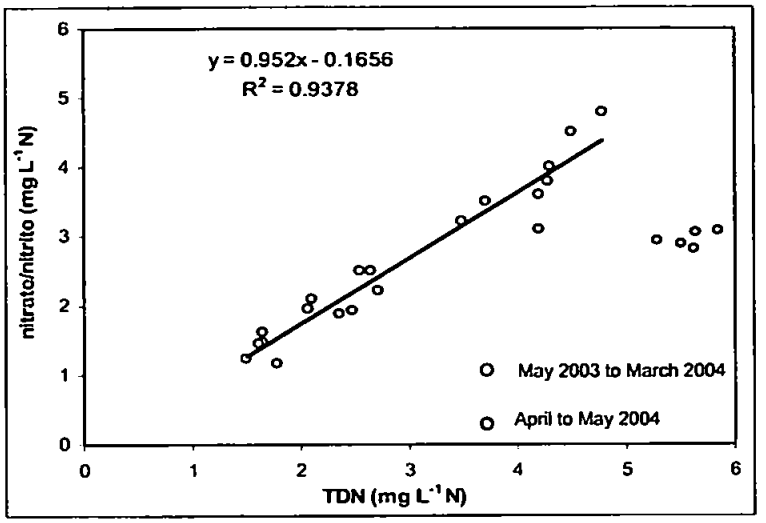


Figure 3.17: Relationship between nitrate/nitrite and TDN in the Tamar River at Gunnislake from May 2003 to May 2004.

temperature and light would encourage increased productivity and uptake of bioavailable N species. Ammonia data available from the EA for April to May 2004 does not indicate significant increase in the ammonia concentrations at Gunnislake, suggesting that the increase in TDN observed from April to May may be due to increased DON in the waters of the Tamar.

3.3.2.3 Hydrological and physico-chemical trends

The median daily rainfall of 3 mm, recorded from May 2003 to May 2004, was lower than the range of median daily rainfall calculated per annum over the period 1961 to 2004 and lower than the 43-yr median daily rainfall of 4 mm. The total rainfall over the sampling period, 1100 mm, was also lower than mean annual rainfall (1255 mm ± 173 mm) calculated from historical data. Mean discharge rates (15 m^3s^{-1}) over the sampling period were just within the range of mean discharge rates calculated per year from 1974 to 2004 (Section 3.3.1.). Therefore the study period, from May 2003 to May 2004, could be considered drier than average.

Historical trends (Section 3.3.1) suggest that mean monthly TP concentration was

positively influenced by the rate of discharge from February to August (r^2=0.898). However, a similar relationship was not observed in this study (r^2=0.095). In addition, N and P concentrations in this study were not correlated with river discharge rates (Table 3.3). Total N and P concentrations in natural waters where nutrients are derived from diffuse sources, such as in rural agricultural catchments, are generally related to rate of discharge (Jarvie et al., 1998). Field trip dates were planned in advance and weather conditions were only considered with respect to safety rather than convenience. Nevertheless, little or no rainfall was recorded on most sampling occasions in this study and spate events, with discharge rates greater than 34.3 m^3 s^{-1}, were encountered on only one sampling occasion (19 November 2003, sampling event 12 in Table 3.4). Therefore, the hydrological conditions encountered in this study are not truly representative of the range of environmental conditions encountered within the catchment and possibly fails to capture some environmental trends such as the relationship between nutrient concentration and discharge. This is a feature of routine monitoring that needs to be considered carefully in the design of sampling schemes.

A positive correlation between SS and river discharge from the Tamar was observed (Table 3.3). Littlewood and Marsh (2005), in a study of annual river mass loads from Great Britain, also reported similar observations for UK rivers. DO concentrations in natural waters tend to increase with increasing discharge rates as observed in the Tamar over the study period (r^2=0.364, Table 3.3). Over this period, DO concentrations also decreased linearly with increasing temperature (r^2=0.842, Table 3.3 and 3.4). pH measurements recorded at Gunnislake during this study (Table 3.4) were within the range expected for the Tamar based on historical data (Section 3.3.1).

3.3.2.4 N:P ratios

Nutrient concentrations as well as elemental nutrient ratios are thought to influence aquatic productivity, especially in freshwaters (Turner et al., 2003; Jusctić et al., 1995a).

Table 3.4: Hydrological and physico-chemical characteristics at Gunnislake for each sampling event

Sampling event	Date	mean daily discharge (m^3s^{-1})	daily rainfall (mm)	pH	Conductivity (µS)	Temperature (°C)	DO ($mg\ L^{-1}$)	SS ($mg\ L^{-1}$)
1	28-May-03	6.0	0.20	7.60	172	15.4	8.3	18
2	20-Jun-03	3.6	0.20	7.22	263	16.0	9.8	9
3	30-Jun-03	3.82	8.00	7.51	420	16.7	10.0	6
4	14-Jul-03	2.6	0.00	7.62	176	19.1	9.2	7
5	01-Aug-03	10.9	13.30	7.24	193	19.2	8.9	15
6	15-Aug-03	3.0	0.00	6.68	130	19.3	9.3	12
7	19-Aug-03	2.8	0.00	7.04	187	17.8	9.6	5
8	29-Sep-03	2.68	0.90	7.31	216	18.0	9.3	8
9	30-Sep-03	2.6	0.60	7.46	232	17.9	9.3	20
10	20-Oct-03	3.1	0.00	7.27	186	13.2	10.6	3
11	30-Oct-03	20.9	4.70	6.9	217	12.0	10.9	40
12	19-Nov-03	17.1	0.50	7.23	127	9.4	11.5	19
13	27-Nov-03	30.2	0.70	6.3	133	7.3	11.5	65
14	03-Dec-03	26.2	2.40	6.95	68	8.7	11.8	19
15	19-Dec-03	17.1	0.00	6.30	308	7.0	12.0	21
16	09-Jan-04	82.2	6.70	7.08	251	6.0	12.6	69
17	27-Jan-04	20.1	0.00	7.28	213	5.6	12.5	35
18	25-Feb-04	10.4	0.50	7.36	283	4.3	13.0	18
19	26-Feb-04	8.9	0.50	7.20	40	4.5	14.0	20
20	11-Mar-04	6.5	0.00	7.22	43	4.3	13.1	13
21	15-Apr-04	8.0	0.00	7.23	160	10.2	10.8	6
22	27-Apr-04	11.1	0.00	7.62	340	12.2	11.2	11
23	27-Apr-04	11.1	0.00	7.51	220	12.7	10.7	13
24	13-May-04	7.9	0.00	7.35	285	13.2	10.0	5
25	27-May-04	4.3	0.00	7.37	225	13.2	10.6	3

Comparison of the relative quantities of major nutrients with the average requirements of phytoplankton cells is frequently used to predict which nutrient will limit productivity. The Redfield ratio, based on the molar ratio of N:P of 16:1, is an empirical expression of this limitation and is generally used as an indicator of the mean elemental composition of cells growing without nutrient limitation (Hecky et al., 1993). Redfield (1958) proposed this ratio based on the elemental composition of phytoplankton and the ratio of the NO_3^- and PO_4^{3-} content of seawater (Falkowski and Davis, 2004). This relationship has become fundamental to the study of the biogeochemistry of natural waters. N:P ratios based on relative quantities of various nutrient species ratios have since been used to predict nutrient limitation: Justić et al (1995 a and b) used DIN:FRP and nitrate:TP in a comparison of large US river systems; Jarvie et al. (1998) used TDN:TDP in a study of east coast British

rivers; and Robertson (1997) used TN:TP to assess Australian rivers. The nutrient species used can sometimes be determined by data availability. The nitrate:FRP ratio represent the ratio of the readily bioavailable species, while TN:TP represents the ratio of the maximum N and P available in the system. However, use of the TDN:TDP ratio may be a more realistic indicator of N:P supply ratio than using DIN:FRP only, as the available portion of the DON pool is included in the consideration of potential N supply to aquatic microbiota. Berman (2001) suggests that unless the available portion of DON is taken into account, the application of N:P supply ratio to predict or explain cyanobacterial blooms can be misleading.

N:P ratios in this study were calculated using TDN:TDP from data collected from May 2003 to May 2004 at Gunnislake. Jarvie et al. (1998) calculated the N:P ratio as the mass ratio of TDN to TDP and divided the ratios into four classes: ≤ 8, 8-15, 16-23 and ≥ 24 based on the Redfield ratio, where ratios less than 8 were indicative of systems that were potentially N-limited and N:P$\geq$24 were indictive of systems that were potentially P-limited. Using this classification system, 88 % of the N:P ratios recorded for the waters leaving the Tamar catchment during the study period were greater than 24 (Figure 3.18). According to the classification outlined by Jarvie et al. (1998), this suggests the organisms in the Tamar River are likely to be P limited. This is not unexpected as the N and P fractions shows that for most of the study period these waters could be considered eutrophic with respect to TN but mesotrophic with respect to FRP. N:P ratios increased from October to May with the highest ratios recorded at the start of the growing season (April). The Tamar catchment is an agricultural catchment, so severe P limitation related to increase N uptake at start of the growing season is not unexpected. The period May-September 2003 was characterised by low flow and N:P ratios<50; from April to May by low flow high N:P ratio (>100) and from October to March by varied flow and N:P ratios. The period from December to February was characterised by high flow and higher N:P ratios, suggesting

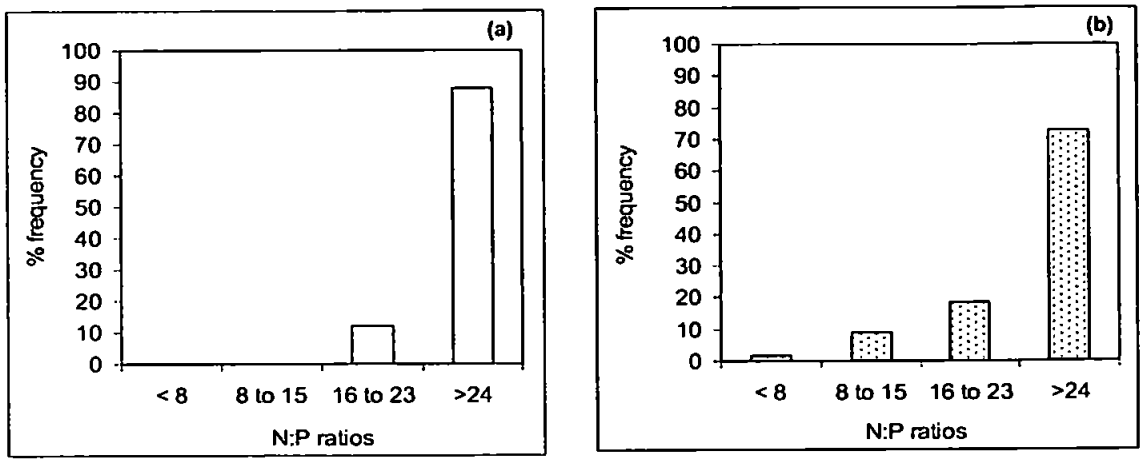


Figure 3.18: Frequency distribution of N:P ratios in the Tamar River at Gunnislake, classified by criteria outlined by Jarvie et al. (1998): (a) from May 2003 to May 2004 calculated from the N:P mass ratio of TDN:TDP; (b) from 1989 to 2004 calculated from the mass ratio of nitrate:FRP.

that during the dormant season more N mobilized than P in higher flow conditions (Jarvie et al., 1998).

The historical dataset provided by the EA for the Tamar River at Gunnislake was also analysed to determine elemental N:P ratios. In this analysis, the N:P mass ratio was calculated from nitrate:FRP as the historical dataset for the Tamar River did not include TDN or TDP data. Historical data indicated that 72 % of the N:P ratios were greater than 24, also suggesting that the system is generally P limited (Figure 3.18 b).

Justić et al. (1995 a) included Si in their analysis of nutrient limitation in natural waters. Their research used molar ratios of N:P, Si:N and Si:P calculated from nitrate, FRP and silicate water quality data to predict limitation based on the Redfield ratio of 16:16:1 (Si:N:P). Their criterion for nutrient limitation was:

P limitation, if Si:P>22 and N:P>22;

N limitation, if Si:N>1 and N:P<10;

Si limitation, if Si:P<22 and Si:N<1.

Ambient nutrient ratios (molar ratios calculated from nitrate, FRP and silicate data) for the Tamar River at Gunnislake over the period 1978 to 2004 for the Tamar were: N:P of 124, Si:P of 43 and Si:N of 0.5, indicating as with other classification systems, that the Tamar is P limited.

Nutrient ratios only suggest potential nutrient limitation and nutrient concentrations must also be considered (Stelzer and Lamberti, 2001). At high environmental N:P ratios benthic algal communities with a high affinity for P may be able to grow faster and monopolise space better than species with that are less efficient at P uptake. Two species with different optimal ratios can coexist only if each species is a better competitor for different nutrients. Robertson (1997) suggests that N:P ratios should be used to predict potential algal blooms only when a catchment is nutrient limited. If the aquatic system is limited by other factors, e.g. light, then the N:P ratio would not be of relevance.

3.3.3 Recommendations for future biogeochemical studies of the Tamar River

The analysis of water quality data collected in this study at Gunnislake, and the review of archival water quality data for this site have shown that monitoring programmes are not infallible and the data that they provide often reflect a compromise between budgetary constraints, current environmental concerns and analytical capabilities. Future monitoring of nutrient species in the Tamar River should include collection of samples for chlorophyll a determination. This would allow comparison of N and P concentrations and N:P ratios with chlorophyll a concentrations. Community structure should be investigated in the event to algal blooms as this would provide useful information with respect to species composition and their nutrient requirement within the Tamar River system. The monitoring of silicate concentrations, particularly in spring, also would be useful in establishing ratios of N:P:Si with respect to diatom blooms. Recommended nutrient species to be monitored are TP, FRP, nitrate/nitrite, TDN and TN, silicate and physico-

chemical and hydrological parameters. TDN:TDP should be used to calculate N:P ratios as it incorporates the possible contributions of DON uptake to productivity within the system.

This study shows that periods of low rainfall are more strongly linked to increased TP from February to August; whilst the nitrate concentration is highest from late autumn to early spring. The N:P ratios indicate that the Tamar River is P limited and therefore increases in the P concentrations are likely to promote increases in biomass. Since flow is linked to a 1-month delay after rainfall at Gunnislake (Table 3.3), periods of prolonged low rainfall could be used as an indicator of periods that require more frequent monitoring.

Manual sampling was undertaken in this study, and the data provided by the EA were obtained from manually collected samples (Littlewood and Marsh, 2005). Manual sampling has inherent flaws such as infrequent sampling, sampling on preferred days of the week, increased sampling frequency when weather is favourable and infrequent sampling when rainfall is high or the temperature is low (Littlewood and Marsh, 2005). Therefore the full range of environmental conditions is not captured. High frequency data collected over regular intervals would more representative of the full range of temporal variability and the associated environmental conditions and nutrient dynamics and mobility within the system. Continuous monitoring using *in stiu* real-time monitors, such as the monitor described in Chapter 5, would make it possible to collect high frequency data from rivers that are more representative (Harris and Heathwaite, 2005).

3.4 Conclusions

Analysis of water quality data for the Tamar River at Gunnislake has highlighted the following trends and observations:

1. Annual mean FRP calculated from 1974 to 2004 indicate a decrease in FRP since the 1990's such that the trophic status of the Tamar was mesoeutrophic from 1974 to 1990

(>60 μg L^{-1} P but <100 μg L^{-1} P) but has been mostly mesotrophic since 1991 (>20 μg L^{-1} P but <60 μg L^{-1} P).

2. Waters of the Tamar River are eutrophic with respect to nitrogen (>1.5 mg L^{-1} N), according to criteria outlined by Dodds et al. (1997). However, the Tamar River at Gunnislake would not be considered polluted according to criteria under the Nitrates Directive for the identification of polluted surface waters (<11.3 mg $L^{-1}NO_3$-N).

3. Nutrients in the in the waters of the Tamar River at Gunnislake were dominated by dissolved species: TDN made up 87 % of TN from May 2003 to May 2004; and TDP made up 66 % of TP over the same period.

4. Nutrients in the dissolved phase (<0.45 μm) of the water column at Gunnislake are dominated by readily bioavailable inorganic nutrients: TDP concentrations are dominated by FRP (77 %) and TDN concentrations are dominated by nitrate/nitrite (82 %) from May 2003 to May 2004. This is indicative of a system dominated by diffuse sources of nutrients such as agriculture rather than point sources.

5. 88 % of N:P ratios, calculated from TDN:TDP mass ratios recorded from May 2003 to May 2004, suggest that the Tamar system is P limited. TN and nitrate concentrations within the catchment are always high (>1.5 mg L^{-1} N), consequently short-term increases in the P concentration, especially of readily bioavailable P species, could promote a rapid increase in productivity within the system. Therefore, FRP can be considered the key nutrient species to be monitored.

CHAPTER 4

An export coefficient approach to modelling nutrient loads in the Tamar catchment

4.1 INTRODUCTION

Legislation concerning water quality, such as the Water Framework Directive (WFD), is moving towards management of water catchments and predictive modelling to inform decision making. Reliable estimates of nutrient export from river catchments are required for freshwater management and environmental research including the assessment of water resources, hydrochemical budgets leading to nutrient mobility within river catchments and hydro-ecological studies (Littlewood and Marsh, 2005). In addition, nutrient export from rivers are of importance to the research and management of water quality of the receiving waters beyond the freshwater 'zone', such as in estuaries, wetlands, coastal waters and ultimately the sea (Littlewood and Marsh, 2005; Salvia-Castellví et al., 2005).

Research linking catchment processes and water quality impact has traditionally been based on simple empirical relationships or on simulation modelling (Harris and Heathwaite, 2005). Models are often catchment specific and need to be developed using high frequency historical data and need be tested or 'ground-truthed' through the collection of environmental data before they can be refined for use. Predictive models allow conservation/remediation measures to be assessed with respect to possible changes in nutrient water quality prior to implementation.

This chapter sets out to use the nutrient coefficient modelling approach to predict nitrogen and phosphorus export from the Tamar catchment, South West England. The model is constructed and calibrated using historical data (discussed in Chapter 3) available for the catchment area and validated using laboratory-generated data (also discussed in Chapter 3).

4.2 METHODS

4.2.1 The export coefficient modelling approach

The export coefficient modelling approach was developed to predict nutrient loading on eutrophic, standing waters (Beaulac and Recknow, 1982) and has been modified to predict nutrient export from river catchments (Johnes and O'Sullivan, 1989). The export

coefficient represents the amount of a nutrient exported from a particular nutrient source within the catchment. This allows the model to be applied to large-scale catchments. The general equation (equation 1) for the annual nutrient load is:

$$L = \left[\sum_{i=1}^{n} E_i[A_i(I_i)]\right] + p \qquad (1)$$

where L is the loss of a particular nutrient, E is the export coefficient for nutrient source i; A is the area of the catchment occupied by land use type i, or of sewage population equivalents (P.E.) i, or number of animal types i; I is input of nutrients to source i; p is input of nutrients from precipitation. The export coefficient is expressed as the mass of a nutrient exported per unit area per year for each land use activity within the catchment (e.g. kg ha^{-1} yr^{-1}) or as the mass of nutrient exported per capita per year with respect to point sources such as human and animal waste (e.g. kg ca^{-1} yr^{-1} or kg $animal^{-1}$ yr^{-1}).

The export coefficient modelling procedure is carried out in four phases: data collection; model construction; model calibration; validation (Figure 4.1). Physiographic, demographic and meteorological data available for the catchment area of study is used to construct and calibrate the model. Validation is carried out by sample collection and comparison of the field data with the modelled data.

4.2.2 Development of a phosphorus export coefficient model for the Tamar catchment

Physiographic, demographic and meteorological data available for 1998 for the Tamar catchment area was used for model construction. The nutrient coefficient modelling approach requires a 1-year period of water quality data (at the point of exit of the catchment) for the model calibration phase. The test year, 1998, was selected because it was the most recent year for which TP data from at least 24 sampling events were available since monitoring of TP at Gunnislake had begun.

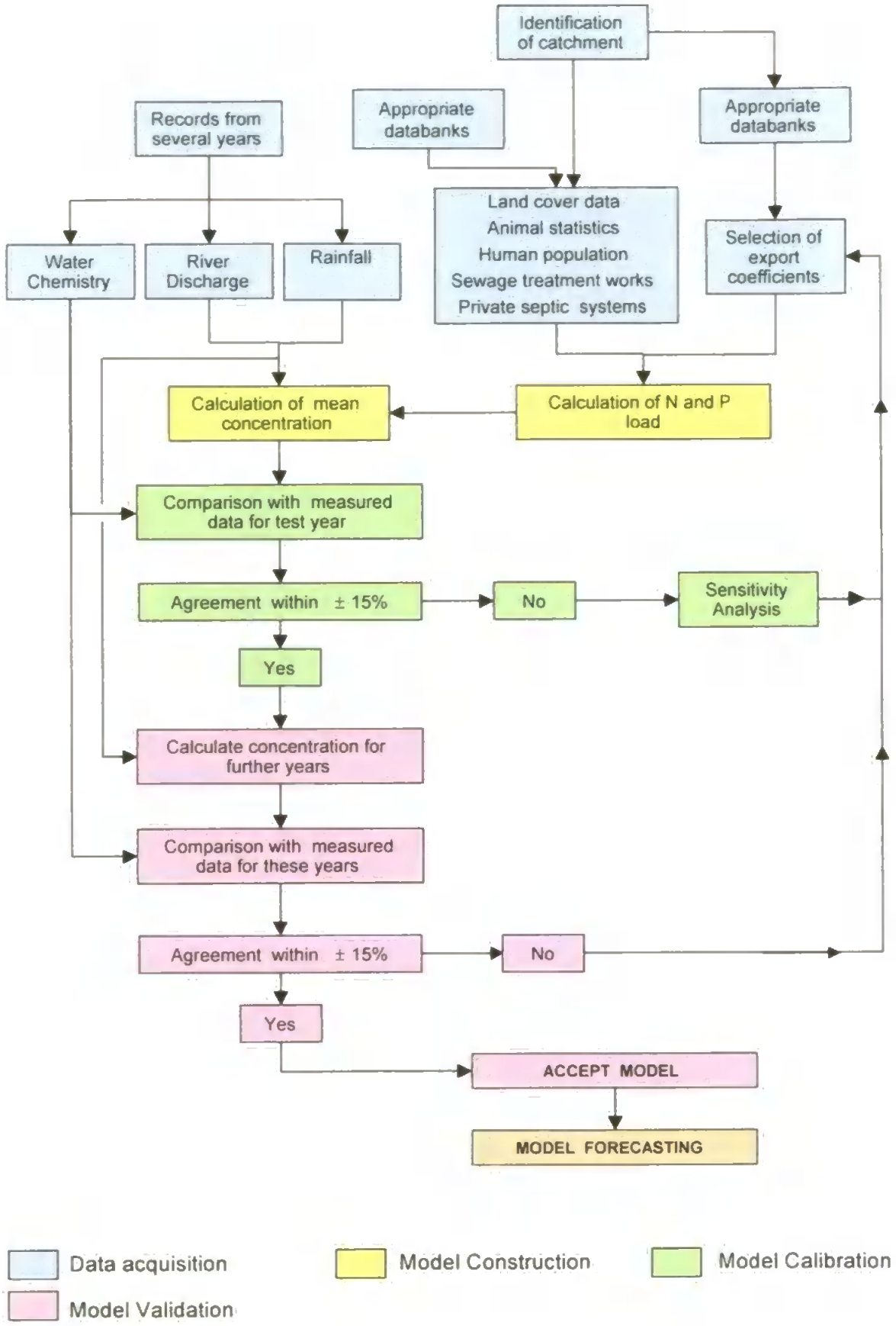


Figure 4.1: Export coefficient modelling procedure

4.2.2.1 Data acquisition

Catchment boundaries: In this study, the Tamar freshwater catchment was identified and digitised using ArcView GIS 3.2 software (Environmental Systems Research Institute, Inc.) from a digital layer of all riverine sub-catchments in South West England provided by the Geography Department, University of Plymouth. Spatial dimensions (such as distance measurement units) were added and the total area of the digitised catchment was calculated.

Water quality and hydrological data: Phosphorus water quality data for 1998, daily rainfall data for 1998 and daily mean river discharge rates for 1998 at Gunnislake were extracted from the datasets described in Section 3.2.3.

Animal statistics: Livestock data for the Tamar catchment was purchased from the Department of Environment, Food and Rural Affairs (DEFRA) as a data subset of the June 2000 Agricultural Census. The data provided pertained to 87 % of the catchment and was extrapolated to cover the entire area of the catchment and also adjusted using the average percentage difference in livestock numbers for Devon and Cornwall between 2000 and 1998 (DEFRA website, 2003) to estimate livestock populations for 1998.

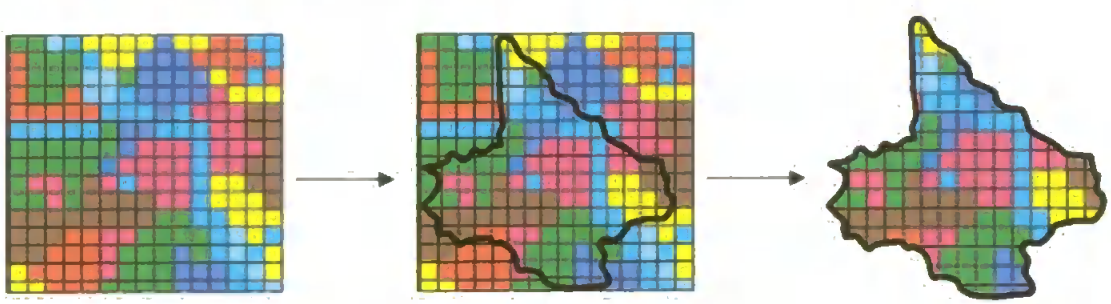
Land use: Land cover data for 1990 was obtained from the Centre of Hydrology (CEH), Monkswood, as a licensed digital data subset (25m spatial resolution) of the LandCover Map of Great Britain and classified into 25 land cover categories (Table 4.1). The digital data was obtained as a grid overlapping the entire catchment and was manipulated with the GIS package ARC/INFO Version 7.0 (Environmental Systems Research Institute, Inc.)* to accurately obtain land cover data for the area within the catchment boundaries, including the area dedicated to each land cover type (Figure 4.4).

Export coefficients were selected from published literature (Table 4.2). Initially, these

* manipulation in Arc/INFO was performed with assistance from Erik Meyles of the Geography Department, University of Plymouth

Table 4.1: The Landsat-derived land cover type classes in the Land Cover Map of Great Britain (25 m spatial resolution), as defined by the Institute of Terrestrial Ecology. Data obtained from from CEH, Monkswood.

Class Code	'target' cover-type classes	General Characteristics
0	Unclassified	Does not fit into the 25 'target' classes
1	Sea / Estuary	Sea, coastal and estuarine waters
2	Inland Water	Fresh water- covered on winter and summer images
3	Beach and Coastal Bare	Intertidal, incl. marine habitats above the tide line
4	Saltmarsh	Marshes up to normal high water spring tides
5	Grass Heath	Inland grasslands often on acidic, sandy soils
6	Mown / Grazed Turf	Managed grasslands (productive swards or mown)
7	Meadow / Verge / Semi-natural	Managed at a lesser intensity than class 6
8	Rough / Marsh Grass	Low herbaceous vegetation of fens, marshes
9	Moorland Grass	Upland swards mostly deciduous grasslands
10	Open Shrub Moor	Shrub species on slopes assoc. with some grazing
11	Dense Shrub Moor	Woody shrubs mixed with herbaceous species
12	Bracken	Dominated by *Pteridium aquilinum*
13	Dense Shrub Heath	High content of heather, broom, and gorse- evergreen
14	Scrub / Orchard	Deciduous shrub and orchards
15	Deciduous Woodland	Deciduous broadleaved trees, no leaves in winter
16	Coniferous Woodland	Maintains leaves all year round - evergreen
17	Upland Bog	Herbaceous wetlands
18	Tilled Land	Arable lands – may be temporarily/seasonally bare
19	Ruderal Weed	Bare ground with annual, short-lived perennial plants
20	Suburban / Rural Development	Mixture of built-up land and permanent vegetation
21	Continuous Urban	Cities, large towns, industrial and commercial sites
22	Inland Bare Ground	Bear of vegetation, surfaced with 'natural' materials.
23	Felled Forest	Recently felled - excepted to be recolonised by 19
24	Lowland Bog	Lowland herbaceous wetlands
25	Open Shrub Heath	Similar to 5 but occurs in lowlands



(a) Digital data grid obtained from CEH for an area overlapping the catchment. Each land cover class is uniquely colour coded and each cell represents land cover information for a 25 m x 25 m area.

(b) The digital data was clipped to fit the digitised catchment boundaries using Arc/Info GIS package. Areas mapped accurately from map coordinates built into data attributes.

(c) Spatial distribution of the land cover classes within the catchment was obtained by counting the number of 25 m x 25 m cells in each class. This step was performed with ArcView.

Figure 4.2: Obtaining land cover data from a digital data set (provided by CEH)

Table 4.2: Export coefficients selected for P

Land cover type	P export Coefficient	Reference
Unclassified	0.48 kg ha^{-1} yr^{-1}	Hanrahan et al., 2001
Inland water	0.00 kg ha^{-1} yr^{-1}	May et al., 2001
Grass heath	0.10 kg ha^{-1} yr^{-1}	Harper and Stewart, 1987
Mown / grazed turf	0.40 kg ha^{-1} yr^{-1}	Johnes, 1996
Meadow / Verge / Semi-natural	0.40 kg ha^{-1} yr^{-1}	Johnes, 1996
Rough / marsh grass	0.07 kg ha^{-1} yr^{-1}	Cooke and Williams, 1973
Moorland grass	0.10 kg ha^{-1} yr^{-1}	Harper and Stewart, 1987
Open shrub moor	0.10 kg ha^{-1} yr^{-1}	Harper and Stewart, 1987
Dense shrub moor	0.10 kg ha^{-1} yr^{-1}	Harper and Stewart, 1987
Bracken	0.10 kg ha^{-1} yr^{-1}	Harper and Stewart, 1987
Dense shrub heath	0.10 kg ha^{-1} yr^{-1}	Harper and Stewart, 1987
Scrub / orchard	0.02 kg ha^{-1} yr^{-1}	Johnes, 1996
Deciduous woodland	0.02 kg ha^{-1} yr^{-1}	Johnes, 1996
Coniferous woodland	0.02 kg ha^{-1} yr^{-1}	Johnes, 1996
Tilled land	0.65 kg ha^{-1} yr^{-1}	Johnes, 1996
Suburban / rural development	0.83 kg ha^{-1} yr^{-1}	May et al., 2001
Continuous urban	0.83 kg ha^{-1} yr^{-1}	May et al., 2001
Inland bare ground	0.70 kg ha^{-1} yr^{-1}	Hanrahan et al., 2001
Felled forest	0.20 kg ha^{-1} yr^{-1}	May et al., 2001
Open shrub heath	0.10 kg ha^{-1} yr^{-1}	May et al., 2001
Cattle [a]	6.89 g $cattle^{-1}$ yr^{-1}	Vollenweider, 1968
Sheep [a]	2.85 g $sheep^{-1}$ yr^{-1}	Vollenweider, 1968
Pig [a]	12.94 g pig^{-1} yr^{-1}	Vollenweider, 1968
Goat [a]	2.85 g $goat^{-1}$ yr^{-1}	Vollenweider, 1968
Horse [a]	13.68 g $horse^{-1}$ yr^{-1}	Vollenweider, 1968
STWs	0.38 kg $capita^{-1}$ yr^{-1}	Johnes, 1996
Private septic tanks	0.24 kg $capita^{-1}$ yr^{-1}	Johnes, 1996

[a] based on average TP produced per 1000 kg live weight by each animal type per year: cattle (17 kg); pig (45 kg); horse (19 kg), sheep and goat (20 kg); the average weight of cattle (450 kg); pig (125 kg); horse (600 kg), sheep and goat (75 kg); based on average export per 1000 kg of TP produced: cattle (0.9 kg); pig (2.3 kg); horse (1.2 kg), sheep and goat (2.85 kg) from Vollenweider (1968):

Vollenweider (1968) reports that 1,000 kg live weight of cattle produces17 kg P yr^{-1};

and 0.9 kg P is exported from every 1000 kg P produced from cattle;

therefore, P export from 17 kg P = (17*0.9)/1000 = 0.0.15 kg P yr^{-1}

since 1 cattle is estimated to weigh 450 kg, 2.2 cattle would weigh 1000 kg and produce 0.0.15 kg P yr^{-1}

therefore, annual P export per cattle = 0.00689 kg P yr^{-1} = 6.89 g P yr^{-1}

coefficients were selected exclusively from Johnes (1996) as these have been used for similar catchments. The Tamar catchment is very similar in geology, soil cover and land use to the Slapton Ley catchment (46 km^2), South Devon, studied by Johnes (1996) but much larger in area (915 km^2). These were later adjusted after the first calibration of the model (see Section 3.3.2.1). In this study, a zone spanning 50 m on either side of all rivers within the catchment was created using ArcView GIS to assess spatial orientation of land

cover types within the riparian zone. Spatial analysis using the ArcView GIS package indicated that the majority of woodlands generally occur near (within 50m) to the rivers while the vast percentage of grasslands do not. Therefore, higher coefficients were selected for woodlands than for arable and grazing lands to reflect this spatial variation. The export coefficients selected for use in the final model are listed in Tables 4.2 and 4.3.

Sewage data: The number of population equivalents served by STWs and the number of private septic tanks within the catchment area was obtained from literature which quoted data based on the 1991 UK census (NRA, 1996).

4.2.2.2 Construction and calibration of a P export coefficient model

The term 'measured load' refers to nutrient export loads calculated from laboratory data, while 'modelled load' refers to the nutrient export predicted from the model.

Modelled loads: The annual TP and TN models for 1998 were constructed using equation 1 and the data collected in the first phase of the modelling procedure.

Measured loads: The measured annual load of nutrients being exported was calculated from P water quality data, river discharges and rainfall data for 1998. Approximately 24 TP readings were obtained at Gunnislake for 1998, which is the least number of statistically viable sampling events necessary for use in modelling water quality (Johnes, 1990). The measured P load for 1998 was calculated using equation 2:

$$L_m = \frac{\left(c_1 q_1 + \sum_{n=1}^{24} \left[\left((c_n q_n + c_{n+1} q_{n+1})/2 \right) * (T_{n+1} - T_n) \right] \right) * d_a}{\sum_{n=1}^{24} (T_{n+1} - T_n)} \qquad (2)$$

where, L_m represents the measured nutrient load calculated from field data (kg yr^{-1}), c represents TP concentration (kg L^{-1}) reported for each of 24 sampling events (n), q represents river discharge volume (L) for each sampling event (calculated from the mean daily discharge rate (m^3 s^{-1})), T represents the sampling event date in Julian days, d_a

represents the number of days in a year and $c_1 q_1$ represents the P load (kg) calculated for the first sampling event recorded for 1998. In 1998, d_a in equation 2 was 365 as 1998 was not a leap year.

Sensitivity analysis: The sensitivity of the Tamar model to nutrients from each source was carried out to determine which model parameters exerted greatest influence on the output of the model. Each export coefficient for each nutrient source was separately adjusted by an arbitrary 10%, as in previous studies (Johnes, 1996, Hanrahan, 2001), whilst all other parameters were held constant. The overall effect on the model was assessed for each parameter and expressed as a percentage change in the model output.

Comparison of measured and modelled P loads for 1998: In the initial calibration of the Tamar freshwater export coefficient model, the modelled P loads were compared with the measured annual P load calculated for 1998. Since the modelled and measured loads for 1998 were not within ± 15% agreement, then the export coefficients were reassessed and model construction repeated. Therefore, according to the export coefficient modelling procedure (Figure 4.1), the export coefficients for each nutrient source were then adjusted to better reflect the conditions of the catchment. This involved acquiring more detailed data for catchment population, parish level livestock statistics, accurate statistics for the number of people served by private septic tanks, fertilizer application rates and classification of 'high risk export zones' to reflect the effect of riparian zones on watercourses. In the final calibration step of the model, the export coefficients were adjusted to the values shown in Table 4.2.

4.2.2.3 Validation of the P export coefficient model

The model validation step was conducted by comparing measured loads calculated from field data collected from May 2003 to May 2004 (Section 3.2.2). In this study, d_a was 366 days as it included part of a leap year (February 2004). The validation step was complete

if the modelled and measured loads differed by <15 %.

4.2.3 Construction and calibration of a nitrogen export coefficient model for the Tamar catchment

Catchment data acquired as described in Section 4.2.2.1 were used to construct an N export coefficient model for the Tamar catchment, using equation 1 (Section 4.2.1). The N model was calibrated as outlined in Sections 4.2.2.2 using water quality data, including TN data, from May 2003-May 2004 for the Tamar at Gunnislake (Section 3.3.2). The export coefficients used in the final calibration step are listed in Table 4.3:

Table 4.3: Export coefficients selected for N

Land cover type	N EXPORT COEFFICIENT	REFERENCE
Unclassified	2.5 kg ha^{-1} yr^{-1}	Royal Society (1983)
Inland water	0 kg ha^{-1} yr^{-1}	Johnes, 1996
Grass heath	6.4 kg ha^{-1} yr^{-1}	Royal Society (1983)
Mown / grazed turf	6.4 kg ha^{-1} yr^{-1}	Royal Society (1983)
Meadow / verge / semi-natural	6.4 kg ha^{-1} yr^{-1}	Royal Society (1983)
Rough / marsh grass	6.4 kg ha^{-1} yr^{-1}	Royal Society (1983)
Moorland grass	6.4 kg ha^{-1} yr^{-1}	Royal Society (1983)
Open shrub moor	6.4 kg ha^{-1} yr^{-1}	Payraudeau et al., 2001
Dense shrub moor	6.4 kg ha^{-1} yr^{-1}	Payraudeau et al., 2001
Bracken	2.5 kg ha^{-1} yr^{-1}	Payraudeau et al., 2001
Dense shrub heath	6.4 kg ha^{-1} yr^{-1}	Payraudeau et al., 2001
Scrub / orchard	13 kg ha^{-1} yr^{-1}	Johnes, 1996
Deciduous woodland	13 kg ha^{-1} yr^{-1}	Johnes, 1996
Coniferous woodland	13 kg ha^{-1} yr^{-1}	Johnes, 1996
Tilled land	13 kg ha^{-1} yr^{-1}	Owens, 1976
Suburban / rural development	2.5 kg ha^{-1} yr^{-1}	Rast and Lee, 1983
Continuous urban	2.5 kg ha^{-1} yr^{-1}	Rast and Lee, 1983
Inland bare ground	6 kg ha^{-1} yr^{-1}	Loehr, 1974
Felled forest	13 kg ha^{-1} yr^{-1}	Johnes, 1996
Open shrub heath	6.4 kg ha^{-1} yr^{-1}	Payraudeau et al., 2001
Cattle[a]	0.81 kg $cattle^{-1}$ yr^{-1}	Vollenweider, 1968
Sheep[a]	0.19 kg $sheep^{-1}$ yr^{-1}	Vollenweider, 1968
Pig[a]	0.15 kg pig^{-1} yr^{-1}	Vollenweider, 1968
Goat[a]	0.19 kg $goat^{-1}$ yr^{-1}	Vollenweider, 1968
Horse[a]	1.21 kg $horse^{-1}$ yr^{-1}	Vollenweider, 1968
Stws	2.14 kg $capita^{-1}$ yr^{-1}	Johnes, 1996
Private septic tanks	2.49 kg $capita^{-1}$ yr^{-1}	Johnes, 1996
Rainfall[b]	11.85 kg ha^{-1} yr^{-1}	Johnes (1983)

[a] based on average weight of cattle (450 kg); pig (125 kg); horse (600 kg), sheep and goat (75 kg) from Vollenweider (1968); average TN produced per 1000 kg live weight per year by each animal type: cattle (156 kg); pig (150 kg); horse (128 kg), sheep and goat (119 kg); based on average export per 1000kg of TN produced: cattle (11.5 kg); pig (8.1 kg); horse (15.8 kg), sheep and goat (21.2 kg) from Vollenweider (1968)

[b] based on annual nitrogen input of 20 kg N ha^{-1} and 59% of rainfall lost to surface runoff in the Tamar catchment

4.3 Results and Discussion

4.3.1 A phosphorus export coefficient model for the Tamar catchment

The UK has approximately 200,000 km of river systems draining in to the sea, via estuaries. Yet, unlike many other EU countries, no single river in the UK accounts for more than 4 % of total runoff (Littlewood and Marsh, 2005). This illustrates the importance of measuring and modelling nutrient export from river catchments within the UK. Data from routine monitoring of nutrients in rivers can be used to calculate annual export from the catchment, which is useful in environmental assessment but not in predicting environmental quality with respect to changes in nutrient inputs to the catchment. Annual export models, such as the export coefficient model, can be used to predict changes in the nutrient export from catchment and therefore they are useful tools for river and catchment management strategies. An export coefficient model for predicting the annual total phosphorus load exported from the Tamar catchment was developed in this study as outlined in Section 4.2.3 and Figure 4.1.

4.3.1.1 Construction and calibration of the P export coefficient model

An export coefficient model for the prediction of TP export from the Tamar catchment was successfully constructed and calibrated in this study and the process is described in this section.

Sensitivity analysis: In the initial model calibration step, the agreement between modelled and measured TP export from the catchment was less than 76% (>15% of the measured TP export). Sensitivity analysis, to determine the model parameters which exert the greatest influence on predicted outcomes indicated that meadows and cattle were the two factors that most influenced the outcome of the initial model. Several steps were taken to improve the model (Figure 4.1): new export coefficients were selected for meadows and grazed/mown turf based on geology and topology; statistics for the number of persons within the catchment that are served by private septic tanks were obtained, and more

refined animal headage dataset based on parish level statistics were obtained from DEFRA (2001).

A sensitivity analysis performed on the revised model indicated that P export from meadows (3.6 %), STWs (1.9 %) and mown/grazed turf (1.4 %) were still the major factors affecting the model outcome (Table 4.4). Meadows account for more than 55% of the all land use (Table 4.5) within the catchment and this spatial dominance appears to be a significant influence on TP export from the catchment. Direct human activities (STWs and septic tanks) also exert considerable influence on the model. It should be noted that although tilled land accounted for only about 6% of the total land use within the catchment, it still had a notable influence (0.89%) on the model output. This strongly suggests that changes in land use would affect TP export from the catchment. A P export coefficient model developed for the Slapton Ley catchment, an intensive livestock catchment, was most sensitive to changes in livestock headage (Johnes, 1996) whereas the Frome P export coefficient model (Hanrahan, 2001 b) was influenced by the same three factors as the Tamar model, but most heavily influenced by export of P from point sources (STWs). The Frome catchment (414 km^2) is mixed agricultural catchment with twice the population density (0.69 persons ha^{-1}) of the Tamar catchment (0.35 persons ha^{-1}) and has a higher animal density than the Tamar.

Uncles et al. (2002) used the export coefficient modelling approach to model N and P loads from the Tamar catchment. However, the Tamar catchment defined in their study was more extensive and included the part of the Tavy, Walkam and Lynher catchments in addition to other sub-catchments as their study aimed at modelling the N and P inputs to the Tamar Estuary, not just the Tamar River. Their export coefficient model was most sensitive to cattle, sheep and grasslands but this data cannot be considered comparable to the findings in our study of the Tamar catchment as the study area is different.

Modelled P export: The catchment characteristics and catchment statistics were used to

Table 4.4 : Sensitivity Analysis (P) for the Tamar catchment

NUTRIENT SOURCE	% Change from base contribution
Meadow / Verge / Semi-natural	3.6
STWs	1.9
Mown/Grazed Turf	1.4
Tilled Land	0.89
Private Septic Tanks	0.62
Suburban / Rural Development	0.34
Unclassified	0.27
Cattle	0.24
Sheep	0.18
Pigs	0.14
Deciduous Woodland	0.060
Bracken	0.039
Rough / Marsh Grass	0.027
Moorland Grass	0.021
Open Shrub Heath	0.010
Coniferous Woodland	0.0090
Horses	0.0059
Felled Forest	0.0059
Continuous Urban	0.0031
Inland Bare Ground	0.0031
Dense Shrub Heath	0.0029
Open Shrub Moor	0.0028
Scrub / Orchard	0.0015
Goats	0.00091
Dense Shrub Moor	0.00010

All values reported to 2 significant figures

predict the annual TP load exported from the Tamar catchment in 1998 (Table 4.6). P export from the catchment was dominated by land use practices, which accounted for 69 % of total modelled TP leaving the catchment annually. Discharge from STWs was the second largest contributor to P export while contributions from livestock were the smallest.

P export from land use: Lands dedicated to grazing (meadows and grazed turf) dominate land use in the catchment and was the chief contributor to P export (73.5%) (Table 4.5). Therefore, the criteria selection of export coefficients for these land use types is important, especially since it exerts the largest influence on the annual modelled P export as discussed in the section on 'sensitivity analysis'.

Table 4.5: Phosphorus export from the Tamar catchment based on land cover types

Land cover type	Area (ha)	Loading (P) (kg yr^{-1})	% P load from each land cover type
Unclassified	2466	1183.7	3.96
Inland Water	11	0.0	0.00
Grass Heath	5946	594.6	1.99
Mown / Grazed Turf	15367	6146.9	20.58
Meadow / Verge / Semi-natural	39553	15821.1	52.96
Rough / Marsh Grass	1670	116.9	0.39
Moorland Grass	900	90.0	0.30
Open Shrub Moor	122	12.2	0.04
Dense Shrub Moor	4	0.4	0.00
Bracken	1696	169.6	0.57
Dense Shrub Heath	127	12.7	0.04
Scrub / Orchard	321	6.4	0.02
Deciduous Woodland	13013	260.3	0.87
Coniferous Woodland	1948	39.0	0.13
Tilled Land	5923	3849.8	12.89
Suburban / Rural Development	1776	1474.3	4.94
Continuous Urban	16	13.5	0.05
Inland Bare Ground	19	13.3	0.04
Felled Forest	128	25.5	0.09
Open Shrub Heath	434	43.4	0.15
Total	**91451**	**29874.0**	

Table 4.6: Contributions to modelled TP export from the Tamar catchment in 1998 from various nutrient sources within the catchment

	TP export (kg)
Land Use	29874
Animals	2748
STWs	8227
septic	2700
Total Modelled Export	**43549**

The steeply sloping valleys and low permeability soils of the Tamar catchment will promote runoff and therefore lower coefficients, such as those selected by Hanrahan et al. (2001), were not used. GIS manipulation of land use data indicated that meadows and grazed lands in the Tamar catchment are generally not within the riparian zone of 50 m for the river and an export coefficient of 0.4 kg ha^{-1} yr^{-1} for grasslands located outside

a riparian zone of 50 m, reported by Johnes (1996) for the Slapton ley catchment, was selected. This coefficient was selected for this catchment based on similarity of geology and topography despite the lower livestock density of the Tamar catchment (estimated 2.1 cattle ha^{-1}; 4.8 sheep ha^{-1}). Johnes and Hodgkinson (1998) quoted coefficients of 0.3 – 0.4 kg ha^{-1} yr^{-1} for extensive upland grazing, and a coefficient of 0.8 kg ha^{-1} yr^{-1} for intensive lowland dairying. It is assumed that less intensive livestock densities would require less intensive fertiliser applications to grasslands and less P produced by the animals per unit area, and therefore the export of P per unit area is lower.

In the Tamar catchment, land use associated with livestock grazing contributes a far greater proportion of the total TP export than the mass of P voided and exported from animals (Table 4.6). Previous literature has shown that total TP export from land use sources is related to catchment size (Hanrahan et al., 2001 b; Diorioz et al., 1998; Johnes and Hodgkinson, 1998). The Tamar catchment data was plotted on a graph of total TP export from land use sources vs catchment size along with other catchments (Figure 4.3 and Table 4.7) and yielded a linear relationship (r^2= 0.796). It has been suggested that this relationship holds true for catchments dominated by diffuse inputs from land use sources (Johnes and Hodgkinson, 1998) and therefore catchment size can be used to estimate TP exports from land use practices (Hanrahan et al., 2001). The linear relationship of the data in Figure 4.3 suggests that in mixed agricultural catchments dominated by P export from diffuse sources approximately 80 % of P export is derived from land use. This relationship can be a useful tool in the estimation of TP export from land use sources in the absence of, or prior to, acquisition of landuse data when P export is known (e.g. from field data), or in the estimation of TP export from catchments if land use is known but animal/sewage data is not available. In addition, this relationship can also provide a good feedback mechanism in the calibration step of the export coefficient modelling procedure. However, this relationship has been established for agricultural and mixed agricultural catchments, caution must be used in applying this relationship to urban and intensively farmed

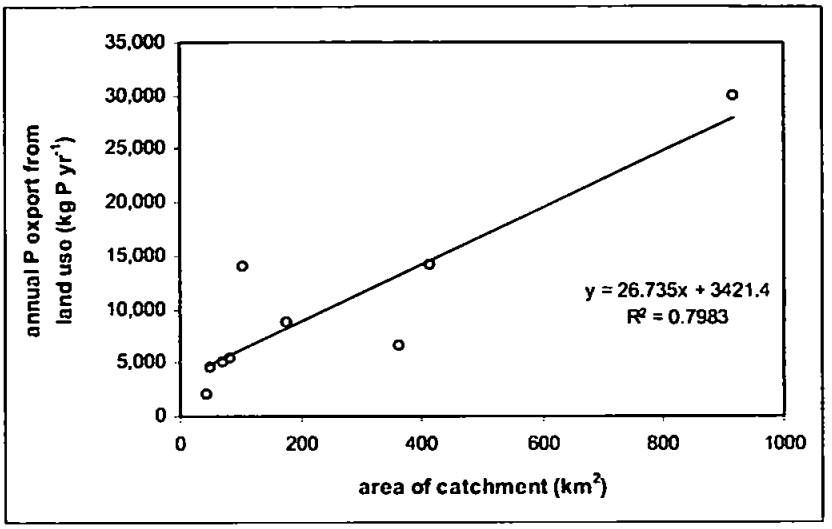


Figure 4.3: Catchment size vs modelled annual TP export from land use for various catchments (described in Table 4.7 below).

Table 4.7: Characteristics of the catchments in referred to in Figure 4.3

Catchment	Size (km^2)	TP load from land use ($kg\ yr^{-1}$)	Catchment characteristics	Reference
Frome	414	14,085	chalk catchment, mixed agriculture, medium population density	Hanrahan et al., 2001 b
Foron (France)	83	5,400	-	Dorioz et al., 1989
Slapton Ley	46	1,970	Lowland, mixed agriculture, medium population and livestock density	Johnes, 1996
Ryburn	51	4,540	Moorland, low population and livestock density	Johnes and Hodgkinson, 1998
Esk	72	5,083	Upland, dominated by grasslands, low population and livestock density	Johnes and Hodgkinson, 1998
Windrush	363	6,500	Lowland, mixed agriculture, medium population and livestock density	Johnes and Hodgkinson, 1998
Waver	104	14,040	Lowland, mixed agriculture, low population density, medium livestock density	Johnes and Hodgkinson, 1998
Cherwell	176	8,759	lowland, agriculture, low population density	Johnes and Hodgkinson, 1998
Tamar	915	29,874	Undulating, mixed agriculture, low population and livestock density	this study

catchments as it is not likely that such catchments would be dominated by diffuse sources (Johnes and Hodgkinson, 1998; Hanrahan et al., 2001 b).

P export from sewage: The Tamar catchment is a rural catchment (0.35 persons ha^{-1})

with the majority of the population are spread over a large area, making it difficult to provide shared amenities for much of the population (NRA, 1996). According to the 1991 UK census, 21,650 people within the catchment were being served by STWs in 1991 (NRA, 1996). Using these population demographics, the estimated TP export from STWs in 1998 was 8,227 kg (Table 4.8).

However, one third of the catchment population is served by private septic systems and the total P load estimated to be exported from septic tanks within the catchment is 2,700 kg per year. This is approximately one third of the total P export from sewage within the catchment. The number of people served by private septic tanks in 1998 is thought to have increased by only a small percentage since the 1991 census. In 1989, changes to regulations governing discharges were altered to include domestic discharge from private septic tanks such that it was necessary to obtain consent to discharge in order to construct private septic tanks. The EA (Sarah Alcock, *pers comm.*, 2001) have reported that 510 consents have been awarded since 1989 for structures within the Tamar catchment.

P export from animals: The total export of P from animals is 2,567 kg (Table 4.9). Cattle and sheep produce the majority of P exported from animal sources within the catchment. However on a 'per animal' basis, horses and cattle produce more P for export than other livestock. Poultry statistics for the catchment was unavailable therefore the P export from this source could not be calculated. However, other researchers have shown that these animals are not a significant contributor to the net P export from animal sources (Hanrahan et al., 2001 b; Johnes, 1996). Cattle and sheep grazing are not as fertiliser intensive as tilled crops, although the production of P per hectare is possibly as high. Stocking density within the Tamar catchment is relatively low when compared with intensive livestock dominated catchments such as the Slapton Ley catchment. As a result, P export from animals within the catchment does not dominate the total TP export from the catchment as it does in the Slapton Ley catchment.

Table 4.8: TP export from human sources within the Tamar catchment in 1998

Human Sources	Population Equivalents[e]	Export Coefficient	TP exported (kg yr^{-1})
STWs	21650	0.38 kg $ca^{-1}yr^{-1}$	8227
Private Septic Tanks	11250	0.24 kg $ca^{-1}yr^{-1}$	2700

Table 4.9: TP produced and exported from animals and sewage population equivalents in the Tamar catchment

Animals	No of animals in the Tamar catchment[a]	TP exported (kg yr^{-1})[d]
Total cattle	152984	1053
Total pigs	49414	639
Total horses	1795	25
Total goat	261	1
Total sheep	361727	1031
Total P export from livestock		**2748**

[a] Animal statistics for the Tamar catchment provided by DEFRA (2001) for year 2000; 1998 and 2000 county statistics (www.defra.gov.uk, 2001) used to obtain 1998 estimate animal popluations

Comparison of measured and modelled P loads for 1998: The final model for the prediction of TP export (43,549 kg P) from the Tamar catchment in 1998 is within 12 % agreement with measured TP export (49,507 kg) over the same period. Recknow and Chapra (1999) suggest that if the model is within 30% agreement with the measured TP loads then it can be considered sufficiently representative of the system to be accepted. In our study, model calibration was set at ± 15 % based on studies by Johnes (1996), Johnes et al. (1996) and Johnes and Hogkinson (1998) on UK catchments. Therefore under criteria outlined in this study, the model can be accepted without further adjustment until it has been validated by comparison with measured TP export from the catchment for another year (Figure 4.1).

4.3.1.2 Model validation: comparison of the export coefficient model with the measured 2003/2004 P load

Prediction error analysis and model validation are still rarely undertaken, although

improvements in computing technology, analytical techniques and water quality databases are clearly supportive of more routine assessments of uncertainty. Model validation involves the evaluation of the model prediction of TP export for more than one year. In predictive error analysis, the error associated with each step of the modelling process is quantified to predict the error of the model outcome. Model validation studies offer an alternative approach to predictive error analysis (Recknow and Chapra, 1999) and allow a model calibrated with data for a particular year to be considered representative of an average year. Successful validation means that the model is transferable and can be used to predict for more than one year.

The annual P export coefficient model developed for the Tamar freshwater catchment (south-west England) using historical data for 1998 was validated, in this study, through comparison with measured loads calculated from TP data collected at Gunnislake from May 2003 to May 2004 (Section 3.3.2). The TP export from the Tamar catchment over this annual period was 40,085 kg P, which is within 8 % of the modelled TP export (43,549 kg P) for the Tamar catchment. Therefore, the model has been successfully extrapolated to another year. This suggests that the export coefficient model for the prediction of annual TP export from the Tamar catchment has been successfully validated using bi-monthly TP data collected over the annual period from May 2003 to May 2004. The total P export from the Tamar was less than P export reported by Neal and Davies (2003) for eastern UK river catchments (both rural/agricultural and urban/industrial) of a similar size in an analysis of water quality fluxes from the rivers of the LOIS study. The P export per unit area for the Tamar catchment, 44 kg P yr^{-1} km^{-2}, was also less than reported for eastern UK rivers (97 - 867 kg P yr^{-1} km^{-2}), including that of the rural/agricultural Tweed (97 kg P yr^{-1} km^{-2}) (Neal and Davies, 2003).

4.3.2 Nitrogen export from the Tamar catchment

Many N models to date are based on the export of inorganic N species only (Johnes, 1996;

Johnes et al., 1996; Uncles et al., 2002), and predict nitrate export only. Nitrogen in the waters leaving the Tamar catchment is predominantly in the form of nitrate (82 %, Section 3.3.2). However, a model that predicts only nitrate export would not allow for dynamic processes within the water column, such as adsorption-desorption of N species onto particulates. It would also ignore the organic N component of the water column which has been shown to be biovailable (Berman, 2001). Therefore, in this study, annual TN export from the Tamar catchment was modelled using the export coefficient approach.

4.3.2.1 Modelled annual TN export

In this study, modelled annual TN export (Table 4.10) from the Tamar catchment was 2028 metric tonnes TN. The largest contribution to modelled N exports, based on annual nitrogen input of 20 kg N ha^{-1} and 59% of rainfall lost to surface runoff in the Tamar catchment (Table 4.3), was from rainfall (53 %), suggesting that rainfall is a major source of N within the catchment. According to this model, diffuse input from land use practices (Table 4.11) was the second largest contributor (33 %) to TN export from the Tamar catchment while animals (Table 4.12) were the third largest (10 %). In the Windrush catchment, a mixed agricultural catchment, rainfall was the second major contributor (after arable agriculture), contributing 20% of the overall TN export (Johnes, 1996). However, runoff from precipitation within the Windrush catchment (35%) (Johnes, 1996) was much lower than for the Tamar (59%). Similarly rainfall was the second largest contributor in the Slapton Ley catchment, where export from animal sources because of the high livestock density dominated the catchment (Johnes, 1996).

The average output of TN from the Tamar catchment modelled in this study was 27.4 kg N ha^{-1}, which was similar to the TN output modelled for the Windrush catchment (28.4 kg N ha^{-1}; Johnes, 1996) but lower than for the Slapton Ley catchment (51.7 kg N ha^{-1}; Johnes, 1996). The Slapton Ley catchment is similar in topography, geology and soil texture to the Tamar catchment as well as in hydrological characteristics,

Table 4.10: Contributions to modelled TN export from the Tamar catchment (91450 ha) in 1998 from various nutrient sources within the catchment

	TN export (kg yr^{-1})
Land Use	702719
Sewage	46331
Septic tanks	28013
Animals	194036
Rainfall	1079117
Total TN export	**2050215**

Table 4.11: Modelled TN export from land use practices

'Target' cover-type Classes	Area (ha)	Loading (kg N yr^{-1})	% export
Unclassified	2466.1	6165.16	0.92
Inland Water	11	0.00	0.00
Grass Heath	5946.3	14865.78	2.21
Mown / Grazed Turf	15367.3	98350.80	14.61
Meadow / Verge / Semi-natural	39552.8	253138.00	37.59
Rough / Marsh Grass	1670.3	10689.60	1.59
Moorland Grass	900.4	2250.94	0.33
Open Shrub Moor	121.9	304.69	0.05
Dense Shrub Moor	4.1	10.31	0.00
Bracken	1696.3	4240.78	0.63
Dense Shrub Heath	127.4	318.59	0.05
Scrub / Orchard	320.8	4169.75	0.62
Deciduous Woodland	13012.7	169164.94	25.12
Coniferous Woodland	1948.4	25329.69	3.76
Upland Bog	8.5	0.00	0.00
Tilled Land	5922.8	76996.56	11.44
Suburban / Rural Development	1776.3	0.00	0.00
Continuous Urban	16.3	4440.78	0.66
Inland Bare Ground	19	40.63	0.01
Felled Forest	127.5	114.00	0.02
Lowland Bog	0.1	1657.50	0.25
Open Shrub Heath	434.3	1085.78	0.16
Total	**91450.6**	**673334.3**	

however the dramatic difference in the modelled TN export may be attributed to the intense livestock density of the Slapton Ley catchment. Uncles at al. (2002) modelled export from the Tamar catchment from 1996 to 1999 was 37 kg N ha^{-1}. However, as previously mentioned in Section 4.3.1.1, their catchment boundaries encompassed a more

Table 4.12: Modelled TN export from animal sources

	No of animals[a]	TN exported (kg yr^{-1})
Total cattle	152984	123504
Total pigs	49414	7505
Total horses	1716	2083
Total sheep	361727	68442
Total goats	261	49
Total TN Export (kg yr^{-1})		**201583**

[a] Animal statistics for the Tamar catchment provided by DEFRA (2001) for year 2000

extensive area including areas of nutrient export >128 kg N ha^{-1} (Uncles et al., 2002) located outside the Tamar catchment as defined in our study.

A ranking of the various land cover types with respect to their contributions to the overall export of N from the Tamar catchment indicates that meadows and woodlands are the most significant source of N from land use. Woodlands account for 16.5% of the catchment area (deciduous – 14.2%, coniferous – 2.3%) but contribute 24 % of diffuse N export and 8.3 % of the total modelled TN export from the catchment. Therefore, they are a major contributor to N export from the catchment, based on the export coefficient of 13 kg ha^{-1} yr^{-1} used in this model and selected from Johnes (1996) and Johnes and Heathewaite (1997).

Sensitivity analysis: The sensitivity analysis was performed on the model and N concentrations in the Tamar River at Gunnislake were found to be most strongly influenced by exports from rainfall (5.3 %) (Table 4.13). This underscores the need for accurate rainfall data, with respect to N concentrations and quanitity of rainfall. It also suggests that if the N content is incorrectly estimated it could have major implications on the reliability of N export calculations. Also, the reliability of the model may not be as robust for storm events. This suggests that models developed for dry years may not agree with models developed for wetter years and the model validation process should include wet and dry years.

Table 4.13: Sensitivity Analysis (N) for the Tamar catchment

Nutrient Source	% Change from base contribution
Rainfall	5.3
Meadow / Verge / Semi-natural	1.2
Deciduous Woodland	0.83
Total cattle	0.59
Mown / Grazed Turf	0.48
Tilled Land	0.38
Suburban / Rural Development	0.38
goats	0.32
STWs	0.23
Grass Heath	0.19
septic tanks	0.14
Coniferous Woodland	0.12
Rough / Marsh Grass	0.052
Unclassified	0.030
Moorland Grass	0.028
Total pigs	0.025
Continuous Urban	0.022
Bracken	0.021
Scrub / Orchard	0.020
horses	0.010
Open Shrub Heath	0.0081
Dense Shrub Heath	0.0040
Open Shrub Moor	0.0038
Felled Forest	0.00056
Total sheep	0.00024
Inland Bare Ground	0.00020
Dense Shrub Moor	0.00013

4.3.2.2 Comparison of modelled and measured annual N export

The export coefficient modelling procedure does not require archival water quality data to model N export but such data are necessary for the calibration of the model. Historical TN data for the Tamar catchment was not available from datasets provided by the EA. Therefore, TN export from the catchment was calculated using the TN data collected during the 2003-2004 monitoring of the Tamar at Gunnislake. TN export over this period was 2053 tonnes N yr^{-1}. Modelled TN would then be within 1 % of this value which would suggest that the model could be accepted without further calibration. This type of model does not provide information as to the proportion of the annual export that is readily

bioavailable. Therefore, it is important to know the N forms present and their proportions within the system being studied. It is also important to base the model on complete, statistically robust datasets. There are two options available for catchments with incomplete datasets: the first would be to use field data to calibrate the model and the second would be to select another similar catchment which has a complete dataset to apply the export coefficient modelling approach.

Total N export for eastern UK river catchments of a similar size to the Tamar was reported by Neal and Davies (2003); total N export ranged from 1673-1781 tonnes N yr^{-1} for rural/agricultural catchments; and 2781-4572 tonnes N yr^{-1} for urban/industrial catchments. The total N export for the Tamar was statistically compared (using Least Squares Difference) to export from these two types of catchments and was found to be significantly different ($p<0.05$) from the TN export of the urban/industrial catchments but not significantly different ($p>0.05$) from the rural/agricultural catchments. Therefore, the total annual TN export can be considered typical of rural/agricultural UK catchments. However, N export per unit area from the Tamar catchment, 2241 kg N yr^{-1} km^{-2} was greater than reported for the rural/agricultural catchments (1807-1814 kg N yr^{-1} km^{-2}, Neal and Davies, 2003) and could not be considered typical of rural/agricultural UK catchments of a similar size. However, TN export per unit are was less than that of the urban/industrial catchments (3096- 4803 kg N yr^{-1} km^{-2}, Neal and Davies, 2003) of a similar size.

Marsh et al. (1980) mapped the nitrate load from major UK rivers collated from the Harmonised Monitoring Scheme data from 1974 to 1977 and reported a nitrate load of 1700 kg N km^{-2} for the Tamar catchment (from nitrate water quality data collected at Gunnislake). This suggests a TN export per unit area of 2070 kg N km^{-2} from the Tamar catchment, since nitrate makes up 82 % of the TN load at Gunnislake (Section 3.3.2). This value is in good agreement with the findings of our study (2241 kg P yr^{-1} km^{-2}).

The N:P ratio calculated from the modelled annual TN and TP exports in this study was

47:1. This ratio, according to criteria outlined by Jarvie et al. (1998) and discussed in Section 3.3.2.4, suggests that waters leaving the Tamar catchment are P limited as concluded from discrete sampling data discussed in Chapter 3.

4.3.3 Recommended strategy for the management of nutrients in the Tamar catchment

A predictive model is only as robust as the data that is used in its construction. House et al. (1997) suggest that the reliability of the calculated measured nutrient export increases as sampling frequency increases. Johnes (1990) collected daily field data for the Windrush catchment to create an export coefficient model, but stated that four sampling events per month were adequate to produce a reliable model, while bi-monthly sampling the least number of data points that could be used to produce an acceptable model. It should be noted that 23 % of the measured annual TP export from the Tamar catchment in this study was attributed to a single spate event in January 2004 (see Table 3.4 for river discharge rates). This highlights the need for high frequency sampling of nutrient and flow data, which would allow better temporal resolution of nutrient fluxes from the catchment.

Sampling regimes are often constrained by economic costs; the development of a validated numerical model for the prediction of nutrient export on the catchment scale would be a valuable management tool allowing a balance between the economics of a smaller number of samples and the reliability of predicted data. The P model constructed and validated in this study used the smallest dataset that could be considered reliable and as such validation before use was essential. However, the validated model can now be used to predict P export and for scenario testing to predict potential P export from the catchment. Management scenarios such as changes in land management practices resulting in reduction in grazing lands, increases in catchment population or reduction in animal numbers such as experienced during the 'foot and mouth crisis' of 2001, will affect potential P export and the P model provides a simple predictive tool.

The approach to modelling N export in this study strictly adhered to the export coefficient approach using export coefficients from the literature. However, modelled precipitation could be calculated based on the nitrogen content of rainfall which would allow the model to distinguish between wet years and dry years, rather that the procedure being static. Coles (1999) collected nitrate and ammonium data for rainfall in Plymouth over a period of four months. The data when converted from concentration to input (in kg) to the catchment suggested an input of 11.2 kg N ha^{-1} for an average year (1230 mm rainfall), which is less than the 20 kg N ha^{-1} suggested by Johnes (1996) and implies that further calibration of the model would be necessary. However, the N content of rainfall data is highly variable; Coles (1999) reported that rainfall from northeasterly winds contained a higher N concentrations than southeasterly and southwesterly winds; the Royal Society (1983) reported widely varying yearly N inputs to gauging stations. Therefore, until detailed field rainfall data is available, the export coefficient approach best represents these variable field conditions.

Currently, the selection of export coefficients is based on land use within the catchment and on general catchment characteristics. However, a further refinement of the model would be to differentiate between export from similar land use based on slope, geology (bedrock), soil type and intensity (with respect to animal and human populations). For example, nutrient export due to runoff and erosion would be expected to be greater on steeply sloping, impermeable land dedicated to pasture than on steeply sloping more permeable land dedicated to pasture. A catalogue of export coefficients for each land use types, suitable to slope, geology, soil type and intensity, could be linked with GIS layers containing digital data for land use, slope, geology, soil, density with respect to agriculture, proximity to riparian zones within the catchment and the appropriate coefficient applied accordingly. Linking datasets to GIS could make the ease of prediction and scenario 'building' more rapid, ease of manipulation and visual impact of predictive modelling can be useful in the dissemination of information to non-scientists.

The development of a generic model whereby catchment statistics would be put in to a spreadsheet already fitted with a standard set of coefficients seems unlikely at this stage. A more likely scenario would be to develop several models, each pertaining to a particular group of catchment characteristics, e.g. lowland, dairying, low livestock density, low population density, such as the Tamar catchment described in this study. The end-user would then choose a 'generic model of best fit' that best describes the catchment of interest.

4.4 CONCLUSIONS

The export coefficient modelling approach was used to model annual N and P export from the Tamar catchment. The P model for the export of TP from the Tamar catchment was successfully constructed using historical land use data and catchment demographics; calibrated with hindcasted water quality data for 1998; and validated by collecting TP field data to calculate the measured annual P export from the catchment:

1. The sensitivity analysis (± 10 %) performed on the calibrated model indicates that the model is most influenced by P export from land use practices, in particular the export of P from lands dedicated to grazing (meadows, 3.6 %; mown/grazed turf, 1.4 %).

2. P export from the catchment is dominated by the export of diffuse P; modelled annual TP export was dominated by P from land use practices (75 %). Comparison of P export from land use with catchment size indicated that in catchments dominated by diffuse P approximately 80 % of the export is from land use. Therefore, export from land use can be used to estimate annual P export in such catchments. 61 % of lands within the catchment is dedicated to grazing (meadows, 44 % and grazed turf, 17 %), which dominate the land use of the area and the export of P from land use sources.

3. The Tamar catchment is a rural catchment (0.35 persons ha^{-1}) with respect to population, yet the model is sensitive to P export from sewage which contributes 25 %

of the modelled annual P export. Increasing population would increase the total P load and the P export per unit area.

4. The P export model was successfully validated with field data collected from May 2003 to May 2004: modelled P (43. 5 tonnes P yr^{-1}) export was within 8 % agreement with the measured P load (40.1 tonnes P yr^{-1}). Therefore, it can be considered to be representative of the annual P export from the Tamar catchment in a typical year.

This study also attempted to refine a model to predict TN export from the catchment. Although TN export was modelled and calibrated, further development of the model was constrained by data access and the availability of good quality data, and complete datasets. The following conclusions could be made:

1. A TN model was constructed and calibrated for the Tamar catchment using the May 2003 to May 2004 field data, with 1 % agreement between modelled and measure TN.
2. The largest contribution to modelled N exports from the Tamar catchment was from rainfall (53 %), suggesting that rainfall is a major contributor to the N cycle within the catchment.
3. The sensitivity analysis (± 10 %) showed that the outcome of TN model would be most strongly influenced by exports from rainfall (5.3 %).
4. Further work to validate the N model is necessary before it can be used for modelling N management scenarios in the Tamar catchment

CHAPTER 5

A portable flow injection analyser for the in situ *determination of FRP in the Tamar catchment*

5.1 INTRODUCTION

Many freshwater systems, including the waters of the Tamar River, as it leaves the Tamar catchment at Gunnislake, are severely P limited. Consequently, monitoring low P concentrations and small changes in the P concentration, especially FRP, which is representative of readily bioavailable P species, is critical to assessing trophic status and water quality. Importantly, there is a lack of high resolution information on short-term changes in FRP concentration and fluxes during spate events such as prolonged rain events and storm events. Therefore, the acquisition of high-quality, *in situ* analytical data with good temporal resolution is necessary to improve our understanding of the biogeochemistry of phosphorus in freshwater systems (Hanrahan, 2002).

The development of reliable *in situ* portable nutrient analysers, capable of continuous or semi-continuous monitoring and providing data in real-time, to determine bioavailable nutrients in natural waters, would provide a valuable contribution to the process of characterising nutrient water quality. Such analysers have the potential to provide high temporal and spatial resolution water quality data for scientists and managers involved the process of assessing and ensuring aquatic ecosystem health (Wiryawan, 2000, Hanrahan, 2002). Flow injection (FI) analysis coupled with spectrophotometric detection has been used by several researchers to monitor FRP in natural waters (Lyddy-Meaney et al., 2002; Hanrahan et al, 2001; Auflitsch et al., 1997; McKelvie et al., 1997; Robards et al., 1994).

The aim of the research described in this chapter is to develop a robust, sensitive, reproducible and portable *in situ* instrumental method for real-time monitoring of FRP in freshwaters. The specific objectives were to configure the FI manifold for accurate determination of FRP in freshwater with a linear range of at least 20-100 $\mu g\ L^{-1}$ P, based on classification of trophic states (Section 1.1.3); to use the instrument to obtain *in situ*, real-time FRP data in the Tamar River; and to validate the instrument performance through comparison with a validated laboratory reference method. The feasibility of using the portable analyser to monitor FRP in estuarine and coastal waters was also examined.

5.2 EXPERIMENTAL

5.2.1 Reagents

All reagents used were of AnalaR grade and purchased from BDH Chemicals (Merck), Poole, unless otherwise specified. An ammonium molybdate solution was prepared by adding 35 mL sulphuric acid to 10 g ammonium molybdate and making up to 1 L using ultra pure water (Elga Maxima, 18.2 M Ω cm^{-1}). A 0.2 g L^{-1} tin(II) chloride solution containing 28 mL L^{-1} sulphuric acid and 2 g L^{-1} hydrazinium sulphate was prepared. Phosphate standards were prepared by serial dilution of a 100 mg L^{-1} P stock solution (Section 2.2.1) using an appropriate carrier to match the working salinity range. Low nutrient seawater (LNS), obtained from Ocean Scientific, Petersfield, Hampshire was mixed with ultra pure water to obtain different salinity matrices.

5.2.2 Cleaning protocol

All laboratory glassware, bottles and containers used in experiments, sampling and sample storage were prepared as described in Section 2.2.2

5.2.3 Instrumentation

The portable flow injection analyser (Figure 5.1 and 5.2) consisted of two battery powered 12 V Ismatec® peristaltic pumps, a 6-port 2-way rotary injection valve (Rheodyne®, Rheodyne LLC, California, USA) with a variable volume sample loop, a 3-port solenoid valve (2 inlets, 1 common outlet) (Biochem Valve Corporation, New Jersey, USA), a 1 m reaction coil, and a flow through solid state detector (Daviron Instruments, Bere Alston, Devon). Figure 5.2 is a schematic representation of the FI manifold. The control and processing boards were housed within the PVC pressure housing or the instrument body, while the FI manifold was mounted on the surface. A 12V power source was required to operate the instrument; in the laboratory the 240 V DC mains supply was converted

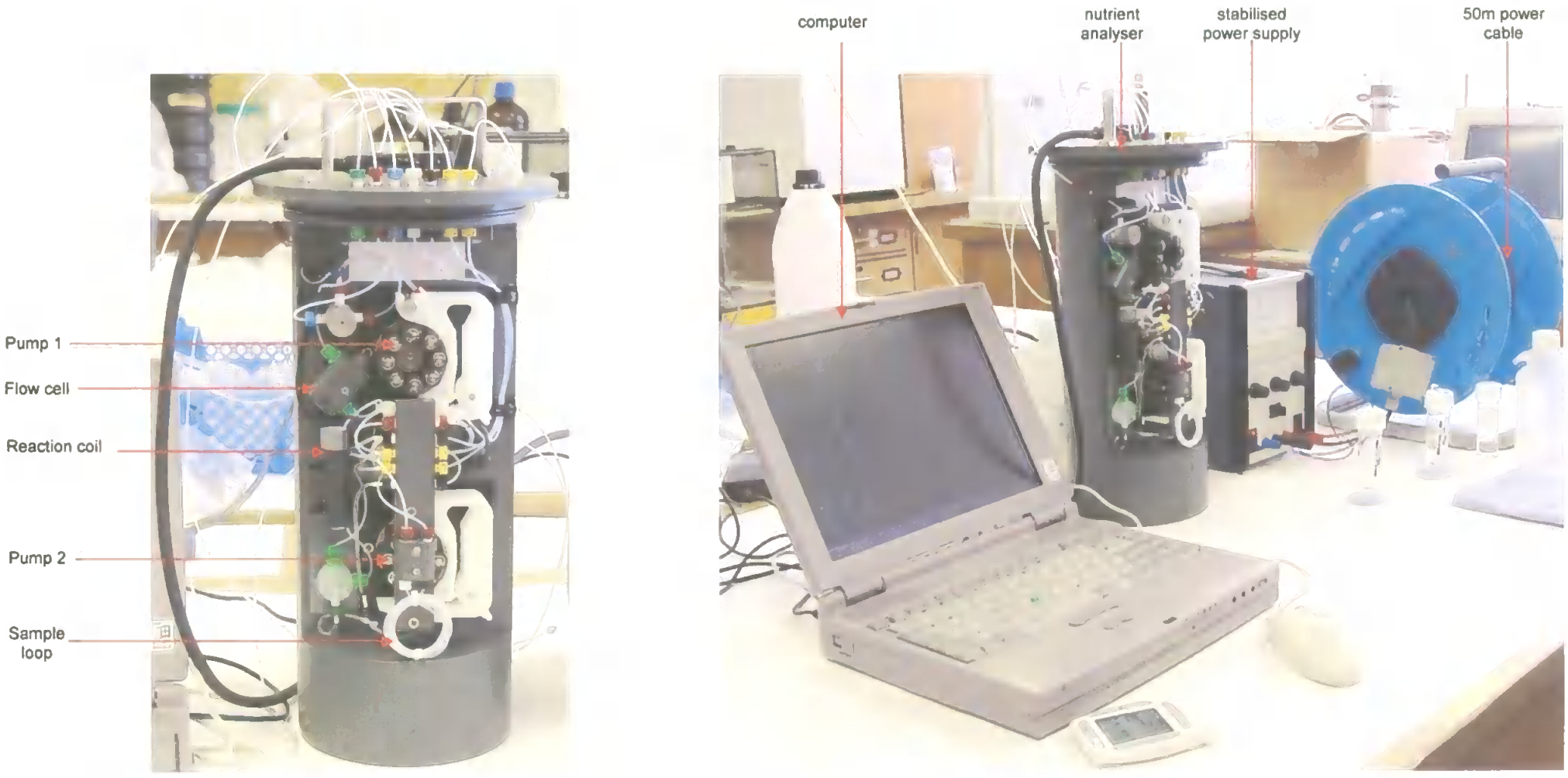
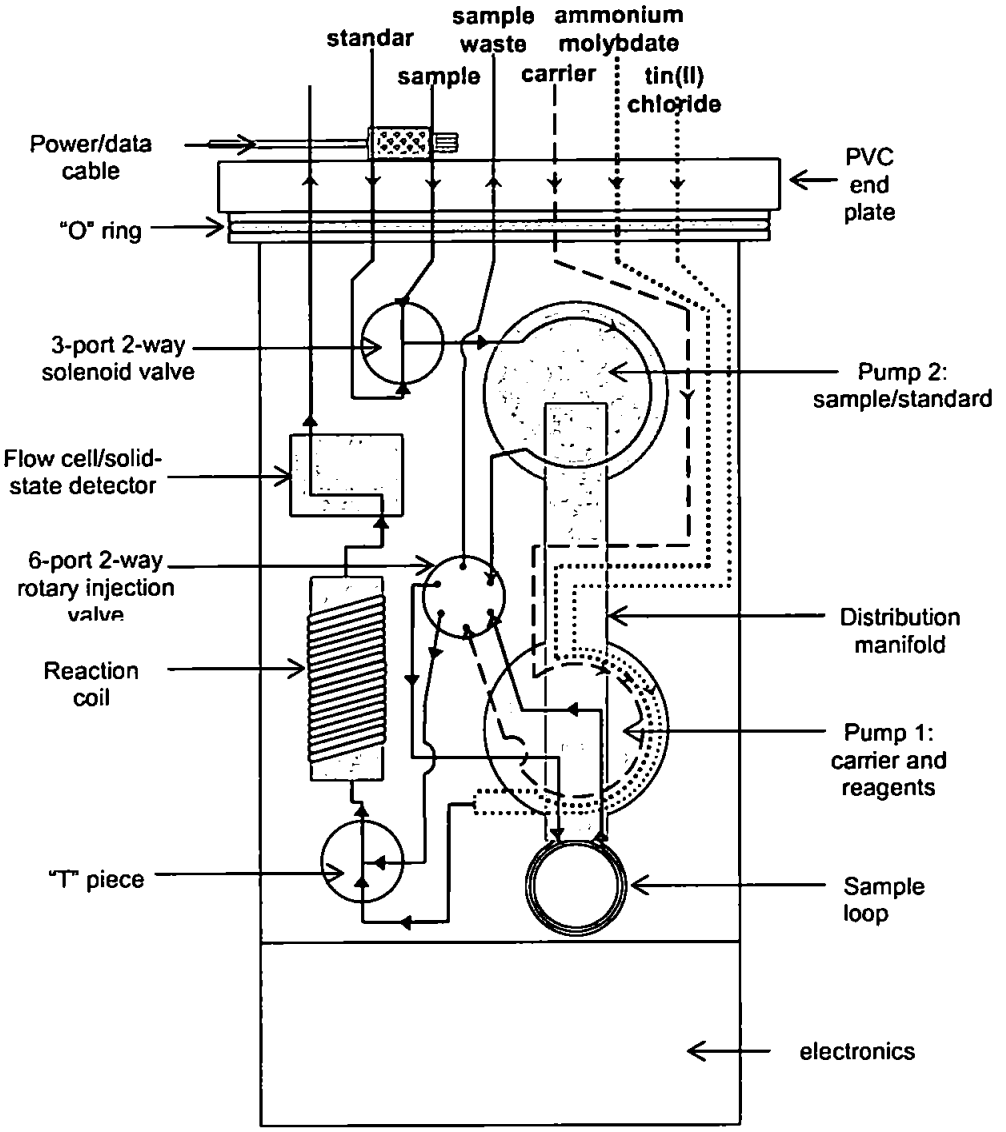
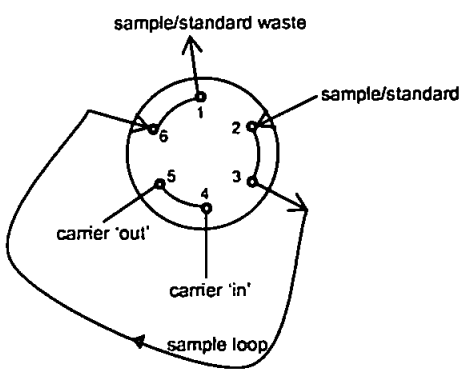
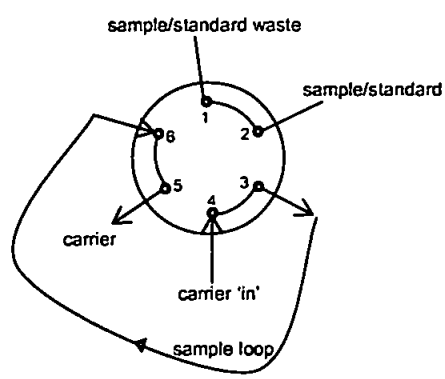


Figure 5.1: The portable FI analyser showing (a) front view of the FI manifold fitted onto the surface of the analyser and (b) the analyser set up as a bench-top instrument for FRP determination





Position 1 – load sample/standard



Position 2 – sample/standard injected to manifold

Figure 5.2: A schematic diagram of the portable Flow Injection Analyser and the 2 positions of the 6-port rotary injection valve.

to 12 V using a stabilised power supply (Farnell Instruments, Leeds) and in the field a 12 V car battery was used as a power supply. Data communication was performed using a RS232 port and a Toshiba notebook PC (Toshiba Information Systems Ltd., Surrey, UK). The programming and operation of the portable flow injection analyser was carried out using Crosstalk for Windows® communication software. The instrument was capable of two modes of operation and data acquisition: manual and automatic. The automatic mode is designed for long term monitoring and was used during portable deployments. The manual mode was used when the instrument was operated in the laboratory for method development.

The FI chemistry was based on the reaction of the FRP with molybdate under acidic conditions (pH < 2) to produce phosphomolybdic acid, which was then reduced by tin(II) chloride to form a blue complex that was detected spectrophotometrically. Ammonium molydate and tin(II) chloride reagents were continuously propelled through the manifold by Pump 1 (Figure 5.2 and Figure 5.3) while the instrument was in operation. When the sample/standard loop was loaded, Pump 2 was activated and at the same time the 6-port rotary injection valve switched to Position 1 (Figure 5.2) and sample/standard was propelled through the sample/standard loop. When the sample/standard was injected, the injection valve valve switched to Position 2 (Figure 5.2), Pump 2 was simultaneously switched off, and the carrier pushed the sample/standard from the sample loop toward the reaction manifold where the sample standard/standard plug mixed with the reagents in the 20-turn reaction coil. The product of the reaction was quantified using a flow-through solid-state detector incorporating an ultrabright red light emitting diode (LED) as the light source and a photodiode (peak response ≈ 635 nm) as the detection device. The detector is capable of providing up to 2 readings per second. In the manual mode, the instrument recorded raw transmittance data which was converted to digital data by 14-bit analogue-digital converter and displayed and recorded on a computer as arbitrary digital units using Crosstalk for Windows®. Instrument response was recorded as peak height. In the

automatic mode, an algorithm programmed into the software processes the data as concentration by reference to an onboard standard. The sample feed tube was fitted with an in-line 0.45 μm cellulose acetate membrane filter and a glass-wool pre-filter during field deployment (Figure 5.3). Reagents, carrier and standard solutions were packed in collapsible 600 mL reagent bags (Daviron Instruments, Bere Alston, Devon) and fitted to the respective reagent streams using 2-way PTFE non-return valves (Omnifit Ltd, Bio-chem Valve/Omnifit, Cambridge) during field deployments.

5.2.4 Manifold optimisation

The FI manifold was optimised with respect to carrier and reagent flow rate, sample injection volume and reagent composition. Manifold optimisation was carried out in the manual injection mode. For each parameter studied, the parameter under investigation was varied while all other conditions were held constant. All reactions were carried out at 20 °C unless otherwise stated. The carrier and reagent flow rates were each varied from 0.3 to 2.0 mL min^{-1} in turn; sample injection volume was investigated over the range 128 to 800 μL loop size; ammonium molybdate concentration was varied from 6 g L^{-1} to 10 g L^{-1}, and the use of ascorbic acid instead of tin(II) chloride as a reducing agent in the formation of the phosphomolybdenum blue reaction was also investigated. A rapid approach to manifold optimisation was adopted wherein experiments were conducted using 0, 50 and 100 μg L^{-1} P standard solutions but the analytical figures of merit for each condition investigated were not exhaustively investigated. The optimised manifold is shown in Figure 5.3.

5.2.5 Phosphorus interference studies

The effect of arsenate (0-50 μg L^{-1} As) and silicate (0–10 mg L^{-1} Si) on the determination of FRP (100 μg L^{-1} P in ultra pure water) was investigated. For each potential interference,

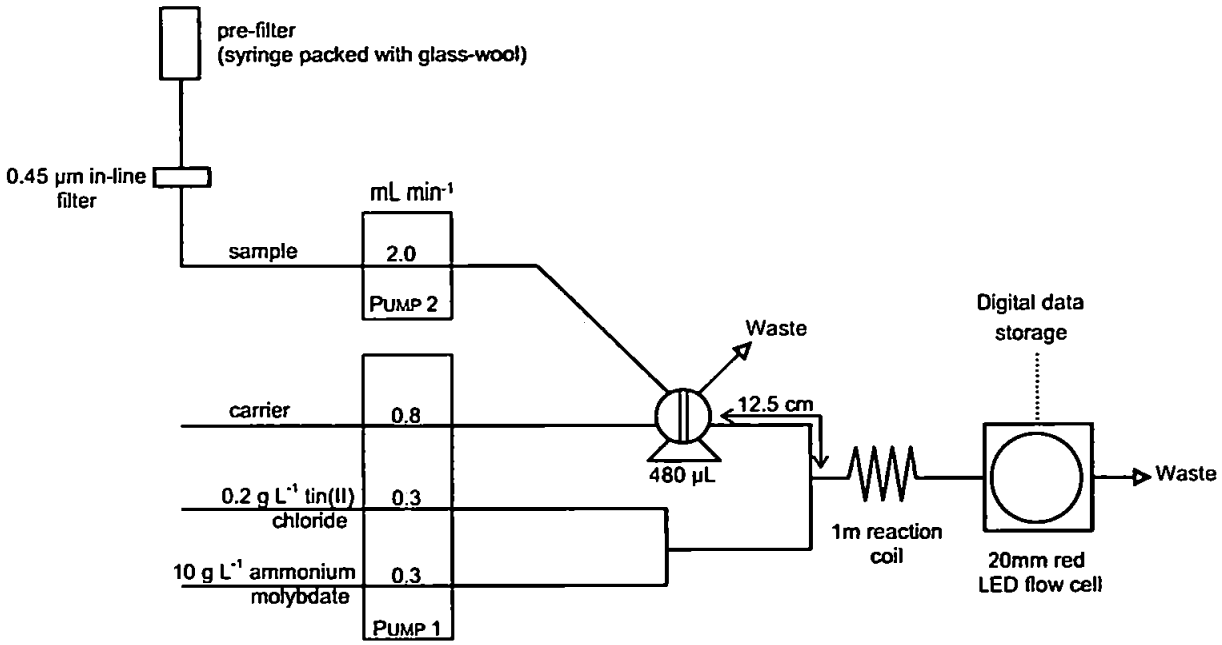


Figure 5.3: Schematic representation of the FI manifold for phosphate determination. Carrier – ultra pure water.

a blank solution containing the interference in the absence of FRP in ultra pure water was also analysed.

5.2.6 Field deployment

The *in situ* nutrient analyser was deployed at Gunnislake (National Grid reference SX4332722) on the bank of the Tamar River on two occasions. Shipboard deployment of the nutrient analyser was conducted along the Tamar estuary, from Morwhellham Quay (SX44756975) to Rumleigh (SX44506850), on two occasions. On each occasion, discrete water samples were collected while the analyser continuously measured FRP. These samples were also analysed for FRP using the Skalar San Plus laboratory reference method (Sections 2.3.1 and 2.3.2).

5.3 Results and Discussion

5.3.1 Manifold optimisation

The ability to obtain long-term, real-time P datasets would provide environmental managers and scientists with data to identify long-term trends in water quality as well as short-term environmental changes such as storm events. The portable nutrient analyser used in the present study has been previously used to monitor nitrate/nitrite concentrations in estuarine and coastal environments in real-time (Gardolinski et al., 2002). This monitor has been shown to be physically robust, submersible to 50 m and portable (David et al., 1998 and 1999; Gardolinski et al., 2002) and the instrument design is such that the FI manifold can be quickly re-configured to suit local conditions. The present study aimed to configure this portable *in situ* analyser to determine FRP in surface waters. The objective was to design a FI manifold to allow the accurate determination of FRP in freshwater with an LOD of 10 μg L^{-1} P and linear range of 10-100 μg L^{-1} P (based on boundary conditions for trophic state described in Section 1.1.3). Within this discussion, *in situ* refers to on-site but movable (not fixed-site) and man-portable is described as portable.

The initial FI manifold conditions (Figure 5.3) were based on a manifold reported by Benson et al. (1996 a and b) and Hanrahan et al. (2001). Flow rates for the carrier, molybdate and tin(II) chloride streams were initially set at 0.6, 0.8 and 0.6 mL min^{-1}, respectively. Initial reagent concentrations were 0.2 g L^{-1} tin(II) chloride and 10 g L^{-1} ammonium molybdate, with ultra pure water used as the carrier reagent. The initial sample loop volume was 295 μL and the reaction coil length was 1 m with 20 turns. Figure 5.4 is an example of a typical instrument output for a calibration run, with 2 replicates of each standard (0, 25, 50 and 100 μg L^{-1} P). The data was transferred to an MS Excel worksheet after acquisition and a Visual Basic macro* was used to separate the unmarked transmittance data into separate peaks. The average initial baseline was subtracted from

* written by Luis Tortajada Genaro, visiting scientist from the University of Valencia, Valencia, Spain.

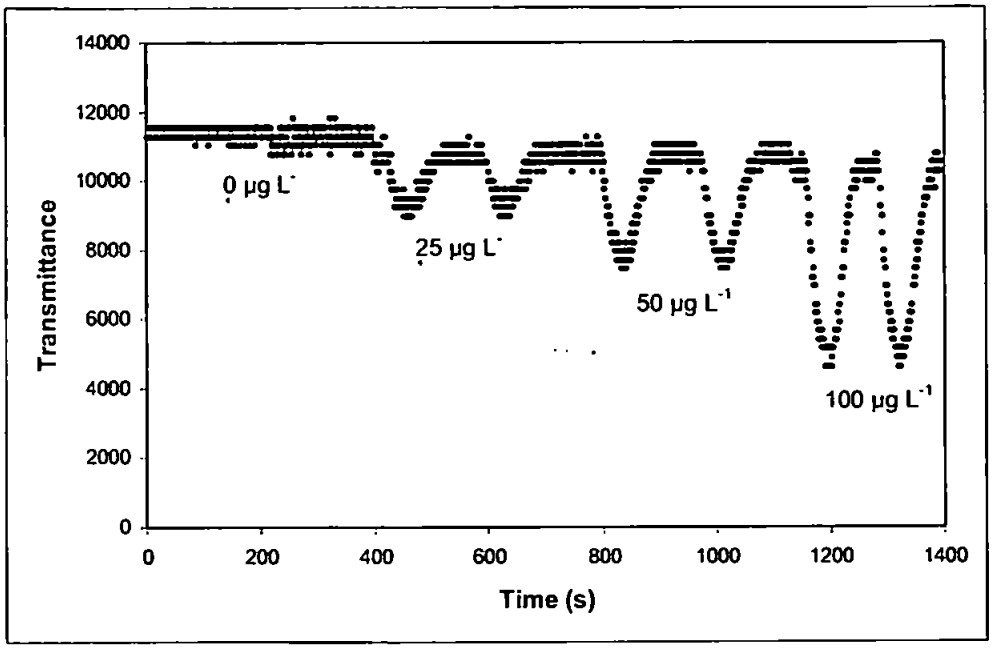


Figure 5.4: A plot of the typical digital response on injection of 0, 25, 50 and 100 µg L^{-1} P into the FI manifold for the determination of FRP.

the peak maximum to obtain peak 'height'.

Flow rate: The flow rate of each carrier/reagent stream was varied by changing the pump tubing (3-bridge, 72 mm), using tubing of different internal diameters, because the reagent pump (Pump 1, Figure 5.3) on the portable analyser was a fixed-speed pump. The reported flow rates have been calculated from the volume propelled by Pump 1 per unit time. The linear relationship ($r^2 = 0.9927$) between the flow rate reported by the manufacturer (for a standard peristaltic pump) and the measured flow rate was:

Measured flow rate across Pump 1 = 2.53*(manufacturer's reported flow) + 0.017

The tin(II) chloride and the molybdate reagent flow rates were varied in turn (0.3 to 2.0 mL min^{-1}) while all other conditions were kept constant. For each reagent investigated, the instrument response increased as reagent flow rate decreased such that the optimal flow rate of both reagents was 0.23 mL min^{-1}, respectively. At these flows, the ratio of ammonium molydate to tin(II) chloride was 10:1. The configuration of the manifold used by Benson et al. (1996 a and b) and by Hanarahan et al. (2002) was such that the

ammonium molydate to tin(II) chloride ratio was 10:1.5.

When the reagent flow rates were kept constant, the instrument response increased as carrier flow rate increased from 0.27 to 0.83 mL min^{-1}. The instrument response remained constant (±5 %) for flow rates ≥0.83 mL min^{-1}. A carrier flow rate of 0.83 mL min^{-1} provided conditions for maximum formation of the molybdenum blue complex, while conserving reagent consumption, and was selected as the optimal carrier flow rate.

Reagent composition: The effect of reducing the ammonium molybdate concentration from the initial 10 g L^{-1} to 6 g L^{-1} on the system response was investigated at 20 °C. Peak response was 50 % lower with the 6 g L^{-1} ammonium molybdate solution than with the 10 g L^{-1} ammonium molybdate solution (Figure 5.5). The kinetics of the reduction of phosphomolybdic acid to the molybdenum blue complex was affected by acidity and at each pH there is a $[H^+]/[MoO_4]$ range over which the rate of reduction is constant. The pH of the final reaction measured just after the flow cell at the start of the waste line was 0.37 in this study. Drummond and Maher (1995) showed that for the pH range 0.36 to 1.06, $[H^+]/[MoO_4]$ ratios of 45 – 80 gave a constant analytical response which indicated that colour formation was complete. The $[H^+]/[MoO_4]$ ratio of the 10 g L^{-1} ammonium molybdate solution was 80 which is optimal for colour formation; the $[H^+]/[MoO_4]$ of the 6 g L^{-1} ammonium molybdate solution was 136. At higher ratios, $[H^+]/[MoO_4]>>80$, formation of molybdenum species which are unreactive with phosphate are formed (Drummond and Maher, 1995). In this study, the molybdate and tin(II) chloride reagent were mixed prior to the point of sample introduction, which indicates that the lower ammonium molydate concentration (6 g L^{-1}), with $[H^+]/[MoO_4]>>80$, was not as reactive as the 10 g L^{-1} ammonium molybdate solution ($[H^+]/[MoO_4]$ of 80). At $[H^+]/[MoO_4]<40$, self reduction of the molybdate reagent is likely to occur (Drummond and Maher, 1995). Therefore, 10 g L^{-1} ammonium molybdate provided conditions for fast, stable colour formation at a final reaction pH of 0.37.

Chloride ions are known to suppress the formation of phosphomolybdenum blue complex

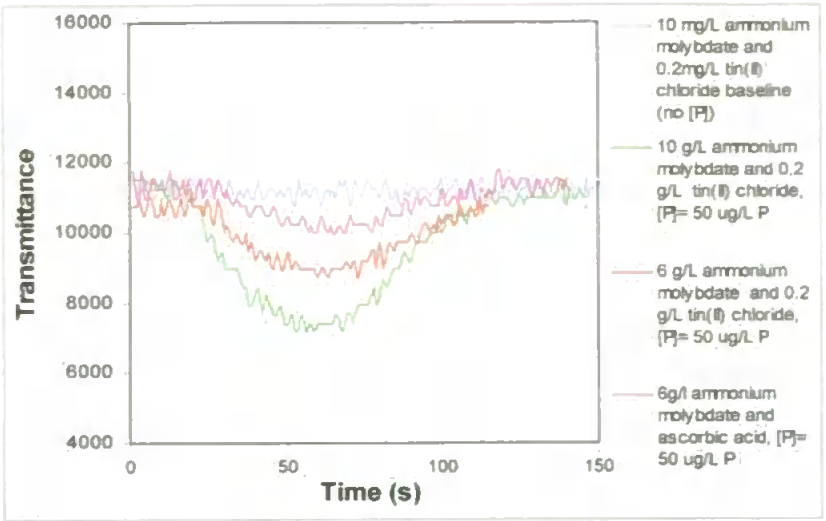


Figure 5.5: Graphs showing system response as ammonium molybdate concentration is varied from 6 to 10 g L^{-1} and as the reducing agent is varied using 0.2 g L^{-1} tin(II) chloride and 10 g L^{-1} ascorbic acid

when tin(II) chloride is used as a reductant (Lyddy-Meaney et al., 2002; McKelvie, 2000).

Ascorbic acid can be used as the reductant to overcome this salt interference (Murphy and Riley, 1962) and was also investigated in this study as a reducing agent in place of tin(II) chloride. The reduction kinetics of phosphomolybdate to phosphomolybdenum blue is slower when ascorbic acid is used as the reductant than when tin(II) chloride is used (Broberg et al., 1988) and antimony is often added to increase the rate of reduction of the complex (Murphy and Riley, 1962).

The Skalar molybdate reagent composition (6g L^{-1} ammonium molybdate with 0.2 g L^{-1} potassium antimonyl tartrate) and ascorbic acid (10 g L^{-1} ascorbic acid) was selected for this study. This reaction was performed at 30 °C, however, the instrument response was lower than with tin(II) chloride at 20 °C. The maximum transmittance of the molybdenum blue product formed was only 65% of that observed using tin(II) chloride/6 g L^{-1} ammonium molybdate and 33% of that obtained with 10 g L^{-1} ammonium molybdate/tin(II) chloride (Figure 5.5). The use of ascorbic acid as a reductant generated a product with a wavelength maximum at 880 nm (Robards et al., 1984). The solid state

detector in this study was fitted with a red light emitting diode light source ≈635 nm; this wavelength was not adjustable and, therefore, was not optimal for the detection of the product of the ascorbic acid reduction. Reaction kinetics increase with temperature; an increase in the temperature of the ascorbic acid reduction would increase the transmittance at ≈635 nm. However, although the portable FI analyser has the option of heating the reaction coil, this step was not considered to be appropriate for long-term field deployment with respect to power requirements (Lyddy-Meaney et al., 2002).

Tin(II) chloride was selected as the reductant for use with the optimised manifold for freshwater applications. It provided more efficient reduction than ascorbic acid and heat was not required. In addition, the use of tin(II) chloride as a reductant generated a product with a wavelength maximum at 710 nm as compared with 880 nm for ascorbic acid (Robards et al., 1984). This allowed greater sensitivity when a solid state detector (using a red light emitting diode light source ≈635 nm) was used (Worsfold et al., 1987) such as in the portable analyser.

Sample loop volume: Sample injection volume was varied from 128 µL to 800 µL. There was a linear increase in peak response and peak width at baseline as the sample loop volume increased from 128 µL to 480 µL (r^2 = 0.996) due to increased sample dispersion (Figure 5.6 a-c), with no further increase in peak height for injection volumes greater than 480 uL (Figure 5.6 a).

Calibrations using 480 µL and 800 µL sample injection volumes, respectively, indicated a decrease in the sensitivity of the system as the injection volume increased, with an increase in the limit of detection (Table 5.1). From an operational perspective, as the injection volume increases from 128 µL to 800 µL there was an associated decrease in sample throughput from 40 to 18 samples h^{-1}, respectively. The system was most efficient with a loop volume of 480 µL with respect to sample throughput and instrument response (linear range: 4-150 µg L^{-1}) and this sample loop volume was selected for all further studies.

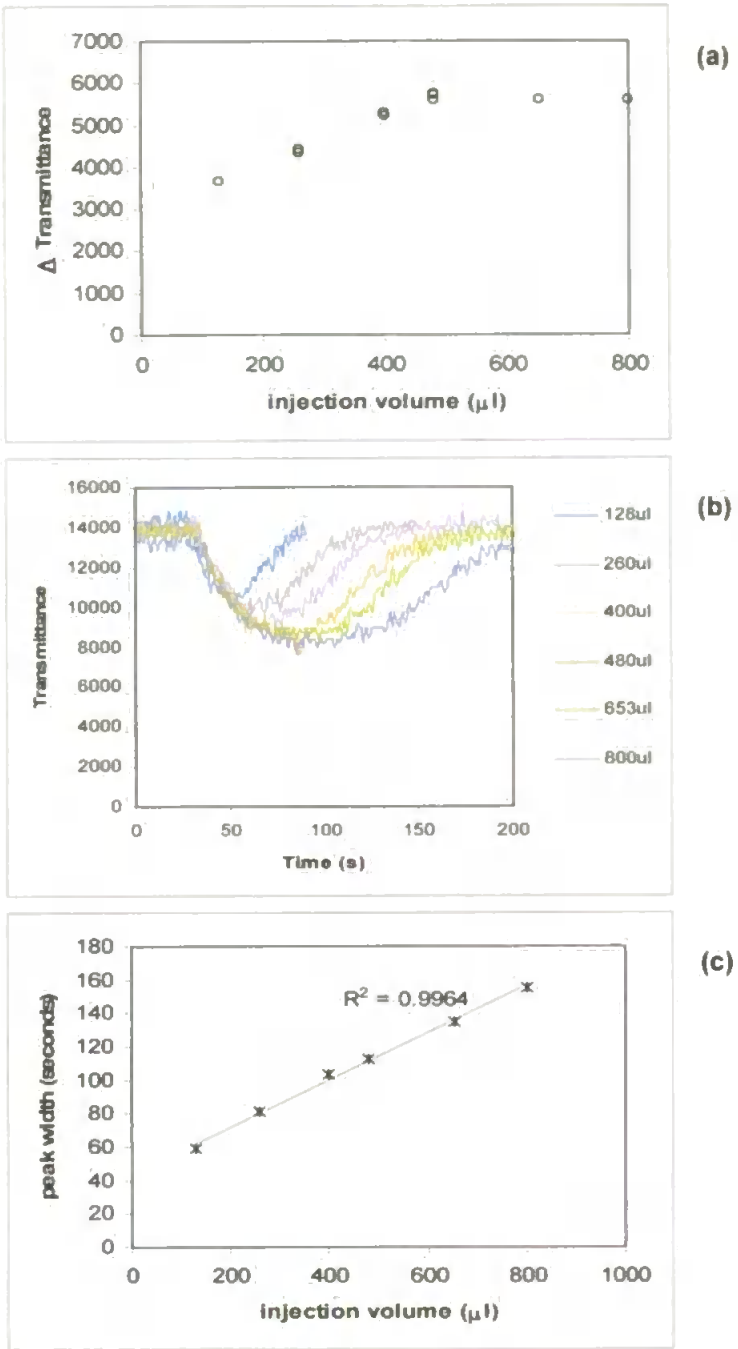


Figure 5.6: Change in instrument response as sample injection volume was varied from 128 to 800 µL: (a) change in peak response, (b) overlays of the FI peaks for each injection volume investigated, and (c) the change in peak width. Carrier – ultra pure water, [P] = 100 µg L^{-1}

Interferences: Silicate and arsenate ions form molybdenum blue complexes in the presence of molybdate under acidic conditions (Worsfold et al., 2005; Zhang et al., 1999). These ions are present in natural waters (Section 2.3.3.2) and could cause interference in the determination of FRP which would result in an overestimation the analyte (Neal et al.,

2000; Zhang et al., 1999). In this study, the potential interference of silicate and arsenate on the determination of FRP in natural waters using the portable FRP analyser was quantified by measuring apparent phosphate concentration in samples containing only the potential interference and in samples containing phosphate and the potential interference. The range of silicate (0 – 10 mg L^{-1} Si) and arsenate concentrations (0 – 50 µg L^{-1} As) selected for this study was the same as for the study of interference with the Skalar SAN Plus flow analyser and were based on concentrations recorded for freshwater systems including the Tamar River (Zhang, 1999; Langston et al., 2003). These silicate and arsenate concentrations selected for this study did not affect the determination of FRP using the portable FRP analyser (Tables 5.1 and 5.2). Arsenate and silicate concentrations recorded for the Tamar River from 1974 to 2004 (Section 3.3.1; Langston et al., 2003) are typically 0.6 - 3.9 mg L^{-1} Si and 1.5 – 5.5 µg L^{-1} As, respectively. Therefore, silicate and arsenate concentrations typically encountered in natural waters would not interfere with the accuracy of FRP determination using the optimized method developed in this study.

Table 5.1: Effect of arsenate on the observed phosphate concentration

[As] added (µg L^{-1} As)	[P] added (µg L^{-1} P)	Mean P measured (µg L^{-1} P)	% P detected
0	0	0	
5	0	0	
10	0	1	
25	0	0	
50	0	0	
0	100	101	100.0 ± 3.5
5	100	98	97.0 ± 3.2
10	100	99	98.0 ± 5.3
25	100	102	101.0 ± 3.6
50	100	98	97.0 ± 4.6

Table 5.2: Effect of silicate on the observed phosphate concentration

[Si] added (mg L^{-1} Si)	[P] added (µg L^{-1} P)	Mean P measured (µg L^{-1} P)	% P detected
0	0	0	
5	0	0	
10	0	1	
0	100	99	100.0 ± 4.2
5	100	102	103.0 ± 3.0
10	100	103	104.0 ± 5.0

Laboratory FI calibration under optimised manifold conditions: Performance characteristics of the optimised manifold are presented in Table 5.3. The optimised manifold conditions (Figure 5.3) provided a 33 % increase in sensitivity and the limit of detection (LOD) was 3 times lower than with the original manifold conditions. Reagent composition was unchanged but reagent flow rates were lower and sample injection volume was larger in the final optimised manifold.

Seven phosphate standards (20-150 μg L^{-1} P) with triplicate injections of each standard gave linear calibration graphs (r^2>0.99) with a gradient of 55 arbitrary transmittance units per μg L^{-1} P and an intercept of 263 arbitrary transmittance units with good (<5 %) RSD between replicate injections. The limit of detection, calculated using 3 standard deviations of the slope as discussed in Section 2.3.1, was 3.8 μg L^{-1} P, with a linear operating range of 4-150 μg L^{-1} P. The final sample throughput under optimised operating conditions was 15 samples h^{-1}.

Table 5.3: Portable FRP analyser performance characteristics

	Operating conditions
Sample throughput (h^{-1})	15
LOD	3.8 μg L^{-1} P
Linear range	4 – 150 μg L^{-1} P
Linear relationship	y = 54.6x + 262.8 (y = transmittance in arbitrary digital units; x = concentration (μg L^{-1} P))
Regression coefficient (r^2)	0.996
Sample loop volume (μL)	480
SD (n=3)	1.66 μg L^{-1} (40 μg L^{-1} P) 1.48 μg L^{-1} (100 μg L^{-1} P)
% RSD (n=3)	3.2 (40 μg L^{-1} P) 1.5 (100 μg L^{-1} P)
Reagent consumption (based on 15 samples h^{-1})	carrier[a]: 25.5 mL h^{-1} tin(II) chloride reagent: 18 mL h^{-1} molybdate reagent: 18 mL h^{-1} standard[b]: <10 mL h^{-1}

[a] carrier does not flow through the system during the sample load cycle

[b] standard injected approximately every 7 samples

The LOD and the linear range of the portable FRP analyser makes it suitable for monitoring FRP in riverine systems. The EA classifies oligotrophic waters as <20 μg L^{-1} P and eutrophic waters as >100 μg L^{-1} P. It is important from an environmental management and regulatory perspective to be able to identify oligotrophic waters since prolonged oligotrophic conditions can adversely affect productivity within aquatic systems. In addition, it is also critical to identify waters that are overly rich in dissolved inorganic P as this could lead to excessive productivity and eutrophication. The analytical figures of merit (Table 5.3) indicate that this portable FRP analyser can reliably measure FRP across this range of environmental concentrations.

The portable FRP analyser has a lower LOD than other field-deployable FI monitors capable of measuring FRP in real-time in freshwaters and a comparable LOD with those reported for marine monitoring (Table 5.4). The portable FRP analyser reported in this study seals into a PVC housing which make it water-tight and submersible. The total weight increased from 5 kg to 12 kg when the analyser was placed in the housing. Alexander et al. (1996) classed >10 kg as transportable, however, Gardolinski et al. (2002), stated that such an instrument is still portable and easily deployed by a single operator. Gardolinski et al. (2002) and David et al. (1998 and 1999) used this monitor, fitted with a nitrate/nitrite manifold, for submersible real-time monitoring in the North Sea and in the Tamar Estuary. Therefore, this analyser has been shown to be robust. Of the few *in situ* real-time FRP analysers reported in the literature, only one system, reported by Thouron et al. (2003) for deep ocean monitoring, is submersible but it is not portable and has a low sample throughput.

5.3.2 Riverine monitoring

The suitability of the portable FRP analyser for monitoring FRP in natural waters was investigated in this study. This included both laboratory analysis of freshwater samples and *in situ* monitoring in the Tamar River and in the upper reaches of the Tamar estuary

Table 5.4: *In situ* FI analysers used for the determination of FRP in environmental matrices

Matrix (sampling environment)	Instrumentation	deployment	LOD	Linear range	RSD	Sample throughput (h^{-1})	Reference
Freshwater - river	FIA, dual wavelength spectrophotometric detection	*In situ*, portable	21 µg L^{-1} P	25-1550 µg L^{-1} P (sample volume - 90 µL, λ = 650 nm)	<3.0 %	38	Hanrahan et al., 2001
				25-250 µg L^{-1} P (sample volume - 130 µL, λ = 710 nm)	<1.3 %		
Natural water - river	FIA, double-beam photometric detector with LED	Portable, *in situ*	12 µg L^{-1} P	12-2000 µg L^{-1} P	0-0.9 %	NR	Worsfold et al., 1987
Natural water - river	FI, microflow injection system, spectrophotometric	Portable, *in situ*	9 µg L^{-1} P	9-310 µg L^{-1} P	0.3 %	24	Motomizu, 1997
Seawater - open ocean	FI, LED/photodiode	Submersible up to 1000 m	3.1 µg L^{-1} P	3-150 µg L^{-1} P	NR	<5	Thouron et al., 2003
Coastal water	FIA, gas pressure for reagent propulsion, multi-reflection flow cell, LED detector	shipboard, portable (5 kg)	4.7 µg L^{-1} P	4.7 – 100 µg L^{-1} P	1.95 %	225	Lyddy-Meaney et al., 2002
wastewater	FIA, LED/photodiode	Fixed-site, *in-situ*	50 µg L^{-1} P	50-20000 µg L^{-1} P	NR	20	Benson et al., 1996

NR – not reported

(Figure 5.7) when the tide was low and the salinity was 0. The waters of the Tamar River at Gunnislake, as it leaves the Tamar catchment, and enters the Tamar Estuary can be considered P limited (Section 3.3.1).

Laboratory analysis of freshwater samples: Freshwater samples collected from Gunnislake from May 2003 to August 2003 as part of the May 2003-May 2004 nutrient water quality monitoring campaign (Section 3.3.2) were analysed in the laboratory using the portable FI FRP analyser. These samples were also analysed using a laboratory reference method (the Skalar SAN Plus continuous flow analyser, Section 2.3.2) and this allowed comparison of instrument performance prior to field deployment. The Skalar FRP method was validated through successful participation in the NOAA 2002 intercomparison study (Clancy and Willie, 2003) as discussed in Section 2.3.2. FRP data obtained from the portable analyser was in good agreement with the laboratory reference method; a 2-tailed paired t-test indicated that there was no significant difference between the results obtained by the two methods ($p>0.05$, Table 5.5), indicating that under laboratory conditions this method can be considered reliable and accurate for the determination of FRP in freshwaters. Therefore, the analytical performance of the instrument was considered suitable for field deployment.

Bank-side deployment: The portable FRP analyser was operated on the bank of the Tamar River at Gunnislake on two occasions. The instrument was calibrated in the laboratory immediately prior to fieldwork. A sample feed was set up from the river to the instrument as shown in Figure 5.8. The sample feed tube was supported within lightweight PVC tubing and clamped to a retort stand and weighted to reduce buoyancy and prevent drift due to the river flow. For this type of deployment, the FRP analyser was operated in the manual mode, with sample injections every six minutes to compensate for the longer sample feed tube (to span the distance from rive to river bank) and sampling frequency was reduced to 8 samples h^{-1} (with standard injection every 30 minutes or 5 samples). The reagents were not submersed in the river in this type of deployment. Manual sample

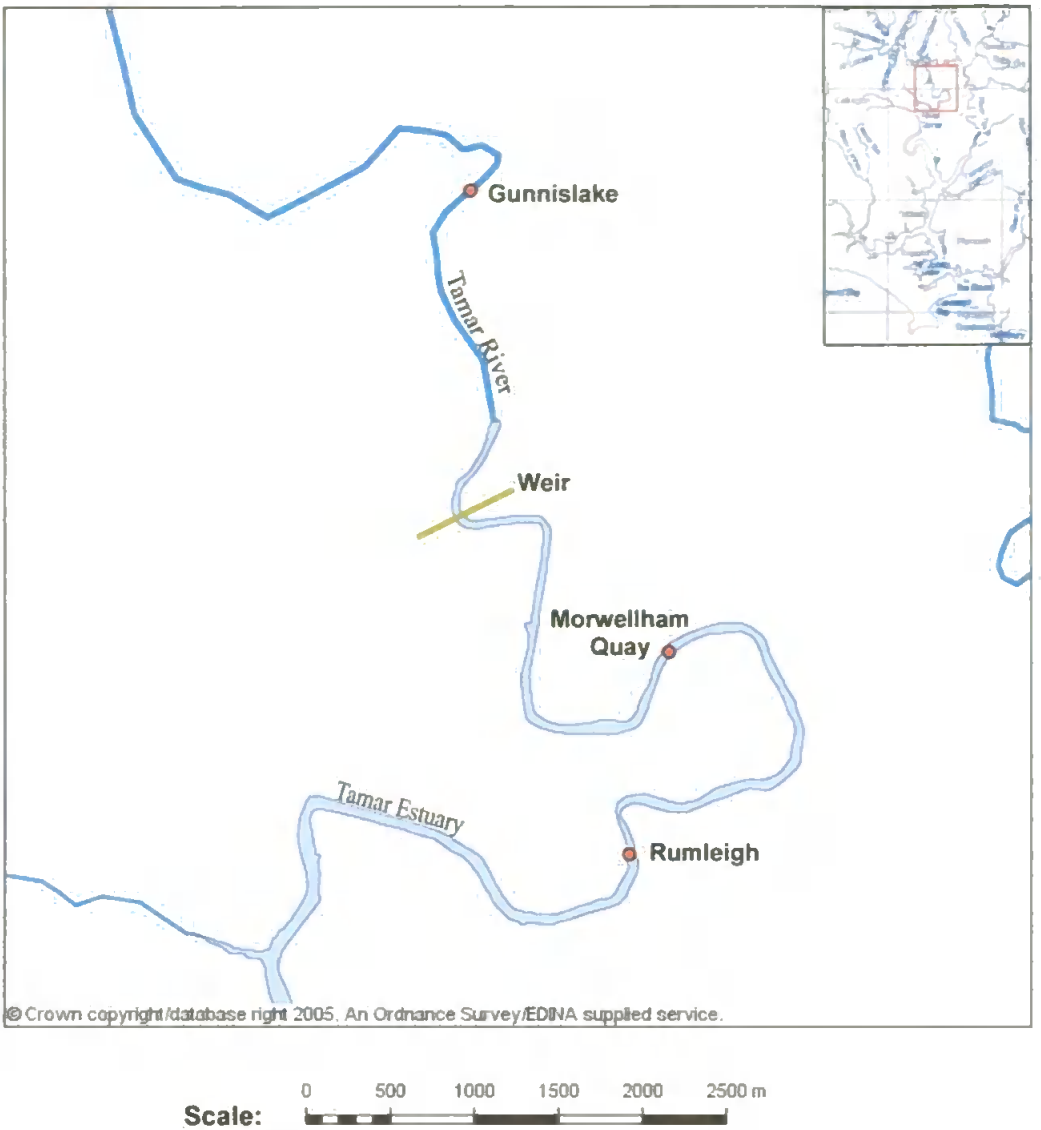


Figure 5.7: Map of Tamar River and Tamar Estuary showing Gunnislake, where bank-side deployments were undertaken, and Morwellham Quay to Rumleigh along which shipboard deployment of the portable analyser was carried out. The Tamar Estuary and the lower Tamar River are shown in the inset. This map was downloaded from Digimap (http://edina.ac.uk/digimap) and altered to indicate sampling sites.

collection for laboratory FRP analysis was carried out simultaneously with each sample injection to allow comparison of the performance of the portable FRP analyser with a validated reference method (Table 5.5). Samples were filtered in the field (0.45 µm, cellulose acetate) within 1 h of collection.

A 2-tailed paired t-test indicates that FRP concentrations measured in the field were not

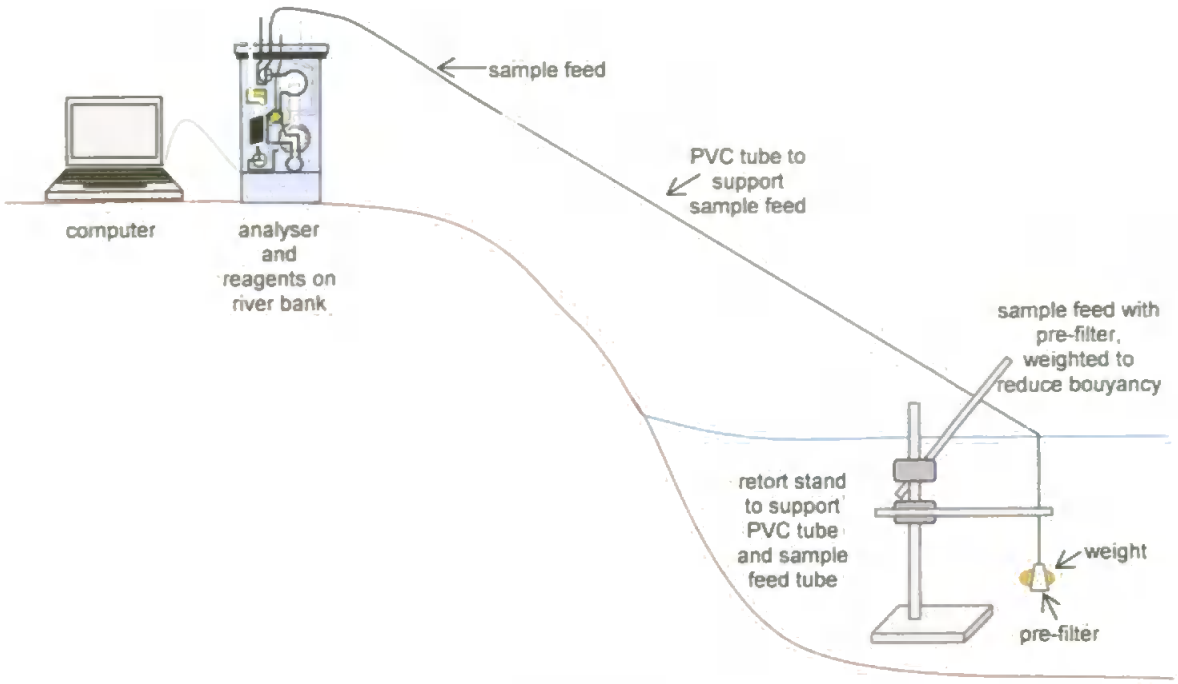


Figure 5.8: Illustration of bank-side deployment and Gunnislake on the Tamar River

statistically different from the FRP concentration measured using the reference method. However, the % bias between concentrations recorded with the portable analyser varied from -21.5 to 20.5 % of the reference method. Analysis of 40 µg L^{-1} P and 80 µg L^{-1} P FRP standards in the field were within ± 4 % of laboratory values and the % RSD obtained in the field were <3 % (n=3 for each standard) for all standard injections. Instrument performance in the field with respect to FRP standards suggests that the bias could be due to changes in P speciation between collection and laboratory analysis. The DOP fraction made up 22 % of the TDP in the waters of the Tamar River at Gunnislake from May 2003 to May 2004. Rapid hydrolysis of organic or condensed phosphates during storage and transport to the laboratory may contribute to higher FRP concentrations recorded in the laboratory (Lyddy-Meaney et al., 2002). The brief time delay (<1 h) between sample collection and filtration in the field may have led to adsorption of dissolved reactive phosphate onto particulates resulting in lower FRP concentrations recorded in the laboratory. This suggests that the time difference between sample collection, filtration and

Table 5.5: Comparison of the portable FRP analyser with the laboratory reference method

Deployment mode		Portable FRP analyser (µg L^{-1} P)	Laboratory reference method (µg L^{-1} P)	% bias	t-value (tabulated values)	p-value (p=0.05)
Laboratory, bench-top analysis (n=7)	28/05/2003	52.36	52.68	-0.61		
	30/06/2003	135.70	136.70	-0.73		
	01/08/2003	63.64	66.60	-4.44		
	15/08/2003	45.97	47.62	-3.46	1.16	0.288
	15/08/2003	47.38	47.65	-0.57		
	19/08/2003	41.20	39.70	3.83		
	30/09/2005	25.02	25.33	1.22		
	20/10/2003	62.00	61.70	0.49		
Bank-side deployment (n=10)	05/08/2004	98.63	102.51	-3.79		
	05/08/2004	120.33	153.33	-21.53		
	05/08/2004	96.96	103.50	-6.32		
	05/08/2004	96.54	106.43	-9.29		
	05/08/2004	104.89	109.10	-3.86	0.25	0.810
	18/11/2004	40.10	40.48	-0.94		
	18/11/2004	46.88	39.27	19.37		
	18/11/2004	46.88	38.56	21.58		
	18/11/2004	43.49	36.07	20.57		
	18/11/2004	37.33	34.99	6.69		
Shipboard deployment (n=17)	20/08/2003	43.10	37.80	14.01		
	20/08/2003	41.10	39.40	4.55		
	20/08/2003	42.80	39.90	7.29		
	20/08/2003	36.00	40.20	-10.49		
	20/08/2003	48.60	41.60	16.67		
	20/08/2003	53.40	47.90	11.49		
	20/08/2003	54.60	46.00	18.76		
	20/08/2003	43.40	42.30	2.67		
	18/08/2003	33.30	38.60	-13.78		
	18/08/2003	34.40	39.40	-12.63	-2.09	0.053
	18/08/2003	35.10	39.70	-11.50		
	18/08/2003	43.90	40.10	9.52		
	18/11/2003	40.10	40.48	-0.92		
	18/11/2003	46.88	39.27	19.37		
	18/11/2003	46.88	38.56	21.57		
	18/11/2003	43.49	36.07	20.58		
	18/11/2003	37.33	34.99	6.68		
All modes (n= 35)					-0.19	0.853

laboratory analysis could have contributed up to 22 % error in analysis. *In situ* analysis of natural waters reduces the risk of sample contamination and negates the need for sample storage. These findings strengthen the case for *in situ*, continuous, real-time monitoring of FRP in natural waters.

Shipboard surveys: The analyser was mounted inside the vessel *Tealia* on two occasions and a sample feed tube fitted with a pre-filter, consisting of a syringe packed with glass wool, was attached to the stern and sample pumped directly to the monitor. The instrument was calibrated in the laboratory immediately prior to the fieldwork. On these shipboard surveys, the sample injection valve to the FI system was operated in the manual mode and a sample was injected every six minutes as in the bank-side deployments (8 samples h^{-1}) with a mid-range standard (40 µg L^{-1} P) characteristic of average FRP in the Tamar River at Gunnislake injected at the start of each survey and subsequently after every fifth sample. As with the bankside deployments, samples were collected simultaneously with each sample injection to allow comparision of the performance of the portable FRP analyser with a validated reference method (Table 5.5) and subsequently filtered (0.45 µm, cellulose acetate) before transport to the laboratory.

Data collected with the portable FRP analyser and the reference method were not significantly different ($p>0.05$) but the FRP concentrations recorded with the portable analyser were generally higher than the reference method. As with the bankside deployments, field analysis of an FRP standard solution (40 µg L^{-1} P) indicated that method was reproducible (<3 % RSD between injections, n=3) and each standard injection was within 4 % of the reference method. This suggested that the bias in the sample results may be due to changes in P speciation occurring handling, transfer and transport and storage prior to laboratory analysis.

General aspects of field deployment: Data generated from each analytical method for all freshwater samples were pooled. The two datasets showed good linear agreement (Figure 5.9). However, the portable FRP analyser reported higher concentrations than the

reference method for [P]<50 μg L^{-1} P and lower concentrations for [P]>80 μg L^{-1} P as indicated by the regression equation:

$$[P]_{\text{portable FRP analyser}} = 0.823\ [P]_{\text{reference method}} + 9.42$$

$$(r^2 = 0.957;\ n = 35)$$

The portable FRP analyser was shown to be reliable and accurate when analysing freshwater samples under laboratory conditions and in the analysis of FRP standards during field deployments. The difference in FRP concentrations reported from the two methods suggests a change in sample integrity between the time of collection and the time of filtration/analysis, possibly due to processes such as adsorption or hydrolysis as previously discussed (Lyddy-Meaney et al., 2002).

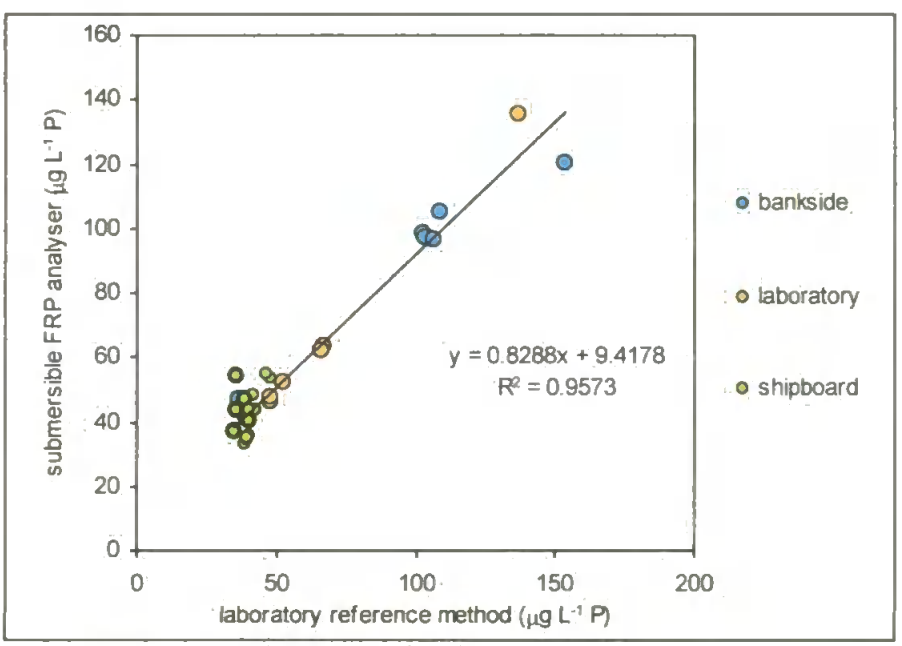


Figure 5.9: Correlation between portable FRP analyser and the laboratory reference method.

The portability of the analyser was illustrated by the easy transfer from laboratory to field in a small field equipment box also containing reagents and bottles for sample collection, carried on a trolley by one person. The analyser has also been designed for submersible

deployment up to 50 m which makes it suitable for vertical profiling and for towed deployment to conduct spatial surveys. The analyser seals into a PVC housing, which make it water-tight and submersible, and is then placed in a metal 'cage' designed to hold the analyser at the centre and the reagents in collapsible sample bags around the analyser. This cage is designed to increase the weight of the analyser from 5 kg to 35 kg which reduces buoyancy and horizontal drift within the water column, which is important when conducting vertical profiles, but makes the analyser transportable rather than portable. Submersible deployment was not necessary in this study as the depth of the waters at each sampling site was <2.5 m.

The portability and submersible capability of the monitor make it appropriate for spatial surveys of FRP concentrations in large rivers and lakes and it can be used as a towed submersible or shipboard monitor, or transported between sites and used as a bank-side monitor. Therefore, it allows one monitor to serve many sites saving in economic cost compared with manual sampling.

This type of monitoring can provide continuous or semi-continuous data. The sampling rate can be adjusted to conserve battery power, memory, to reduce reagent consumption and to coincide with data collection of other physico-chemical parameters such as river discharge rate, temperature, dissolved oxygen and pH.

Continuous monitoring is not limited by the number of hours of daylight or days of the week assigned for work and allows high frequency data to be collected over regular intervals that are more representative of the full range of temporal variability. Therefore, continuous monitoring of dissolved nutrient species such as FRP and associated physico-chemical conditions can provide valuable data to describe nutrient dynamics and mobility in natural waters, especially of potentially P-limited waters such as the Tamar.

The portable FRP analyser could be used in routine monitoring and it is recommended that the sampling rate is adjusted to collect a sample synchronous with flow data collection;

flow data is logged by the EA at 15 minute intervals at Gunnislake (Section 3.3.1). Data obtained from such continuous monitoring could be also be used to refine the export coefficient model for the Tamar catchment described in Chapter 4. This would provide a sound basis toward predictive modelling tool for use in strategic catchment management. Such resolution could also provide data toward developing a seasonal export co-efficient model as proposed by House et al. (1997) and Hanrahan et al. (2002).

5.3.3 Adaptation to estuarine monitoring

Current EU environmental legislation (e.g. Water Framework Directive, Habitats Directive, Nitrate Vulnerable Zone) is concerned with the water quality status of all natural waters and acquiring reliable, accurate nutrient data to assess status with respect to trophic status. The feasibility of using the portable nutrient analyser to monitor FRP in estuarine and coastal waters was, therefore, investigated in this study. However, the use of conventional FI methods for the determination of phosphorus in estuarine waters is affected by the different refractive indices of waters at different salinities. If the ionic strength of the sample is sufficiently different from the carrier stream it is being injected into, parabolic interfaces are set up at either end of the sample zone under laminar flow conditions causing a "lensing effect" and producing a displacement of the base line (Auflitsch et al., 1997, McKelvie et al., 1997; Liu and Dasgupta, 1994; Fang, 1993). This effect is known as the Schlieren or refractive index effect and can cause large errors in quantitation, especially at low analyte concentrations (McKelvie et al., 1997).

The Schlieren effect can be eliminated by matching the refractive index of the sample and carrier streams (McKelvie et al., 1997; Fang, 1993; Růžička and Hansen, 1988). In the present study, the portable analyser was used for the laboratory determination of phosphate in 0, 17.5 and 35 salinity matrices (using water/LNS) to simulate freshwater and saline waters; carrier salinity (water/LNS) was matched to that of the standards. Good linear calibrations were obtained at each salinity investigated (Table 5.6). However, the

efficiency of tin(II) chloride as a reductant is lowered by increasing chloride ion concentration (Lyddy-Meaney et al., 2002; McKelvie, 2000), which resulted in an increase in the LOD and decreased sensitivity with increasing salinity in this study (Table 5.6).

However, when sample and carrier salinity are not matched, the Schlieren effect observed can distort the sample response peak (see Figure 5.10). The range of salinities over which each carrier could be used was, therefore, investigated (Table 5.7). A mid-salinity carrier (LNS/ultrapure water, 15 salinity) was used with samples varying from 10 to 20 salinity with little bias (< 3 %) (Table 5.7). However, neither ultra pure water nor LNS were effective over a wide range of salinity, with the bias increasing as the salinity difference between the carrier and sample increased (Table 5.7).

Various strategies reported by previous researches to minimise the Schlieren effect (Fang, 1993; Fogg et al., 1990; Auflitsch et al, 1997) have been investigated in this study, including the introduction of a knotted or knitted mixing coils before the final measurement; increasing the injected sample volume and developing a reverse configuration manifold into which reagent is introduced into a sample stream.

Mixing coils: Liu and Dasgupta (1994) introduced a mixing coil in the sample/ carrier line before it meets the reagents. This approach was investigated with the current analyser. A knitted coil was introduced into the manifold of the current system to provide maximum radial mixing over the shortest distance but no reduction in the Schlieren effect was observed by this addition. Other measures attempted in this study included an increase in the length of the mixing coil (from 1 m to 2 m and 3 m, respectively), introduction of an inert solid phase mixing device (controlled pore glass beads, 120-200 mesh) immediately before the flow cell to promote radial mixing and the introduction of a mixing chamber with an aspect ratio of 1 (5 mm x 5 mm id) containing glass beads. None of these measures reduced the Schlieren effect.

Table 5.6: Typical analytical performance of the FRP manifold in the portable FI analyser, under optimised operating conditions, at salinity 0, 15, and 35.

Salinity	Detection limit [a] ($\mu g\ L^{-1}$)	Regression equation [b]	Linear range ($\mu g\ L^{-1}$)	Carrier
0	3.8	y = 54.6x + 262.8	4-150	Ultra pure water
15	8.5	y = 44.1x - 2716.1	9-150	LNS:ultra pure water (75:100 v/v)
35	10.0	y = 26.0x - 1193.4	10-150	LNS

[a] operationally defined as the sum of the blank value plus three times the standard deviation of the data obtained over the linear range (Miller and Miller, 1992).

[b] y = transmittance in arbitrary digital units ; x = concentration ($\mu g\ L^{-1}$ P).

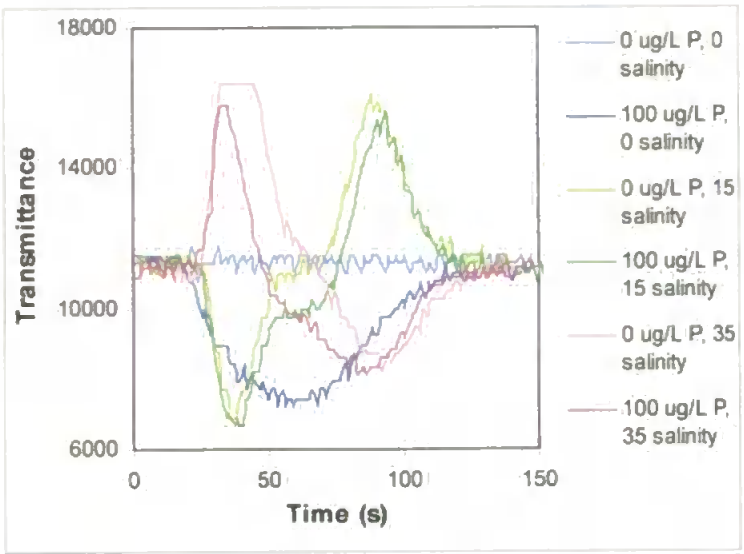


Figure 5.10: Graph showing the Schlieren effect observed when 0 and 100 $\mu g\ L^{-1}$ P samples, at 0, 15 and 35 salinity, are injected into a 0 salinity carrier stream.

Reverse configuration: Auflitsch et al. (1997a) successfully designed a reverse flow manifold for the determination of phosphate in waters of varying salinity. Reverse flow manifolds allow the reagent to be injected into a continuous flowing sample stream. This approach was investigated with the current analyser. Ammonium molybdate was injected into sample/tin(II) chloride stream, with ultra pure water as the carrier (Figure 5.11). No reduction in the Schlieren effect was observed with this configuration and carrier. However, when the carrier/molybdate refractive index was matched with that of the

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[b] y = transmittance in arbitrary digital units ; x = concentration (µg L^{-1} P).

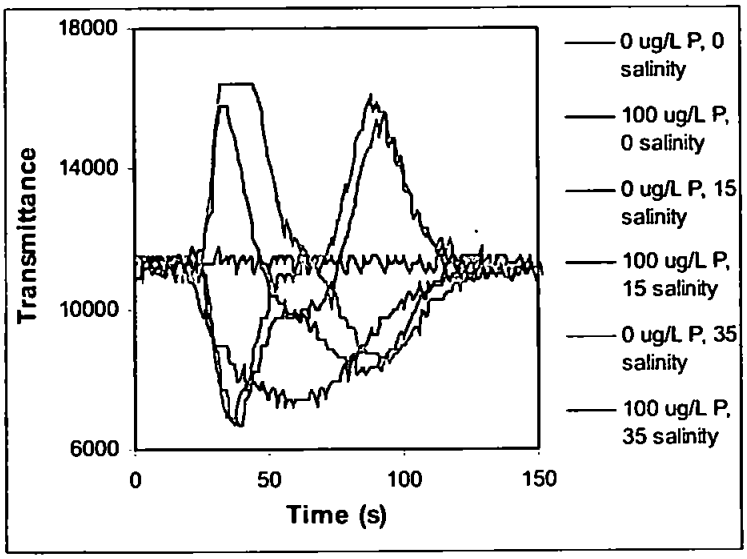

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Table 5.7: Effective range of salinities over which a particular carrier system can be used. Samples of known [P] were analysed using the FI analyser and the experimental [P] obtained by interpolation from calibration graphs generated at different carrier salinities.

Carrier	Carrier salinity	salinity of standard	Theoretical [P] ($\mu g\ L^{-1}$)	Experimental [P] ($\mu g\ L^{-1}$)	Bias ($\mu g\ L^{-1}$)
Ultra pure water	0	5	0	4.5	4.5
		5	100	89.0	-11.0
		10	0	9.3	9.3
		10	100	81.6	-18.4
LNS:ultra pure water (75:100 v/v)	15	10	0	5.9	5.9
		10	100	97.8	-2.2
		20	0	-2.1	-2.1
		20	100	99.7	-0.3
LNS	35	25	0	-17.9	-17.9
		25	100	61.4	-39.6
		30	0	-7.3	-7.3
		30	25	10.5	-14.5
		30	50	43.5	-6.5
		30	100	75.8	-24.2
		30	150	107.0	-43.0

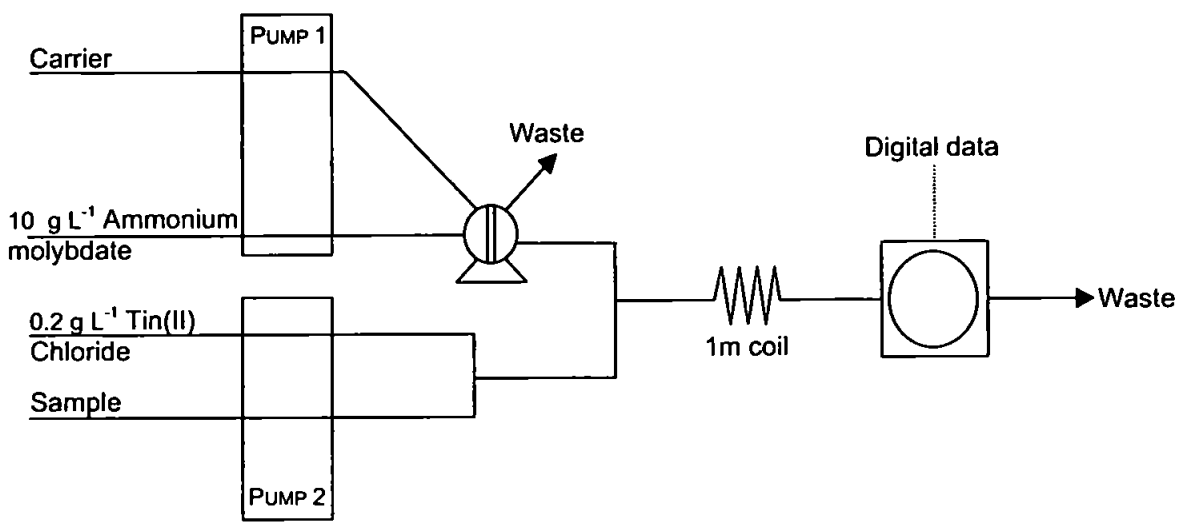
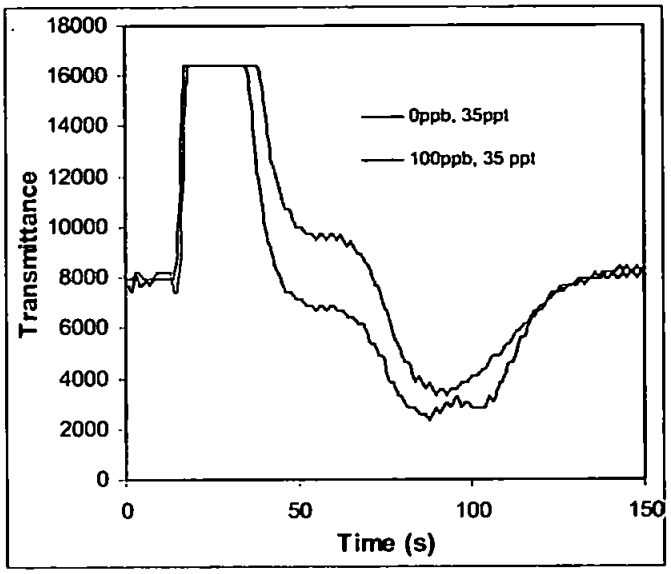


Figure 5.11: Reverse configuration of the FI manifold

sample/tin(II) chloride stream using sulphuric acid, there was an observable reduction in the Schlieren effect but the effect was not eliminated. Therefore, the use of this manifold configuration with the portable analyser for estuarine monitoring was not considered feasible.

Time-based injections: Some researchers have used large volume injections to isolate the dispersion effects at the front and rear boundaries of the injected sample bolus (Fogg et al., 1990) and the 'plateau' formed is proportional to concentration (Figure 5.12). Their work suggests that although the refractive index effect may still be present in observed signal when the sample and carrier ionic strengths are not matched, calibration graphs are still valid as long as the standard and sample solutions are similar in composition. This time-based injection approach (Figure 5.12) was used with the portable FRP analyser to generate calibration curves for 3 salinities to characterise the system. A sample loop volume of 800 µL was selected based on ease of measure measurement if the transmittance of the plateau; 800 µL sample volume provided a plateau that was of 10 s duration, while the 480 µL sample volume provided a plateau of 1 s. As previously observed, sensitivity decreased and the detection limit increases as salinity increased (Table 5.8).

Adapting the FRP manifold for estuarine and marine monitoring: The current manifold design and chemistry can be used for monitoring FRP in open ocean conditions (10-150 µg L^{-1}, Table 5.6) where the salinity is constant and the carrier can be easily matched to the salinity of the samples. However, none of the measures investigated in this study were effective in totally eliminating the Schlieren effect, therefore limiting the system for use within a limited salinity range (±5 salinity units) of the carrier environment. The rapidly changing salinity gradients, both spatial and temporal (tidal cycles) in estuarine systems, means that the portable FRP monitor would need further refining before it could be used effectively in estuarine field conditions. The measures that could be explored are general modifications to the hardware such as the introduction of a reference cell to mathematically subtract the interference (Liu and Dasgupta, 1994) or the use of a dual



Peak response, ΔT, was calculated as the difference of the average transmittance from t = 56 s to t = 63 s and the average baseline, calculated from t = 4 s to t = 20 s:

$$\Delta T = \left(\frac{\sum_{t=11s}^{t=20s} T}{10} \right) - \left(\frac{\sum_{t=56s}^{t=63s} T}{8} \right)$$

Figure 5.12: Graph of instrument response using an 800 µL sample volume (salinity 35) to allow time–based injections when the Schlieren effect is present (carrier, LNS/ultrapure water, salinity 15)

Table 5.8: Linear operating ranges of the phosphate monitor, using time-based injections

Salinity (ppt)	Detection limit (µg L^{-1})	Linear range (µg L^{-1})	Regression equation	Carrier
0	6.2	6-150	y = 41.0x - 149.3	ultra pure water
0	6.4	6-150	y = 41.4x -1350	LNS:ultra pure water (75:100 v/v)
15	9.7	10-150	y = 33.7x - 1144.8	LNS:ultra pure water (75:100 v/v)
35	12.5	13-150	y = 25.5x - 1142.3	LNS:ultra pure water (75:100 v/v)

wavelength detector (Liu and Dasgupta, 1994; Hanrahan et al, 2001). However, this would entail re-designing the monitor as extra space would be required for the additional components.

5.4 Conclusions

An FRP manifold for use with a portable FI spectrophotometric analyser was optimised in this study. The optimised method can be used for the determination of FRP in freshwater

environments (4-150 μg L^{-1}) and in coastal waters (10-150 μg L^{-1}), using a sample injection volume of 480 μL and 0.2 g L^{-1} tin(II) chloride and 10 g L^{-1} ammonium molybdate to generate the molybdenum blue complex. The analyser is capable of sampling with high temporal resolution (up to 15 samples h^{-1}), which is necessary to measure short-term changes in FRP concentration. Silicate (0-10 mg L^{-1} Si) and arsenate (0-50 μg L^{-1} As) did not interfere with the determination of phosphate. Therefore, environmental concentrations of silicate and arsenate in natural waters are unlikely to interfere with the determination of FRP using this method.

FRP data generated from *in situ* (bank-side and shipboard) deployment of the analyser were biased towards reporting higher concentrations than the reference method as indicated by the regression equation. However, the portable FRP analyser was reproducible and accurate when analysing freshwater samples under laboratory conditions and in the analysis of FRP standards during field deployments. The bias between the two methods for field samples suggests loss of FRP due to hydrolysis of filterable organic and/or condensed phosphates during sample collection and laboratory analysis.

The method was modified by increasing sample loop size to 800 μL to allow time-based injections in order to facilitate FRP measurements across a range of salinity. This modification allows a single carrier (LNS/ultrapure water, 15 salinity) to be used across a salinity gradient of 0-35 salinity (linear range 12 – 150 μg L^{-1}, LOD varies from 6.4-12.5 as salinity varies from 0 to 35, respectively). This method requires calibration over a range of salinity and is suited to shipboard deployment, although further refining of the chemistry/hardware is recommended for effective use as an FRP monitor in estuarine waters.

CHAPTER 6

An ecotoxicological assessment of sub-lethal nutrient concentrations on Anodonta cygnea

6.1 INTRODUCTION

Recent EU legislation (Water Framework Directive, Section 1.2) has outlined long-term qualitative targets to maintain and improve the quality of aquatic ecosystems within the EU based on ecological status as well as chemical water quality. *In situ* chemical monitoring techniques, such as described in Chapter 5 for FRP, can provide specific chemical data which allows characterisation of nutrient water quality; historical trend analysis, such as discussed in Chapter 3, can establish possible trends in nutrient species, ratios, mobility and dynamics; and predictive modelling (Chapter 4) can provide a basis to predict change in nutrient species and status. These techniques provide information to describe chemical water quality but they do no relate water quality to ecological status. The ability to monitor nutrient species coupled with knowledge of the possible nutrient concentrations that can have an adverse effect on the component species within an aquatic ecosystem would be a valuable tool in assessing nutrient water quality with respect to ecosystem health.

This chapter examines the possibility that high environmental nutrient concentrations within the water column can directly affect the health of ecosystems by causing physiological stress within individual organisms. This study is carried out in two stages (Figure 6.1); the first stage examines present literature to determine the range of nitrate and nitrite concentrations toxic to invertebrates and conducting laboratory experiments to establish nitrate and nitrite concentrations toxic to the selected test organism (LC50), the freshwater mussel *Anodonta cygnea* (Figure 6.2). The second part of the study uses established ecotoxicological tests to investigate the effect of sub-lethal concentrations and high environmental concentrations of nitrite on the physiological health of the test organism.

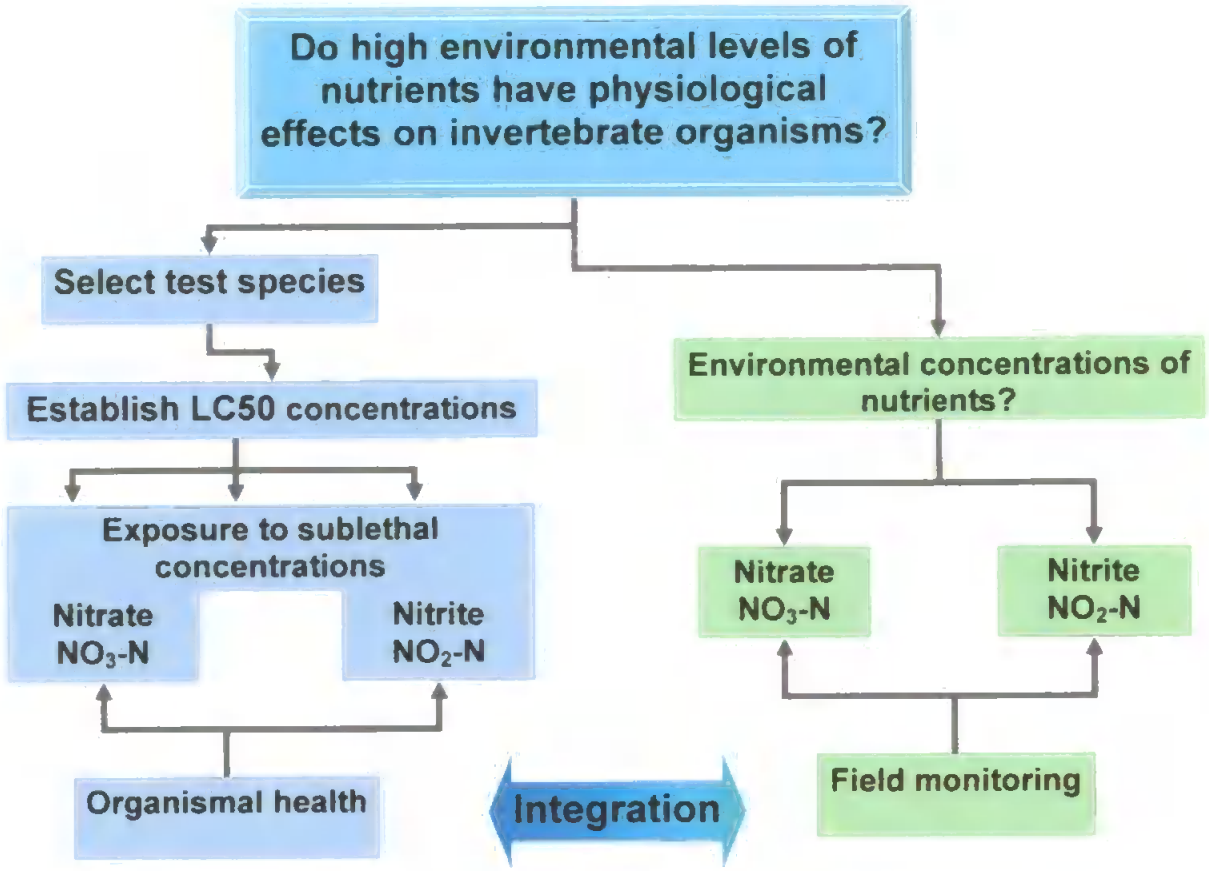


Figure 6.1: Schematic representation of experimental design.

6.2 EXPERIMENTAL

6.2.1 Selection of the test organism, *Anodonta cygnea*

Biomarkers of physiological stress have been shown to be useful indicators of environments stressful to aquatic organisms and have been used to relate environmental stress to organism health (Robillard et al., 2003; Riffeser and Hock, 2002; Baker and Hornbach, 2001; Nicholson, 1999; Bloxham et al., 1999; Lowe et al., 1995). Bivalve molluscs are useful biomarkers of water pollution (Naimo, 1995) and have several characteristics which make them useful sentinels for chemical pollution (Robillard et al., 2003; Naimo, 1995). They are relatively stationary organisms and obtain food principally through filter-feeding and are consequently exposed to contaminants that are dissolved in water and associated with suspended solids (Naimo, 1995). Therefore, mussels may be exposed to large pollutant loads even when concentrations are quite low (Robillard et al.;

2003 Naimo, 1995). As filter feeders, freshwater mussels are ecologically and economically important (Naimo, 1995): they play a role in recycling nutrients and controlling seston and are consumed as food and provide a trophic link between primary producers and predators (Baker and Hornbach, 2001; Naimo, 1995).

The swan mussel, *Anodonta cygnea* (Figure 6.2), is a member of the unionidae family and is a typical lowland species (Figure 6.2 a and b). This freshwater mussel is native to Britain (Figure 6.2 c) and can be found throughout Europe (Pfleger, 1998). It is found in large bodies of quiet or slowly moving water in lakes, drainage dykes, canals and rivers (Kerney, 1999). Adult swan mussels are typically 95 to 200 mm in length and 30 to 60 mm in breadth. A unique physiological feature of bivalve molluscs is their ability to filter large volumes of water; unionidae bivalves filter between 60-490 mL of water per individual per hour (Naimo, 1995). The distribution and physical characteristics of the swan mussel potentially make it a useful freshwater biomarker species with respect to the WFD.

6.2.2 Reagents

Neutral Red and BSA protein standards were obtained from Sigma Chemicals, Poole. The protein assay reagents were obtained from Pierce and Warriner, Chester, UK. All other reagents were of AnalaR grade and obtained from BDH Chemicals (Merck), Poole.

Physiological buffer: 0.1M sodium phosphate buffer, pH 7.4, made up in distilled water was used as a physiological buffer.

Neutral Red Solution: A 0.004 % neutral red solution was prepared in 100 mL distilled water. This solution was prepared daily as needed.

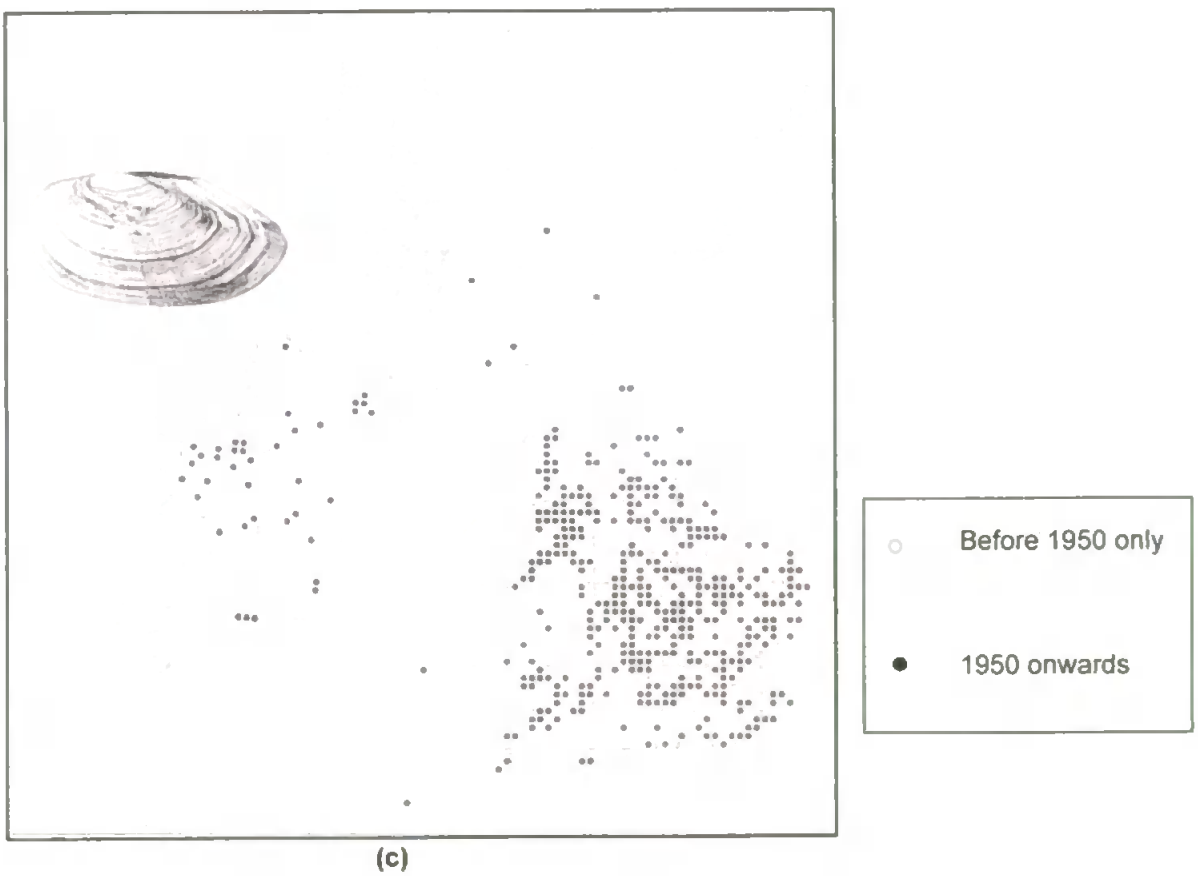
Acidified Ethanol: A 1 % glacial acetic acid/50 % ethanol was made up in distilled water. This solution was stored up to 1 month at 4 °C when not in use.

Anodonta cygnea:

Phylum: *Mollusca*
Class: *Bivalvia*
Order: *Eulamellibranchiata*
Sub-Order: *Schizidonta*
Super Family: *Unionacea*
Family: *Unionidae*
Genus: *Anodonta*
Species: *Anodonta cygnea*

(a)

(b)



(c)

Figure 6.2: (a) Biological classification of *Anodonta cygnea* (Linnaes, 1758) (Source: Pfleger, 1998); (b) adult *Anodonta cygnea*, with height and breadth measurements shown in inset; (c) map showing the distribution of *Anodonta cygnea* within the UK (Source: Kerney, 1999)

Protein reagent: 500 µl of BCA (bicinchoninic acid) Protein Assay reagent B was added to 25 mL of BCA protein reagent A and mixed to form a green solution. Reagents A and B, based on the Lowry method, were patented by Pierce Biotechnology (U.S. Patent # 4,839,295).

6.2.3 Acute toxicity studies

Adult swan mussels (*Anodonta cygnea*, 12 ± 2 cm shell length) were obtained from World of Water, Reading (www.worldofwater.com). On receipt, they were stored at 18 °C in 50 L aquaria (maximum of 12 organisms per aquarium) containing dechlorinated water and continuously aerated. Short term (96-h) static toxicity tests without water replacement were conducted to estimate the acute toxicity concentrations of nitrite and nitrate. Mussels were not fed for the 12-h period preceding the tests. Groups of 4 swan mussels were randomly selected and transferred to 20 L glass aquaria containing dechlorinated tap-water at 15 °C, aerated throughout the exposure period, with a light-dark cycle of 12 h. For each analyte, six experimental concentrations were investigated for each bioassay. Death was assumed when gaping shells remained unclosed after mechanical stimulus. The 96 h LC50 (50 % mortality) was obtained from plots of % mortality vs toxicant concentration.

6.2.4 Sub-lethal exposure studies

These studies were conducted as outlined for the acute toxicity studies using nitrite concentrations ranging from 0 to 22.2 mg L^{-1} N (0, 0.1, 1.0, 22.2 mg L^{-1} N), except that cardiac activity was monitored during these exposure studies.

6.2.4.1 Cardiac monitoring

The cardiac activity of *Anodonta cygnea* was monitored throughout exposure to sub-lethal

concentrations of nitrite using the Computer-Aided Physiological Monitoring (CAPMON) system (Figure 6.3 a and b). Collarless CAPMON sensors were constructed in this study according to protocol outlined by the Ecotoxicology Laboratory, University of Plymouth (Appendix I). They were constructed from twin-core screened cable (Farnell Instruments, Leeds) attached to a sealed free 3-way plug (Farnell Instruments, Leeds) on one end and to an infrared emitting diode/phototransistor (Farnell Instruments, Leeds) on the other. The infrared emitting diode/phototransistor end of a sensor was positioned on the shell over the heart of each test organism; the heart is usually situated close to the edge of the shell just forward of the shell-hinge in bivalves (Figure 6.3 c). The sensor was attached directly to the shell of each mussel using one drop of waterproof cyanoacrylate glue (Figure 6.3 d). The infrared emitting diode/phototransistor allowed cardiac activity to be measured (through the shell) as the difference of the infrared radiation emitted and detected: the emitted radiation is constant, but the radiation reflected by the animal's heart varies at each stage of contraction. Each sensor was linked to a CAPMON unit which is essentially an amplifier, and the detected signal was transferred to a computer, via an analogue-to-digital converter, where the data was continuously displayed and recorded as the number of heartbeats per minute. Sensors were attached to the mussels at least one hour before exposure to allow acclimatisation (Handy and Depledge, 1999). The cardiac activity of each test organism, including control organisms, was monitored for at least 1 h before exposure and during the 96 h exposure period.

6.2.4.2 Extraction of haemolymph

A fixed scalpel blade was used to open up the valves of the mussel (Figure 6.4). A needle attached to a syringe was carefully inserted approximately 2 mm into the posterior adductor muscle (Figure 6.3 d) of the organism and haemolymph withdrawn as slowly as possible. The needle was then removed and the haemolymph sample transferred to a

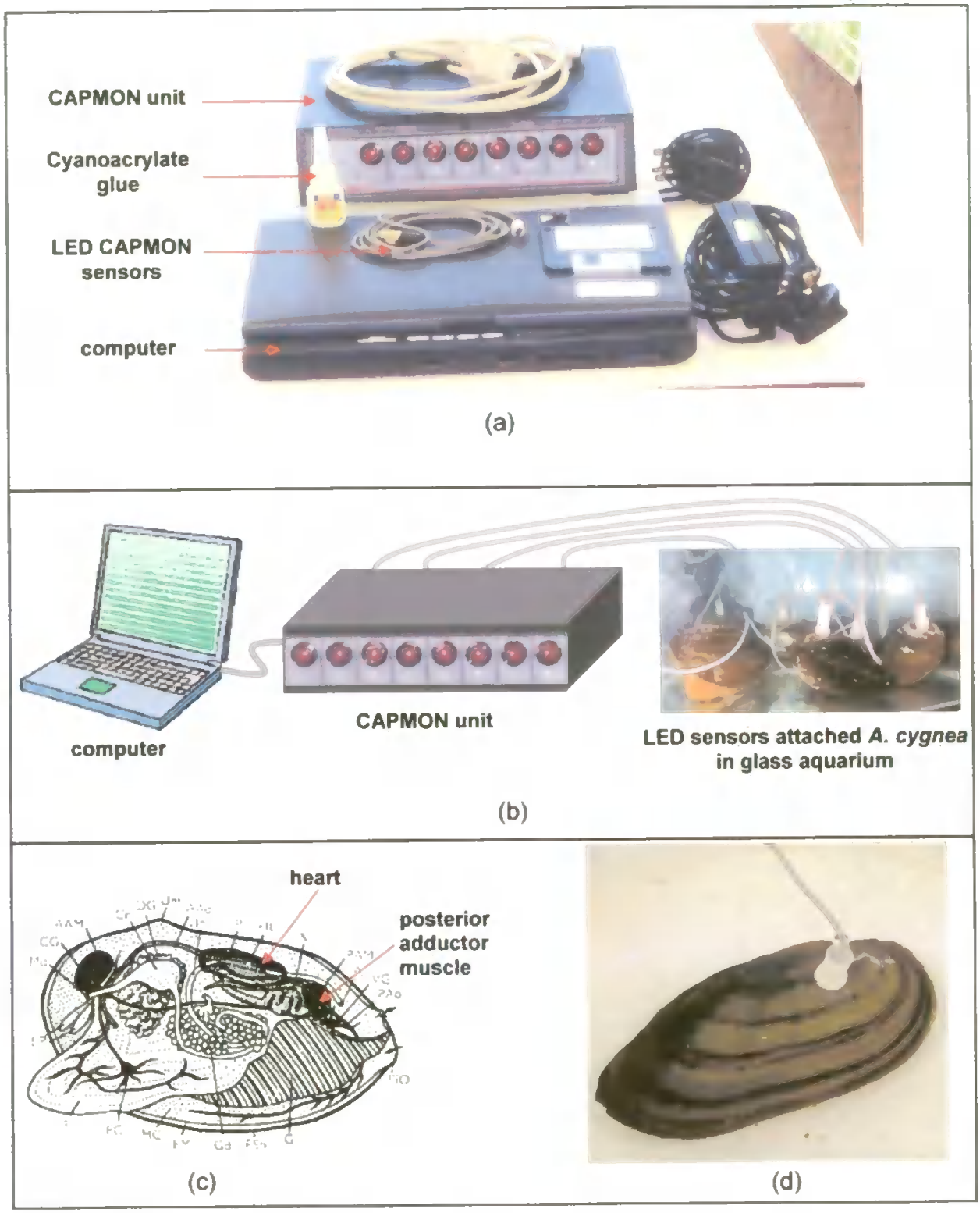


Figure 6.3: (a) Apparatus needed for monitoring cardiac activity using the CAPMON; (b) set-up of CAPMON to monitor cardiac activity of *A. cygnea*; (c) schematic diagram of location of the mussel heart (source: Janus, 1965); (d) CAPMON LED sensor attached to A. cygnea, placed over heart

Figure 6.4: Photograph showing the extraction of haemolymph from an adult *A.cygnea*

siliconised microcentrifuge tube and kept in ice to reduce the possiblity of cell lysis while pipetting onto microplates.

6.2.4.3 Neutral red retention assay

The neutral red retention assay in the present study was carried out according to protocol outlined by the Ecotoxicology Laboratory, University of Plymouth. A 200 µL aliquot of each haemolymph sample was transferred to a flat-bottomed microplate. Each haemolymph sample was analysed in triplicate. The plate was agitated at 400 × *g* for 60 s then covered and incubated at room temperature for 50 minutes to allow adhesion of cells to the plate. After incubation, the liquid from each cell was carefully discarded using a micropipette and 200 µL neutral red solution added to each well. The plate was covered once more and incubated at room temperature for 3 h to allow staining of cells. After the second incubation period, the neutral red solution was removed from the wells and each well rinsed three times with physiological buffer solution, by carefully introducing the buffer down the sides of the wells to minimize disturbance of the cells, until all residual neutral red solution was removed. A 200 µl aliquot of acidified ethanol mixture was then

added to each well to initiate cell lysis. The plate was incubated at 10 °C for 10 minutes and then read at 550 nm using a plate reader and agitated for 30 s before reading. The residual liquid was then carefully discarded, to minimise disturbance of the cells, leaving approximately 10 µL in each well to perform the protein assay. Neutral red retention was calculated as the absorbance of the neutral red retained per unit mass (mg) of protein:

$$\text{Neutral red retention} = \left(\frac{\text{Absorbance of haemolymph sample at 550 nm}}{\text{protein concentration in haemolymph sample (mg mL}^{-1}\text{)}} \right)$$

Protein assay: 10 µL of each haemolymph sample was added, in triplicate, to the wells of a microtitre plate. Protein standards (0, 0.5, 1.0 1.5 and 2.0 mg mL^{-1} protein) were prepared from a 2.0 mg mL^{-1} BSA protein standard stock and 10 µL of each standard was also added, in triplicate, to the plate. Blank samples were prepared by adding 10 µL physiological buffer, in triplicate, to the plate. 200 µL of protein reagent was then added to each well on the plate. The plate was covered and agitated for 1 min at 400 × *g* using a plate vortex mixer and then incubated for 30 minutes at 37 °C. After incubation, the plate was read at 562 nm. Protein concentrations of the haemolymph samples were calculated by interpolation of the calibration curve produced from the protein standards.

6.2.4.4 Determination of condition index

After the extraction of haemolymph, each individual mussel was measured for shell length, then dissected and shell volume and wet mass of entire soft tissue recorded. The entire soft tissue was frozen then freeze dried to constant weight for 48 - 72 h to determine dry tissue mass. The condition index was calculated as the ratio:

$$\text{Condition Index (\%)} = (\text{dry mass of soft tissue/shell length}) \times 100$$

6.2.4.5 Statistical analyses

All cardiac activity, neutral red retention assay and condition index data were plotted as box and whisker plots (Section 3.2.2) and were subjected to one-way analysis of variance (ANOVA) using STATGRAPHICS Plus 5.1. The Kruskal-Wallis test was performed on data where outliers were identified. All statistical significance tests were at the $p<0.05$ level.

6.3 Results and Discussion

Of the various N species, nitrite and ammonia are known to be particularly toxic to aquatic species: nitrite is linked to methaemoglobemia; ammonia is linked to O_2 depletion. Nitrite and nitrate are known to diffuse into the blood of fish and crustaceans (Cheng et al., 2002; Chen and Cheng, 2002; Chen and Cheng, 1999, Jensen, 1996; Stormer et al., 1996) and under some circumstances, nitrite concentrations may be high enough to alter haemoglobin and become toxic to aquatic organisms (Lewis and Morris, 1986). This suggests that nitrate and nitrite concentrations that may not be acutely toxic to organisms could have an adverse effect on the health of individual organisms. Therefore the acute toxicity and possible sub-lethal effect of these ions on freshwater invertebrates was investigated.

6.3.1 Acute toxicity studies

Biochemical and physical responses of organisms are often modified by exposure to toxicants, reducing their probability of survival or ability to tolerate additional environmental changes (Alcaraz et al., 1997). Therefore, it is important to establish the concentrations of chemicals that are toxic to aquatic organisms. The LC50 is a typical toxicity endpoint and is defined as the amount of toxicant present per litre of aqueous solution that is lethal to 50% of the test organism population within the stated study time (PAN pesticide database, 2004). In this study, the acute toxicity of nitrate and nitrite to

Anodonta cygnea was investigated and 96 h LC50 was used as the endpoint. With respect to nitrite, generally 24 h is required for maximum uptake but Lewis and Morris (1986) suggest that 96 h LC50 provides the most conservative estimate of short-term nitrite tolerance of fish under specified conditions and therefore is used in this study.

6.3.1.1 Nitrate toxicity

The toxicity of nitrate to invertebrates has not been widely investigated and the established nitrate LC50 toxicity data in the present literature are limited, with one exception, to marine organisms (Table 6.1). Published 96 h LC 50 nitrate toxicity data ranges from 97 mg L^{-1} N in the rotifer *Hydropsche occidentalis* early instar (juevenile life stage) to 2604 mg L^{-1} N in the adult american oyster *Crassostrea virginica*. (Table 6.1). This limited body of available data suggests that nitrate is less toxic to molluscs and crustaceans than to aquatic insects and rotifers: rotifers>aquatic insects> crustaceans>molluscs. Epifanio and Srna (1975) investigated the acute toxicity of nitrate to two marine bivalves, the american oyster *Crassostrea virginica* (Table 6.1) and the hard clam *Mercenaria mercenaria*, they concluded that nitrate was not acutely toxic to either species. Nitrate concentrations up to 4500 mg L^{-1} N were not acutely toxic to either juvenile or adult *Mercenaria mercenaria* (hard clam), while a 96 h LC50 of 2604 mg L^{-1} N was established for *Crassostrea virginica* (Table 6.1). Research by Tsai and Chen (2002) with prawns indicated that nitrate toxicity increased as salinity decreased (Table 6.1). If this trend is the same in all in vertebrates, then nitrate should be more toxic to freshwater bivalves than to marine bivalves. Established nitrate toxicity to freshwater invertebrates is limited to one study on pond snail eggs. The 96 h LC 50 established for pond snail eggs was comparable to that obtained for the juevenile american oyster .

The present literature does not cite any investigation of acute toxicity in freshwater bivalves. Such a study would provide useful information toward identifying whether high

Table 6.1: Nitrate toxicity data for molluscs, crustaceans, and aquatic insects

Organism	Salinity	Duration of static exposure (h)	LC50 value (mg L^{-1})	Reference
Crassostrea virginica (adult, 4.6 – 6.2 cm) (American oyster)	NR	96	2604	Epifanio and Sma (1975)
Crassostrea virginica (juvenile, 1.3 – 1.7 cm) (American oyster)	NR	96	3794	Epifanio and Sma (1975)
Lymnea sp. eggs (pond snail)	NR	48	6460	Dowden and Bennett (1965)
		72	5950	
		96	3251	
Penaeus monodon jueveniles (giant tiger prawns, black tiger prawns)	15	48	2876	Tsai and Chen (2002)
		72	1723	
		96	1449	
	25	48	3894	Tsai and Chen (2002)
		72	2506	
		96	1575	
	35	48	4970	Tsai and Chen (2002)
		72	3525	
		96	2316	
Hydropsche occidentalis (Banks) Early instar (cadisfly)	NR	72	149	Camargo and Ward (1992)
	NR	96	97	
		120	66	
Hydropsche occidentalis (Banks) Late instar (cadisfly)	NR	72	184	Camargo and Ward (1992)
	NR	96	109	
		120	77	
Cheumatopsyche pettiti (Banks) Early instar (cadisfly)	NR	72	191	Camargo and Ward (1992)
	NR	96	114	
		120	107	
Cheumatopsyche pettiti (Banks) Late instar (cadisfly)	NR	72	210	Camargo and Ward (1992)
		96	166	
		120	119	
Ceriodaphnia dubia neonates (water flea)	NR	48	374	Scott and Crunkilton (2000)
Daphnia magna neonates (water flea)	NR	48	462	Scott and Crunkilton (2000)

NR – not reported

environmental concentrations of nitrate can have a toxic effect on freshwater bivalves. Nitrate concentrations typically range between 1-100 mg L^{-1} N in freshwaters although the Nitrates Directive has identified water with >11.3 mg L^{-1} NO_3-N as polluted with respect

to nitrate. However, in cases of extreme pollution, concentrations may be as high as 500 mg L^{-1} NO_3-N in areas of high N fertiliser application (Chapman, 1998).

The acute toxicity of nitrate to the swan mussel was investigated in the present study. The results of this study indicate that nitrate concentrations up to 1625 mg L^{-1} N were not acutely toxic to swan mussels (Figure 6.2). Although a 96h LC50 was not established in this study, the concentrations investigated were well above both normal environmental conditions and levels expected in extreme cases of nitrate pollution (Chapman, 1998; Lewis and Morris, 1986). Therefore, nitrate concentrations in natural water would not be acutely toxic to the freshwater mussel, *A. cygnea.*

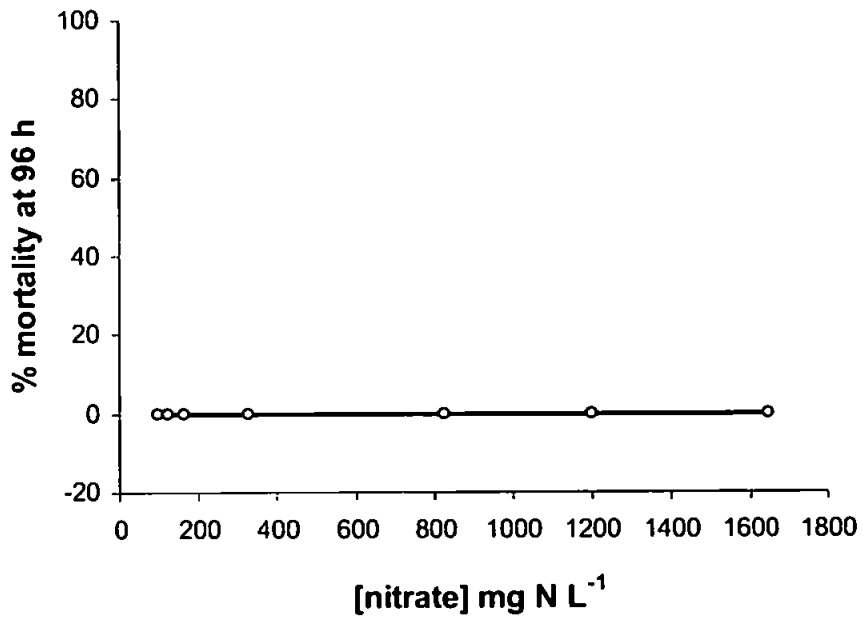


Figure 6:5: Exposure-response curve of *A. cygnea* (n=4) to nitrate.

6.3.1.2 Nitrite toxicity

The toxicity of nitrite to invertebrates has been much more widely investigated than that of nitrate owing to its greater toxicity to aquatic organisms. The economic importance of regulating nitrite water quality in aquaculture and mariculture systems as well as the ecological relevance of monitoring nitrite in natural waters has made this type of data

significant. However, the literature indicates that although nitrite toxicity is well-studied in a wide variety of fish, there is limited toxicity data for freshwater invertebrates (Table 6.2). The established LC50 data reported in the present literature for the toxicity of nitrite to invertebrate organisms are summarised in Table 6.2.

Lin and Chen (2003), Chen and Lee (1997) and Chen and Lin (1991), using different species of shrimp, successfully demonstrated that nitrite toxicity decreased as salinity increased (Table 6.2). Nitrite ion uptake in molluscs is inhibited by increasing salinity (Jensen, 2003). Therefore, as salinity increases, the uptake of nitrite is reduced and hence nitrite toxicity decreases, which indicates that nitrite is potentially more toxic to freshwater organisms than marine organisms. This is demonstrated in the present literature which indicates that nitrite is more toxic to the giant river prawn (freshwater crustacean) than to marine prawn species (Table 6.2).

Freshwater invertebrates, such as swan mussels, are hyperosmotic relative to their environment and their internal osmotic pressure is regulated by the constant uptake of ions via the gills and loss of ions via urine and via the gills (passive efflux). Chen and Cheng (1999) found that although nitrate and nitrite both diffuse into the organism via the same mechanism, concentrations of nitrite in crustacean haemolymph was up to 6 times higher than that of the surrounding environment while nitrate concentrations were one sixteenth that of the surrounding environment when exposed to elevated nitrate and nitrite concentrations. Therefore, excessively high environmental concentrations of nitrite could be acutely toxic to freshwater invertebrates and was further investigated (Section 6.3.2).

A 96 h LC50 for the exposure of *Anodonta cygnea* to nitrite was established in this study as 222 mg L^{-1} N (Figure 6.6). Such levels are unlikely to occur either naturally or under extreme pollution scenarios in natural waters. Nitrite concentrations in natural waters typically range from 1-100 µg L^{-1} N, but in cases of extreme pollution concentrations may be as high as 1 mg L^{-1} N such as in waters strongly influenced by industrial effluent (Chapman, 1998). Maximum admissible concentration of nitrite for the protection of

Table 6.2: Nitrite toxicity data for molluscs and crustacaens

Organism	Salinity	Static exposure time (h)	LC50 value (mg L^{-1})	Reference:
Corbicula manilensis 1.0-2.7 g (Asiatic clam)	NR	96	51	Chandler and Marking (1979)
Callinectes sapidus late premolt (blue crab)	NR	96	71	Ary and Poirrier (1989)
Callinectes sapidus intermolt (blue crab)	NR	96	92	Ary and Poirrier (1989)
Litopenaeus vannamei (Boone) juveniles (white shrimp)	15	24	188	Lin and Chen (2003)
		48	142	
		72	93	
		96	77	
		144	61	
	25	24	274	Lin and Chen (2003)
		48	244	
		72	225	
		96	178	
		144	152	
	35	24	521	Lin and Chen (2003)
		48	424	
		72	375	
		96	322	
		144	257	
Macrobrachium rosenbergii Juvenile (giant river prawn)	[Cl⁻] = 15 mg L^{-1}	96	8	Chen and Lee (1997)
	[Cl⁻] = 24 mg L^{-1}	96	11	
	[Cl⁻] = 34 mg L^{-1}	96	13	
Metapenaeus ensis juveniles (greasyback prawns, sand prawns)	NR	24	113	Chen et al (1990 a)
		48	80	
		72	57	
		96	51	
		120	41	
		144	31	
		168	25	
		192	19	
		216	17	

NR – not reported

Table 2 continued: Nitrite toxicity data for crustacaens and rotifers

Organism	Salinity	Static exposure time (h)	LC50 value (mg L^{-1})	Reference:
Penaeus monodon juveniles (giant tiger prawns, black tiger prawns)	NR	24	216	Chen and Lei (1990)
		48	185	
		72	89	
		96	55	
		120	18	
Penaeus monodon adolescents (giant tiger prawns, black tiger prawns)	NR	24	218	Chen et al (1990 b)
		48	193	
		72	171	
		96	140	
		120	128	
		144	106	
Penaeus-Penicillatus juveniles (redtail prawns)	34	24	93	Chen and Lin (1991)
		48	80	
		96	41	
		144	26	
	25	24	83	Chen and Lin (1991)
		48	53	
		96	39	
		144	26	
Penaeus setiferus post larvae (northern white shrimp)	NR	24	268	Alcaraz et al. (1999)
		48	249	
		72	167	
Rotifer *Brachionus-Plicatilis*	NR	24	733	Ostrensky (1993)
		96	169	

NR – not reported

course freshwater fish have been set by the EU as 9 μg L^{-1} N. In this study, nitrite concentrations typically encountered in the environment cannot be considered acutely toxic to freshwater swan mussels (Figure 6.6).

Nitrite in the Tamar River: Nitrite concentrations in the Tamar River are typical of the nitrite concentrations described for natural waters, ranging from 0 to 110 μg L^{-1} N over the past 30 years (1974-2004) (Section 3.3.1). However, the long-term mean nitrite concentration over this period, 20 μg L^{-1} N, exceeds the concentration set by the EU for the protection of course freshwater fish (9 μg L^{-1} N). 92 % of all nitrite data recorded at Gunnislake was ≥9 μg L^{-1} N. Therefore, the question arises as to whether high

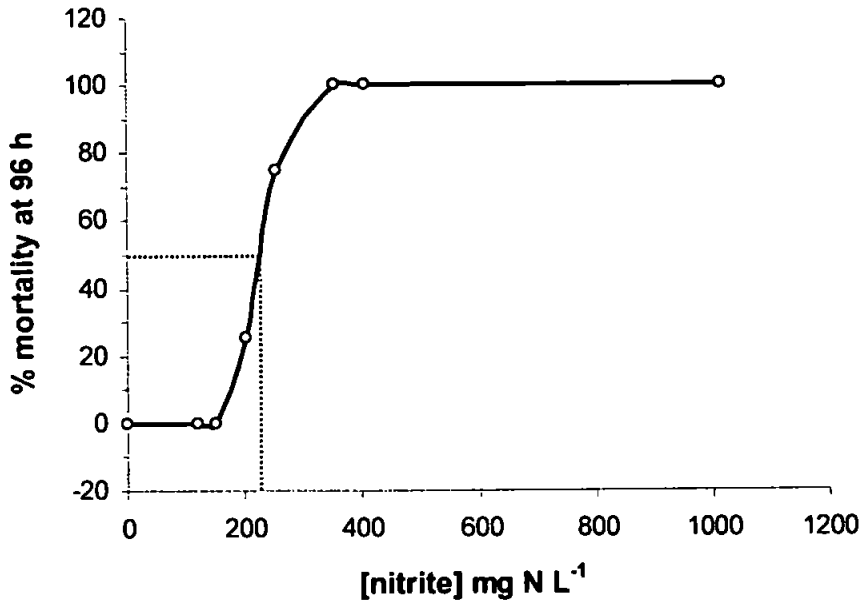


Figure 6.6: Exposure-response curve of *A. cygnea* (n=4) to nitrite. 96 h LC50 obtained graphically (·····)

environmental nitrite concentrations, which are not acutely toxic and lethal, could cause a measurable physiological stress to freshwater invertebrates. The swan mussel has been used to further investigate this question in this study.

6.3.2 Sub-lethal nitrite toxicity studies

Physiological assays attempt to measure organism response to a pollutant/toxicant exposure (Handy and Depledge, 1999). Such assays can be used to identify possible environmental circumstances that could lead to deterioration in organism and ecosystem health. In this study, a suite of generic indicators of physiological stress have been selected to determine whether sub-lethal nitrite concentrations, including environmental concentrations, have any effect on the heart rate, lysosomal cell stability and the condition index. *A.cygnea* were exposed to various sub-lethal nitrite concentrations representative of freshwater systems: 0.1 mg L^{-1} N, high end of nitrite concentrations encountered in freshwaters; 1.0 mg L^{-1} N, encountered under conditions of extreme nitrite pollution; and 10 % of the 96 h nitrite LC50 concentration (22.2 mg L^{-1} N). Control

organisms were kept in tanks with 0 mg L^{-1} N.

6.3.2.1 Condition index and protein concentration

Condition index is a measure of nutritive status and is calculated as a function of the shell length and dry tissue mass. It is used as a general indicator of health in invertebrates and it is a non-specific physiological indicator of pollution/environmental stress. The digestive diverticula, which is responsible for food-processing in bivalves, has a well-developed lysosomal system. Accumulation of toxicants taken in by an organism can cause chronic impairment of the lysosomal system of this digestive apparatus, which can lead to a change in nutritive status, measured as a change in the condition index (Nicholson, 1999).

Condition index was successfully used by Robillard et al. (2003) as one of a suite of biomarkers to distinguish between study sites in an *in situ* assessment of the impact of abiotic factors (temperature, pH and dissolved oxygen) and pesticide concentrations (atrazine was used as the sentinel pesticide) on *A. cygnea* (natural population) over a period of 6 weeks. In their study, the change in condition index was attributed to change in tissue mass as shell length did not change over the study period. In this study, the condition index of mussels (n = 8) was recorded after 96 h exposure to sub-lethal nitrite concentrations, (Figure 6.7). Statistical analysis of the data indicated that there was no significant difference ($p<0.05$) between the condition index recorded for control organisms (0 mg L^{-1} N), organisms exposed to environmental nitrite levels (0.1 mg L^{-1} N), or organisms exposed to high or extreme levels of nitrite (1.0 and 22.2 mg L^{-1} N). This indicates that although condition index can be used as an indicator of physiological stress in A. cygnea (Robillard et al., 2003) it is only a useful indicator over longer-term exposures. Condition index recorded for the organisms in this study, 38.0 ± 4.3, n=16, were higher than recorded by Robillard et al. (2003) for their reference site (17.3 ± 3.0, n=15) for similar sized organisms with respect to shell length.

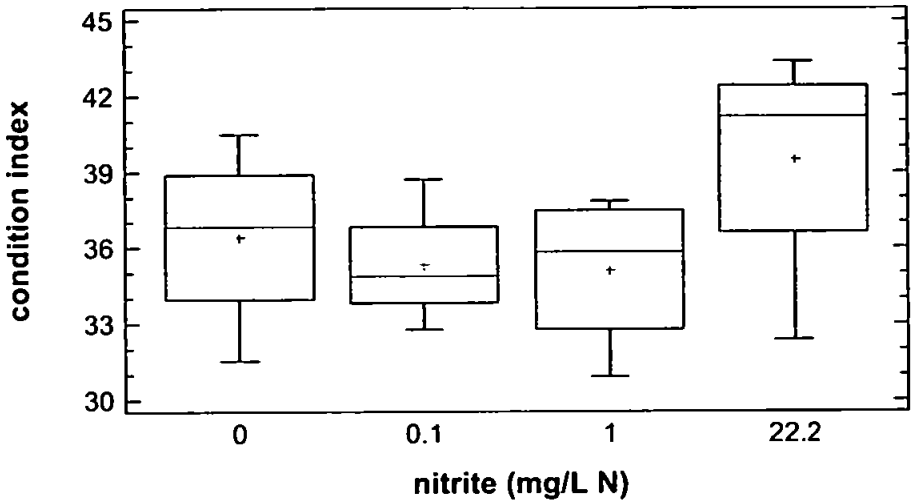


Figure 6.7: Condition index as a ratio of dry whole tissue weight as a function of shell length (n=8).

6.3.2.2 Cardiac monitoring

Once a bivalve mollusc is immersed, its rate of cardiac activity is fairly constant other than during valve closure (Helm and Truman, 1967; Coleman and Trueman, 1971). Any major perturbation of heart rate is therefore likely to be attributed to alterations in environmental conditions such as exposure to toxicants and changes in cardiac activity have been related to many environmental, physiological and behavioural factors (Nicholson, 1999). Bradycardia, a decrease in the rate of cardiac activity, has been linked to elevated metal concentrations (Grace and Gainey, 1987). Tachycardia, an increase in heart rate, is usually associated with elevated metabolism associated with elimination of the toxicant.

The CAPMON system has been successfully used to monitor the heart rate of many crustaceans and molluscs (Aguzzi et al., 2004). This technique benefits from being quick, easy to use and non-invasive, therefore limiting the stress caused to the test animals. Studies using the CAPMON have examined and positively correlated the effects of pollutants such as metals including mercury and copper, (Camus et al., 2004; Curtis et al., 2000; Styrishave and Depledge, 1996), ammonia (Bloxham et al., 1999), whole effluent (Bloxham et al., 1999) and abiotic conditions (temperature; light) with heart rate (Aguzzi et al., 2004).

The cardiac activity of *A. cygnea* has not been previously reported in published literature. In this study, prior to nitrite exposure, the heart rate of *A.cygnea* (n=9) was recorded to determine natural variations and cycles. The thin shell of the *Anodonta cygnea*, which is characteristic of freshwater mussels, assisted in the ease of detecting the hearbeat using the CAPMON system. No diurnal cycle was observed (Figure 6.8), however, the number of beats per minute varied widely amongst individual organisms (10 to 160 beats per minute). This was also observed in the control organisms used during the sub-lethal exposure experiments (Figure 6.9 a).

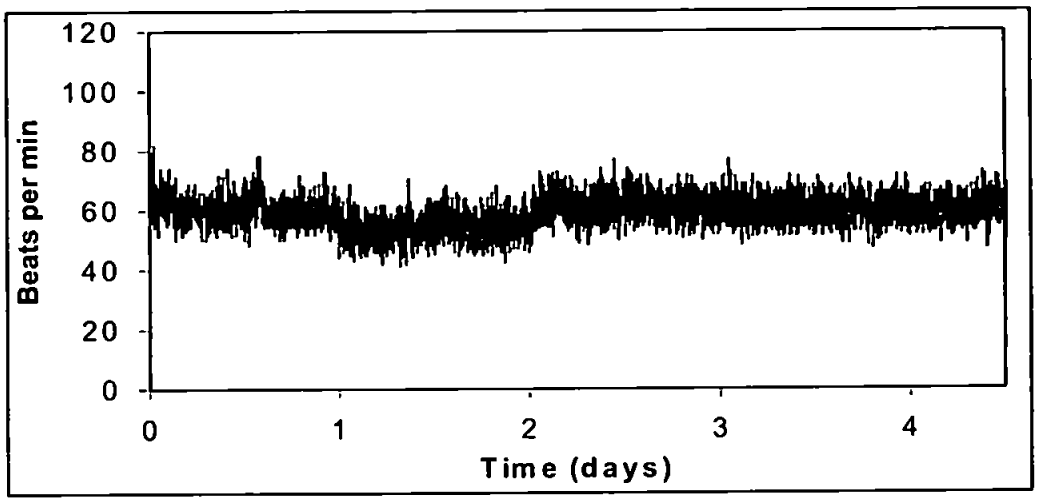


Figure 6.8: Cardiac activity recorded for *A.cygnea* over a 4.5 days using the CAPMON

In this study, no significant change in the cardiac activity was observed when *A. cygnea* was exposed to various concentrations of nitrite (0 - 22.2 mg L^{-1} N) over 96 h (Figure 6.9). In bivalve molluscs, including *Anodonta*, serotonin is thought to be a cardioexcitatory neurotransmitter (Wollemann and Rozsa, 1975). However, in some bivalves such as *Anodonta*, serotonin causes an increase in cyclic AMP levels, which in turn mediates the excitory effect of the serotonin (Wollemann and Rozsa, 1975). Such a mechanism would ensure than non-lethal triggers to heart-rate such as increased metabolism to eliminate excess nitrite would not result in tachycardia when *A. cygnea* was exposed to high nitrite

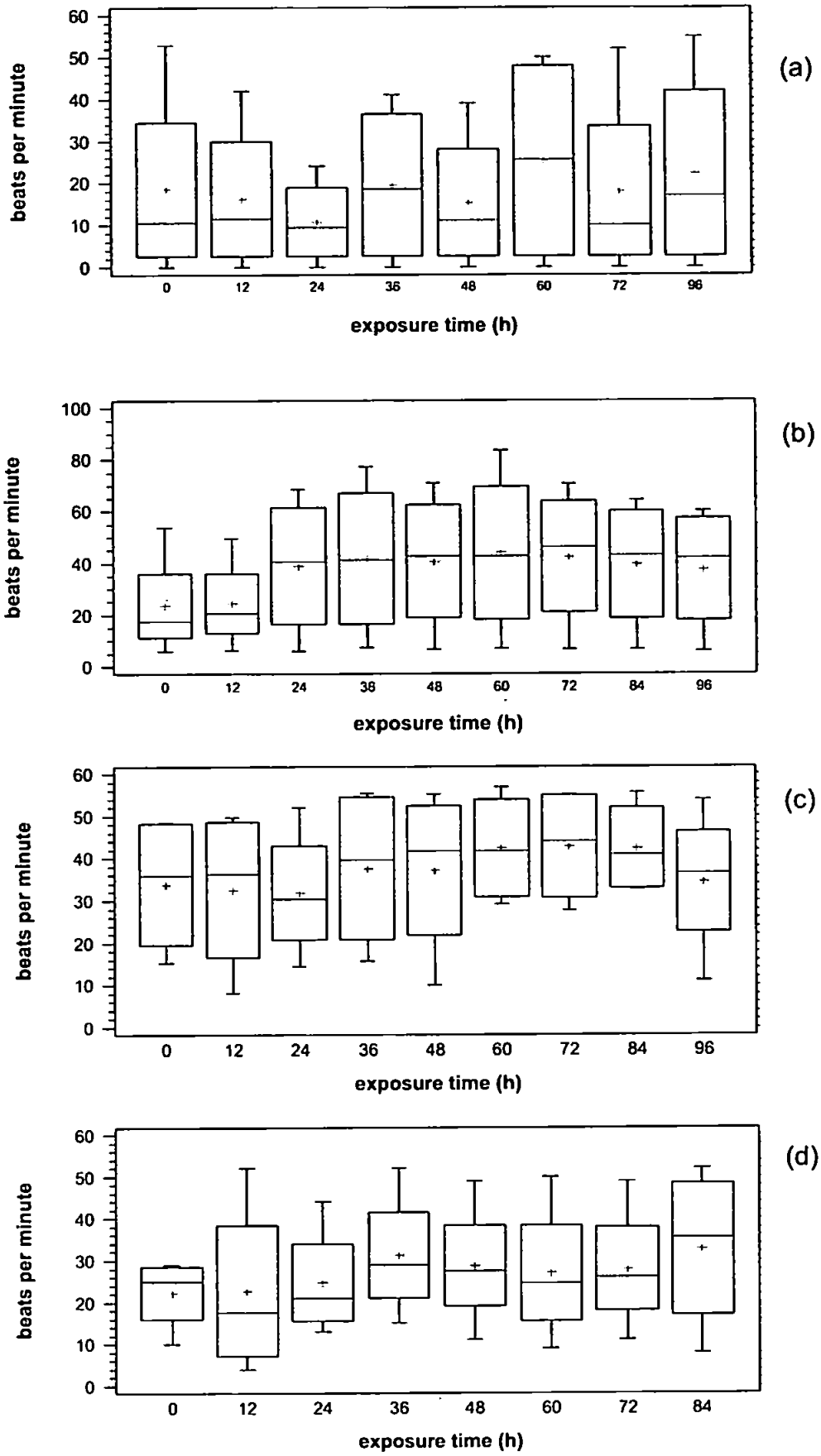


Figure 6.9: Cardiac activity of *A.cygnea* (n=8) exposed to (a) 0 mg L^{-1} NO_2-N (control); (b) 0.1 mg L^{-1} NO_2-N; (c) 1.0 mg L^{-1} NO_2-N; (d) 22.2 mg L^{-1} NO_2-N concentrations for 96 h

concentrations. Therefore, the cardiac activity would appear to be unchanged at various levels of nitrite exposure.

Although tachycardia as a measure of coping with increased metabolism was not observed in this study, excretion of faecal matter appeared to decrease with increasing nitrite concentration. Visual inspection of the aquaria after exposure indicated that faecal matter excreted by organisms exposed to ≤0.1 mg L^{-1} N were similar, but decreased noticeably with organisms exposed to 1.0 and 22.2 mg L^{-1} N, such that tanks used for 22.2 mg L^{-1} N exposures were virtually free of faecal matter. This observation has not been previously reported in bivalves. It indicates that some metabolic functions are being impaired at sub-lethal nitrite concentrations including high environmental concentrations of nitrite (1.0 and 22.2 mg L^{-1} N) and warrants further investigation in future studies.

6.3.2.3 Neutral red retention assay

Haemolymph or the 'blood' of bivalves consists of dissolved haemoproteins and 'blood cells' called haemocytes (Furuta and Yamaguchi, 2001). Haemolymph is responsible for the distribution of oxygen, nutrients, hormones and haemocytes within these organisms (Jaenicke et al., 2003). Lysosomes, organelles within the haemocytes, are components of the immune system and have the ability to take up a diverse range of toxicants (Moore, 1990). This leads to a concentration of toxicants within lysosomes where the acid hydrolases are released to degrade the toxicant. This can result in enhanced toxicity and cell damage via damage to the lysosomes. Damaged lysosomes can release acid hydrolases into the cytoplasm of the haemocytes, which can result disruption to the cell membrane. Neutral red retention assay, is based on dye uptake and retention by lysosomes, has been used as a measure of lysosomal stability and therefore of cell health (Lowe et al., 1995). Greater retention of the dye is expected in healthy cells than in damaged or stressed cells (Svendsen and Weeks, 1995; Lowe et al., 1992).

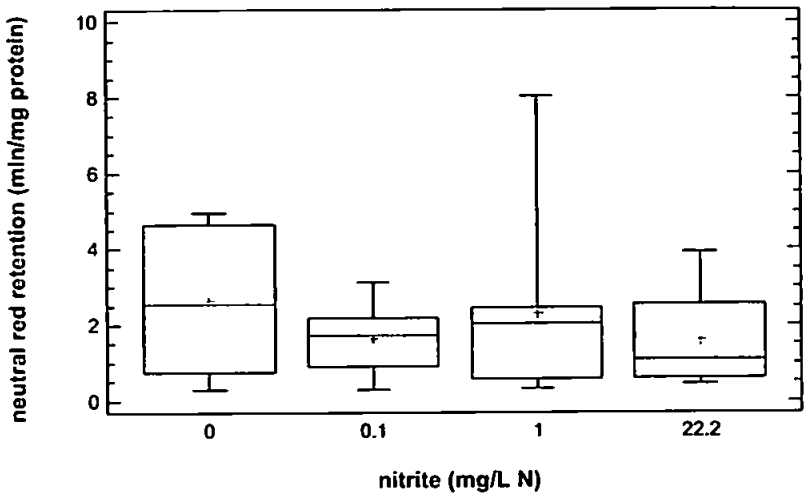


Figure 6.10: Neutral red retention in *A.cygnea* (n=8) exposed to varying nitrite concentrations for 96 h.

In this study, the neutral red retention assay was used to assess whether short-term (96 h) exposure to sub-lethal nitrite concentrations (0-22.2 mg L^{-1} N), including high environmental concentrations (0.1 and 1.0 mg L^{-1} N), would affect the lysosomal stability and cell health in *A. cygnea*. Results (Figure 6.10) indicate that there was no significant difference ($p>0.05$) in neutral red retention in lysosomes of *A. cygnea* exposed to varying concentrations of nitrite (0-22.2 mg L^{-1} N). Therefore, physiological stress indicated by lysosomal instability was not induced by 96 h exposure to up to 10% of the 96 h LC50 nitrite concentration. Lewis and Morris (1986) suggest that as yet there is no evidence that nitrite concentrations (NO_2-N) less that 10% of the LC50 value would be detrimental to freshwater fish. The findings of this study suggest that this also applies to *Anodonts cygnea*, a freshwater mussel.

6.4 Conclusions

A 96 h LC50, 222 mg L^{-1} N, for the exposure of *A. cygnea* to nitrite has been established in this study. Studies also indicate that nitrate concentrations <1625 mg L^{-1} N nitrate are not acutely toxic to *A. cygnea*. Therefore, nitrate and nitrite concentrations in the environment

cannot be considered acutely toxic to swan mussels and are not likely to be acutely toxic to most freshwater mussels.

The cardiac activity of *A. cygnea* was observed and reported to in this study. No diurnal cycle was observed and the number of beats per minute varied widely amongst individual organisms (10 to 160 beats per minute). Condition indices recorded in this study using farmed mussels (38.0 ± 4.3) were higher than reported in the literature for natural populations (17.3 ± 3.0, n=15) of a similar shell length.

Established indicators of physiological stress were used to determine the effect of environmentally high and extreme concentrations of nitrite on *A. cygnea*. There was no significant difference observed in either cardiac activity, condition index or lysosomal stability between control organisms (exposed to 0 mg L^{-1} N) and organisms exposed to sub-lethal concentrations of nitrite (0.1, 1.0, 22.2 mg L^{-1} N). Nitrite concentrations as high as 10% of the 96 h LC50 value did not induce measurable levels of physiological stress in the freshwater mussel *A. cygnea* upon 96 h exposure. Therefore, nitrite concentrations encountered in typical freshwater catchments such as the Tamar catchment are unlikely to induce physiological stress in freshwater mussels.

CHAPTER 7

Conclusions and Future Work

7.1 CONCLUSIONS

This chapter presents some general conclusions derived from this research and proposes some general guidelines for studying the biogeochemistry of nutrients in the Tamar catchment.

7.1.1 Quality assurance and quality control in analytical procedures for the determination of nutrients in the Tamar catchment

Data quality is critical in any biogeochemical study. QA/QC measures to ensure accurate results should include the use of certified reference methods and participation in intercomparison studies. The Skalar air-segmented flow analyser is an accurate analytical reference method for the determination of FRP (3 – 150 μg L^{-1} P) and nitrate/nitrite (0.5 – 5.0 mg L^{-1} N) in aqueous solution and in natural waters. Accuracy was demonstrated by successful participation in the 'NOAA/NRC 2nd annual intercomparison study for nutrients'; *z*-scores of 0.22 and -0.21 for the analysis of FRP and nitrate/nitrite, respectively, were achieved. In the analysis of freshwater, estuarine or seawater samples, the standard and blank solutions should match sample salinity. Under these conditions, the sensitivity and limits of detection of the Skalar air-segmented flow analyser remain unchanged at 0-35 salinity and no other modifications to the respective manifolds, the carrier stream or the buffer reagent are needed. Quantitative conversion of N and P model compounds to nitrate and phosphate, respectively, were achieved using alkaline persulphate/autoclave digestion. Therefore, this digestion protocol followed by FRP and nitrate/nitrite determination using the Skalar air-segmented flow analyser is recommended for the simultaneous determination of TN and TP in the freshwaters leaving the Tamar catchment. TP and FRP concentrations determined in any environmental survey should be checked; if [TP]<[FRP] for a sample, the silicate and arsenate concentrations should be determined to ascertain whether they are above the threshold concentrations of 10 mg L^{-1} Si and 50 μg L^{-1} As, respectively.

7.1.2 Environmental trends from time series data

The waters of the Tamar River at Gunnislake as it leaves the Tamar catchment are mesotrophic with respect to phosphorus (>20 μg L^{-1} P but <60 μg L^{-1} P, calculated from mean annual FRP). Annual mean FRP (1974-2004) has decreased since 1991, coincident with measures introduced by the Urban Wastewater Directive (UWWD) for improved treatment of wastewater. The sampling strategy for collection at Gunnislake by the EA has not been consistent over the past 30 years (1974-2004): all parameters were not monitored with the same frequency; and frequency of data collection of nutrient data decreased from three sampling occasions per month in 1976 to six times per year in 2004.

The Tamar freshwater system is dominated by dissolved nutrients, and the dissolved N and P fractions are dominated by dissolved inorganic species (FRP (77 %); nitrate/nitrite (82 %)). This is indicative of a freshwater catchment dominated by diffuse sources of nutrients such as agriculture rather than points sources. The Tamar River, as it leaves the Tamar catchment at Gunnislake, is severely P limited. *In situ*, real-time monitoring of FRP, which is representative of measurable bioavailable P, is recommended. This would provide high frequency data collection representative of the full range of temporal variability and the associated environmental conditions necessary for assessing trophic status with respect to phosphorus in this catchment.

Future monitoring of nutrient species in the Tamar catchment should include measurement of TP, FRP, nitrate/nitrite, TDN and TN and silicate. A minimum of twice monthly sampling is recommended for routine monitoring to identify environmental trends. Physico-chemical data and chlorophyll a concentrations should also be included in any biogeochemical study of the catchment as this would allow comparison of N and P concentrations and N:P ratios with chlorophyll a concentrations. TDN:TDP should be used to calculate N:P ratios. In the event of algal blooms, community structure should be investigated to provide information with respect to species composition of the bloom and their nutrient requirement within the Tamar River system. The monitoring of silicate

concentrations, particularly in spring, would also be useful in establishing ratios of N:P:Si with respect to diatom blooms. River discharges rates should also be monitored along with nutrient concentration as this would allow export of various nutrient species from the catchment to be calculated.

7.1.3 Export coefficient modelling of P and N

The export coefficient modelling approach was successfully used to develop an annual model to P export from the Tamar catchment. Modelled P (43. 5 tonnes P y^{-1}) export was within 8 % agreement with the measured P load (40.1 tonnes P y^{-1}). This model provides a decision support tool that can be used to predict changes in P export based on proposed changes in land use, population density and livestock intensity. P export from the catchment was dominated by the export from diffuse P sources and 75 % of the modelled annual TP export was from land use practices. P export from land use practices, in particular lands dedicated to grazing, exerts the most influence of the annual P model, based on a sensitivity analysis (± 10 %).

An annual TN export model for the Tamar catchment was also constructed and calibrated in this study predict from the catchment. A 1 % agreement between modelled and measure annual TN export was obtained. The sensitivity analysis (± 10 %) showed that the outcome of TN model would be most strongly influenced by exports from rainfall (5.3 %).

7.1.4 Development of a portable *in situ* FRP analyser

A portable FI analyser for *in situ*, continuous, real-time monitoring of FRP (4-150 μg L^{-1} P) capable of providing high temporal resolution (up to 15 samples h^{-1}) data has been optimised for use in riverine monitoring. Data from the portable FRP analyser was in good agreement (RSD ≤5 %) with the reference method when analysing freshwater samples under laboratory conditions and in the analysis of FRP standards during field

deployments. *In situ* analysis of freshwater samples from the Tamar River/upper Tamar Estuary was biased towards reporting approximately 20 % higher concentrations than the reference method. This suggests that sample degradation may be occurring between sample collection and sample analysis and requires further investigation (see Section 7.2). Continuous monitoring of nutrient species should also include regular collection of a discrete sample for comparison of instrument performance with another validated method.

The use of such an instrument in environmental monitoring can provide data to describe FRP water quality under a range of environmental conditions, to identify short-term changes in FRP concentration and also to provide data for the annual P export model. It is recommended that the sampling rate should be adjusted to collect a sample every 15 minutes, synchronous with the river discharge data collection. This would provide data toward refinement of the export P coefficient model developed in this study to predict annual P export from the Tamar catchment.

7.1.5 Physiological stress in freshwater invertebrates exposed to high environmental nutrient concentrations

Nitrate and nitrite concentrations in the natural waters are not acutely toxic to swan mussels. The 96 h LC50, established in this study, for the exposure of *A. cygnea* to nitrite was 222 mg L^{-1} N, while nitrate concentrations <1625 mg L^{-1} N nitrate were not acutely toxic to *A. cygnea*.

Nitrite concentrations as high as 10% of the 96 h LC50 value did not induce measurable levels of physiological stress in the freshwater mussel *A. cygnea* upon 96 h exposure. Therefore, nitrite concentrations encountered in typical freshwater catchments such as the Tamar catchment are unlikely to induce physiological stress in the freshwater swan mussel.

7.2 SUGGESTIONS FOR FUTURE WORK

Suggestions for future work arising from this research include:

- Validation of the N export coefficient model developed in this study by collecting data (at least twice monthly sampling) to compare measured and modelled loads for another year. This process would evaluate the suitability of the model for modelling and predicting N export for a typical year. This is necessary before the N model can be used for modelling N management scenarios in the Tamar catchment.
- Modify the portable nutrient analyser so it can be used monitor FRP in the rapidly changing salinity gradient encountered in estuaries. Measures such as introduction of a reference cell to mathematically subtract the interference or the use of a dual wavelength detector could eliminate the Schlieren effect.
- Sample degradation between collection and filtration (<1 h) observed during this study could be investigated by analysing discrete samples on-site with using the portable FRP analyser to determine whether there is a change in FRP concentration over short periods. Samples should be collected at the same time as a real-time sample, then a portion of a discrete sample filtered and analysed every 6 minutes for at least 1 hour. Laboratory investigations could include mesocosm studies.
- Investigation of the effect of nitrite on the metabolism of the freshwater mussel, *A. cygnea*. Excretion of faecal matter by *A. cygnea* appeared to decrease with increasing nitrite concentration in the present study, which indicated that some metabolic functions may have been impaired at sub-lethal nitrite concentrations including high environmental concentrations. Measurement of another metabolic process such as oxygen uptake could indicate whether metabolism is compromised as nitrite concentration increases. Oxygen uptake in bivalves is quantified by measuring the oxyhaemocyanin to protein ratio and by quanitative separation of oxyhaemocyanin and deoxyhaemocyanin (by HPLC).

- Determination of the 96 h LC50 for the exposure of selected freshwater invertebrates, such as *A. cygnea*, to determine if ammonia is acutely toxic. An investigation of the effect of sub-lethal ammonia concentrations on the physiological health of such an organism would also provide information as to whether typical ammonia concentrations in freshwater catchments such as the Tamar catchment are likely to induce physiological stress

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APPENDIX

APPENDIX 1: PROTOCOL FOR MAKING CAPMON SENSORS

MATERIALS

Twin-core screened cable (Farnell 4621-EZZD), sealed free 3-way plugs (Farnell 99-0405-00-03), and infrared emitting diode/phototransistor, also known as reflective opto-switches (Farnell HOA13972) were obtained from Farnell Instruments, Leeds (Figure A1). Glue gun, glue sticks and PVC tubing (0.8 cm i.d., 1.0 cm e.d.) were purchased from Antics, Plymouth . Apparatus such as soldering iron and solder, bench vice, end cutters and wire strippers were available at the University of Plymouth.

Figure A1: Materials used in the fabrication of the CAPMON sensors

METHODOLOGY

'Plug' end: The cable to was cut to the required length (1 m) then passed through the main plug components shown in Figure A2 (parts a-d) to assemble the main body of plug. Then approximately 10 mm plastic from the outer-insulation from cable-end was stripped: the copper insulating wire was twisted and 3-4 mm plastic insulation was stripped from each of the core wires (red and blue). (Figure A3 a). The exposed copper cores were coated with a little solder (Figure A3 b and c), then the blue wire was soldered to prong 1 of plug, the copper wire was soldered to prong 2 and the red wire to prong 3 (small numbers are embossed next to

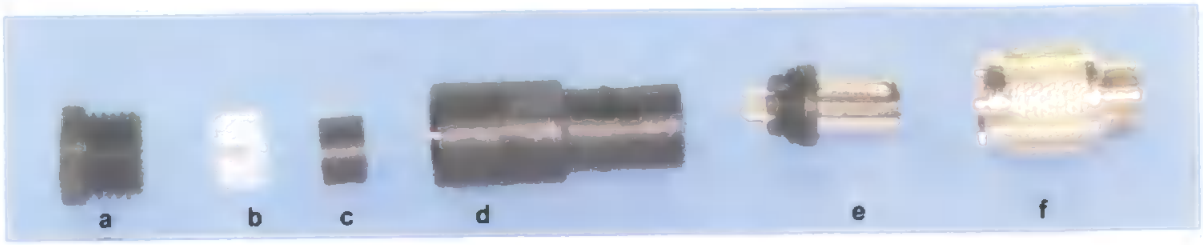

Figure A2: Sealed free 3-way plug components

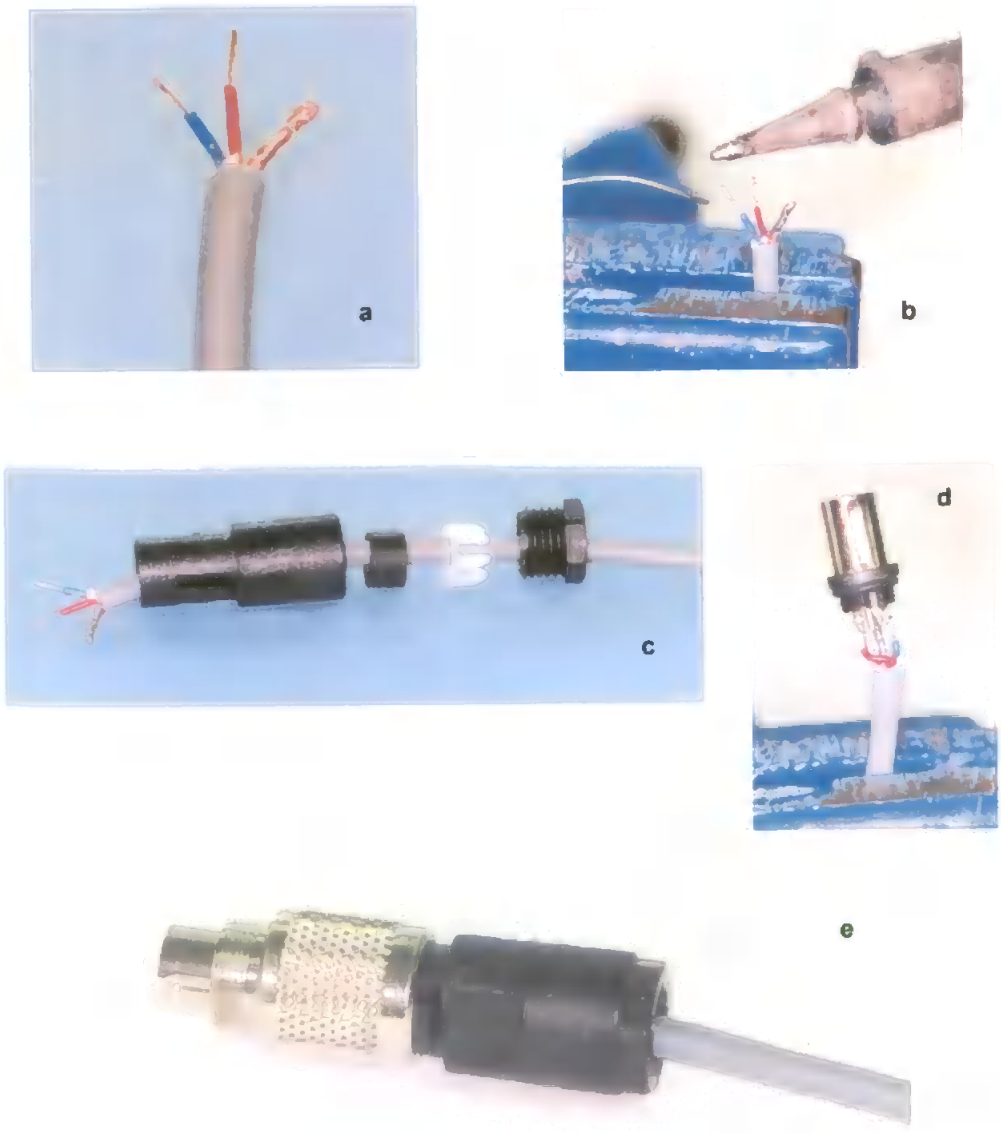

Figure A3: Assembly of the 'plug' end of the CAPMON sensor

prongs) (Figure A3 d). The plug parts were then screwed together (Figure A3 e).

'Infrared emittor/detector' end: Approximately 10 mm plastic from the outer-insulation from cable-end was stripped: the copper insulating wire was twisted and 5 mm plastic insulation from each of the core wires (red and blue) was stripped. The exposed copper cores were coated with a little solder. The opto switch was positioned with lettering (HOA MEX) facing away then the the bottom left prong was pushed diagonally across the switch assembly as shown in Figure A4 a and b. The lengths of the vertical prongs were trimmed to about 3 mm and the horizontal prong was trimmed such that it lay neatly along the opto switch. All the pins were lightly coated with solder at the ends (Figure A4 b). The copper screen wire was then soldered to the two joined pins, the blue wire to bottom-right prong and the red wire to top-left prong (Figure A4 a and c). Soldered connections were as small and neat as possible. Soldered joints were inspected to ensure they were not touching or too close to other joints. Joints that were too close were carefully teased apart to maximise the space between them.

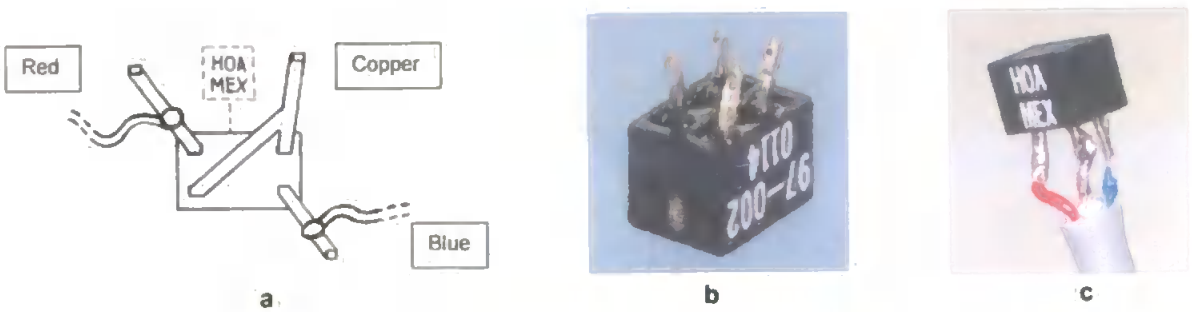

Figure A4: Wiring plan for the infrared emittor/detector and assembly of the infrared emittor/detector end of the CAPMON sensor

The sensor was then connected to CAPMON unit and the end of the sensor tapped with a finger to check if the sensor was functional. The sensor was accepted as functional if the signal on the screen mirrored the tapping (Figure A5).

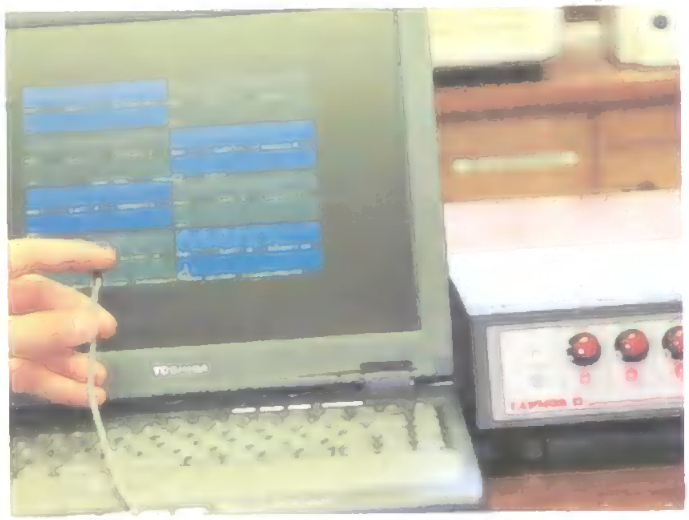

Figure A5: Testing of the CAPMON sensor

Waterproofing the Sensors: Glue, using a hot glue gun, was carefully dripped onto the soldered connections (Figure A6 a). Glue was also allowed to flow between connections while the sensor/cable assembly was gently rotated to cover all areas with glue, adding small amounts of glue to gradually build up a thicker layer. The glue was extended along the cable to a length of around 20 mm((Figure A6 b).).

When the glue was set (dry) and the sensor assembly was placed in the PVC tubing (1 cm length, each) and excess glue trimmed away, taking care not to cut through to the wires. Additional glue was then placed around the existing sensor assembly to hold the PVC tubing in place ((Figure A6 c).). Excess was again trimmed away from the end of the sensor ((Figure A7 a). The finished sansor is shown is Figure A7 b.

Figure A6: Waterproofing of the CAPMON sensor

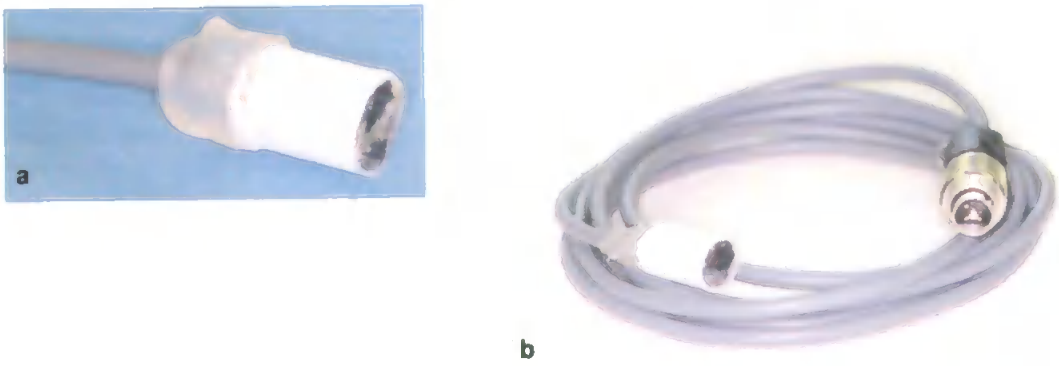

Figure A7: The completed, waterproofed CAPMON sensor.

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Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils

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Abstract

Phosphorus is an important macronutrient and the accurate determination of phosphorus species in environmental matrices such as natural waters and soils is essential for understanding the biogeochemical cycling of the element, studying its role in ecosystem health and monitoring compliance with legislation. This paper provides a critical review of sample collection, storage and treatment procedures for the determination of phosphorus species in environmental matrices. Issues such as phosphorus speciation, the molybdenum blue method, digestion procedures for organic phosphorus species, choice of model compounds for analytical studies, quality assurance and the availability of environmental CRMs for phosphate are also discussed in detail.

Keywords: Phosphorus: Natural waters: Soils: Sampling: Sample treatment: Sample digestion: Quality assurance

1. Introduction

The determination of phosphorus species in environmental matrices provides essential data for assessing the health of ecosystems, investigating biogeochemical processes and monitoring compliance with legislation. At the catchment scale, for example, phosphorus export from both point and diffuse sources can result in increased primary production and eutrophication, with the potential for seasonal development of toxic algal blooms, which can have a major impact on global water quality [1]. For accurate measurements, knowledge of phosphorus speciation is required as environmental behaviour is often critically dependent on its physico-chemical form. In aquatic systems, for example, phosphorus species are found in "dissolved", "colloidal" and "particulate" fractions, as inorganic and organic compounds and in biotic and abiotic particles [2]. The common operationally defined aquatic forms of phosphorus and the various terms used to describe them are shown schematically in Fig. 1. The reliability and comparability of data for any of these fractions will depend on the operational protocols used and the accuracy of the method.

Most manual and automated methods of phosphorus determination are based on the reaction of phosphate with an acidified molybdate reagent to yield phosphomolybdate heteropolyacid, which is then reduced to an intensely coloured

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Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils

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Abstract

Phosphorus is an important macronutrient and the accurate determination of phosphorus species in environmental matrices such as natural waters and soils is essential for understanding the biogeochemical cycling of the element, studying its role in ecosystem health and monitoring compliance with legislation. This paper provides a critical review of sample collection, storage and treatment procedures for the determination of phosphorus species in environmental matrices. Issues such as phosphorus speciation, the molybdenum blue method, digestion procedures for organic phosphorus species, choice of model compounds for analytical studies, quality assurance and the availability of environmental CRMs for phosphate are also discussed in detail.

Keywords: Phosphorus; Natural waters; Soils; Sampling; Sample treatment; Sample digestion; Quality assurance

1. Introduction

The determination of phosphorus species in environmental matrices provides essential data for assessing the health of ecosystems, investigating biogeochemical processes and monitoring compliance with legislation. At the catchment scale, for example, phosphorus export from both point and diffuse sources can result in increased primary production and eutrophication, with the potential for seasonal development of toxic algal blooms, which can have a major impact on global water quality [1]. For accurate measurements, knowledge of phosphorus speciation is required as environmental behaviour is often critically dependent on its physico-chemical form. In aquatic systems, for example, phosphorus species are found in "dissolved", "colloidal" and "particulate" fractions, as inorganic and organic compounds and in biotic and abiotic particles [2]. The common operationally defined aquatic forms of phosphorus and the various terms used to describe them are shown schematically in Fig. 1. The reliability and comparability of data for any of these fractions will depend on the operational protocols used and the accuracy of the method.

Most manual and automated methods of phosphorus determination are based on the reaction of phosphate with an acidified molybdate reagent to yield phosphomolybdate heteropolyacid, which is then reduced to an intensely coloured

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Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils

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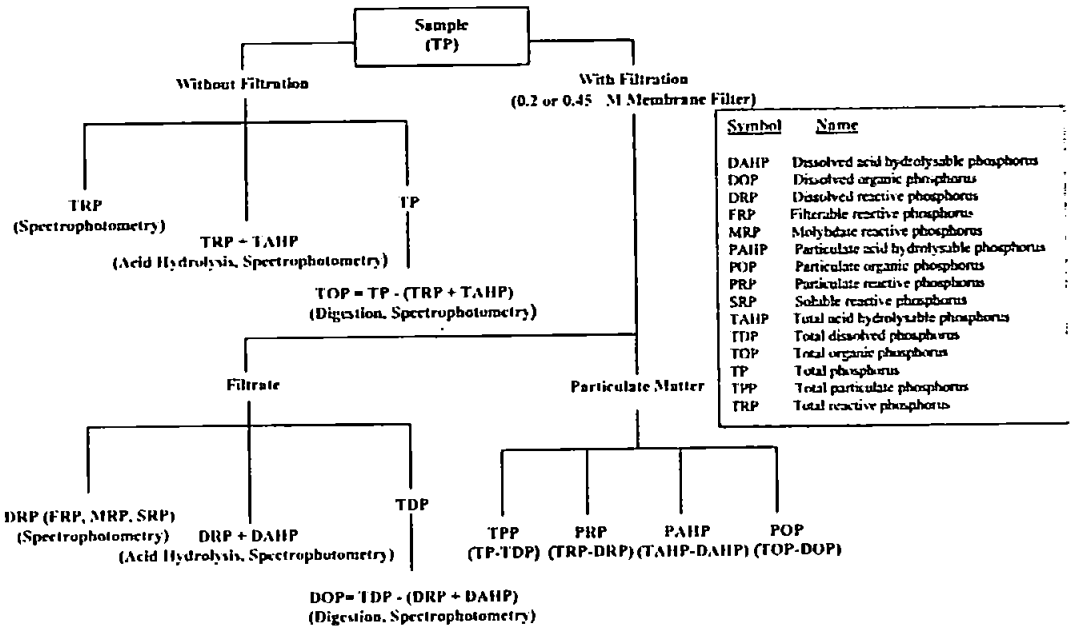


Fig. 1. Operationally defined aquatic P fractions (adapted from [2]).

blue compound and determined spectrophotometrically [3].

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

$$H_3PO_4(MoO_3)_{12} + \text{reducing agent} \rightarrow \text{phosphomolybdenum blue [Mo(VI)} \rightarrow \text{Mo(V)]}$$

There are many modifications of the original Murphy and Riley method [4], particularly the use of different reductants (e.g. ascorbic acid, tin(II) chloride) and acid strengths. As shown in the above reaction scheme, the phosphomolybdenum blue complex is formed in an acidic environment and its absorbance spectrum is dependent on the acidity, type of reductant and phosphate concentration. Under low acidity conditions, for example, non-linear colour development [5] and non-phosphate sensitized reduction (self-reduction of the molybdate) can occur. A variety of $[H^+]/[MoO_4^{2-}]$ ratios have been reported in the literature, with a ratio of 70 and a pH range of 0.57–0.88 suggested for optimum sensitivity (maximum rate of colour formation) [6].

Ascorbic acid and tin(II) chloride are the most commonly used reductants when determining phosphate concentrations in natural waters. Ascorbic acid acts as a 2-electron reductant [7] with the major advantages being that it is less salt sensitive and colour development is fairly independent of temperature [6]. Ascorbic acid on its own however has the major disadvantage of slow colour development [8], but the addition of antimony as a catalyst increases the rate of reduction of the complex [4]. Using tin(II) chloride generates a product with a wavelength maximum at 690–700 nm as compared with 882 nm for ascorbic acid [2]. This allows greater sensitivity when a solid state detector (using a red light emitting diode light source) is used [9]. However, disadvantages include unstable colour development, a considerable salt error, temperature dependence and unsatisfactory performance at high phosphorus concentrations [10].

Interferences in the formation of the phosphomolybdenum blue complex include arsenate, silicate, chromium, copper, nitrite, nitrate and sulphide [11]. However, arsenate interferences can be eliminated by reducing As(V) to As(III) prior to measurement [6], e.g. by the addition of sodium thiosulphate [12]. The acid/molybdate ratio can be altered to enhance the selectivity for phosphate relative to silicate [4]. In addition, use of an appropriate extraction solvent, e.g. *n*-butanol, is an efficient way of eliminating interference from silicate [13].

The phosphorus determined in the filtered fraction using the above reaction is defined as "molybdate reactive" phosphorus (MRP) or dissolved reactive phosphorus (DRP). It has also been called soluble reactive phosphorus (SRP) and filterable reactive phosphorus (FRP). However, this method also determines acid labile phosphorus containing compounds (organic and condensed phosphorus species) which can lead to overestimation of free phosphate [3.6]. Similar problems have been reported in the determination of total reactive (unfiltered) phosphorus (TRP) [3]. Methods have been developed to minimise this overestimation including a critical timing technique ('the 6 second method') in which the acid strength is adjusted prior to the formation of the complex [14] and complexing excess molybdate with a citrate–arsenate reagent [15]. Phosphorus containing organic compounds and con-

densed phosphates can also be determined using the molybdate reaction following chemical, photochemical, thermal or microwave digestion (see Section 4).

2. Natural waters

Phosphorus concentrations in natural waters fluctuate with changes in physico-chemical conditions and biological activity. In chalk-based catchments, for example, phosphorus is influenced by seasonal fluctuations in pH, dissolved carbon dioxide and total dissolved calcium concentrations [16]. Hydrological conditions also play an important role in aquatic phosphorus concentrations. The majority of phosphorus transport to catchments, from both diffuse and point sources occurs during short periods of increased discharge (e.g. storm events) [17,18], which demonstrates the importance of high temporal resolution monitoring during such events. Submersible or field-based instrumentation is desirable for monitoring dissolved phosphorus because it eliminates the need for sample collection and storage and, although such instrumentation is available [19,20], it is not used on a routine basis. Therefore, a comprehensive and effective sampling, sample treatment and analysis protocol must be adopted in order to minimise the physical, chemical and biological processes that can alter the physico-chemical forms of phosphorus during storage.

2.1. Sampling protocol

It is essential that the scientific objectives (e.g. determining bioavailable phosphorus, measuring seasonal phosphorus loads), safety issues and budgetary constraints are clearly identified prior to undertaking any sampling programme. Having established the scope of the exercise, an essential requirement of any sampling protocol is for the sample to be representative of the body of water from which it originates. It is therefore essential to adopt a well-organized protocol, which retains, as closely as possible, the original composition of the water body of interest. The protocol should be kept as simple as possible while minimizing the possibility of contamination or interferences. In rivers and streams, for example, samples should be collected from the water column at a series of depths and cross-sectional locations as individual grab samples or through the use of automated samplers for time series acquisition. Monitoring stations can be constructed to provide high quality supporting data (e.g. pH, dissolved oxygen, temperature, turbidity) in a judicious fashion via data acquisition/telemetry technology. It is also vital to avoid boundary areas, e.g. at the confluence of streams or rivers and below sewage treatment works, unless their impact on the system is being investigated. Point source phosphorus contributions from sewage treatment works, for example, can have a major affect on the overall water quality of freshwater systems [21]. Globally, phosphorus loading into receiving waters still occurs even though tertiary treatment measures (e.g. based on the reduction of phosphate by precipitation with iron chloride) are being implemented in some countries [22]. Other water bodies pose additional complications and these must be considered when designing a sampling protocol. In lakes and reservoirs, representative sampling is often difficult due to environmental heterogeneity, both spatial and temporal (e.g. seasonal thermal stratification). In order to study biogeochemical cycling in stratified water bodies appropriate depth profiling is required. For a complete study high spatial resolution sampling at the sediment–water interface is also essential but is not discussed further in this paper.

Location and frequency must also be considered when designing a sampling protocol. Site selection will ultimately depend on the problem to be addressed and safety and accessibility are of paramount importance. The frequency of sampling, from continuous to seasonal, will depend on the scientific objectives but will often be constrained by cost. For example, the highest phosphorus loadings in rivers and streams are generally correlated with intense, short-term discharges during autumn and winter months, while the lowest loadings occur in the summer months when discharge is low and biological activity is high [23,24]. In-water processes that affect phosphorus concentrations that must also be considered include plant, algal and bacterial turnover, anthropogenic inputs (e.g. sewage effluent), matrix considerations (e.g. water hardness) and resuspension of bottom sediments from increasing river discharge [21,25].

Prior to any sampling campaign it is essential to adopt an efficient cleaning protocol for all sampling equipment and storage bottles and continue this throughout the study. The walls of sample containers, for example, are excellent substrates for bacterial growth and therefore rigorous cleaning of all laboratory ware is necessary. For phosphate determination, it is recommended that containers be cleaned overnight with a nutrient free detergent, rinsed with ultrapure water, soaked in 10% HCl overnight, and then rinsed again with ultrapure water [26]. Containers should be rinsed at least twice with the water of interest prior to sample collection. In addition, sampling blanks should be taken to monitor and control the sampling process.

2.2. Sample preservation and storage

The overall effectiveness of any sample preservation and storage protocol depends on various factors including the nature of the sample matrix, cleaning procedures for sample containers, container material and size, temperature, chemical treatment (e.g. addition of chloroform) and physical treatment (e.g. filtration, irradiation of sample and pasteurization) [27–29].

Preliminary treatment often involves filtration which differentiates between the dissolved phase (operationally defined as that fraction which passes through a 0.45 or 0.2 μm filter) and suspended matter (that fraction collected on the filter) [30]. It is essential that filtration is carried out im-

mediately after the sample is collected to prevent short-term changes in phosphorus speciation. Polycarbonate or cellulose acetate membrane filters are recommended for dissolved constituents in natural waters [31]. Filtration with a 0.2 μm filter is preferred as it removes the majority of bacteria and plankton that would otherwise alter dissolved phosphorus concentrations during storage [30]. It should be stressed however that some bacteria, as well as viruses, will pass through a 0.2 μm filter. As with sample containers, the filtration apparatus (including individual filters) must be cleaned prior to use with a similar acid wash/ultrapure water rinse procedure. The filtration procedure can be conducted under positive pressure or vacuum. However, excessive pressure gradients should be avoided as rupture of algal cells and the subsequent release of intracellular contents into the sample could occur. In samples of high turbidity it is important to minimise the sample loading to prevent clogging of filter pores.

Table 1 shows a summary of reported storage/preservation methods for phosphorus determination. Physical (i.e. refrigeration, freezing and deep-freezing) and chemical (i.e. addition of chloroform, mercuric chloride and acidification) preservation techniques have been used to help maintain the original phosphorus concentration during storage. It should be noted however that the use of chloroform is now discouraged in some countries because of toxicological risks. In addition, a variety of sample containers have been used including quartz, borosilicate glass, polyethylene, polypropylene, high-density polyethylene (HDPE) and polytetrafluoroethylene (PTFE).

For phosphorus determinations, however, it is difficult to select a generic treatment protocol due to the different effects of specific matrix characteristics (e.g., phosphorus concentration, hardness, salinity, dissolved organic matter and bacterial nutrient uptake) of the sampling location. In chalk catchments, for example, studies have shown that freezing samples is not the best treatment due to the possibility of phosphate being coprecipitated with calcite when thawing the samples [26,46]. Fig. 2a demonstrates this effect, showing an immediate (after 1 day) and continuing (up to 250 days) decrease in DRP concentration in samples analysed for phosphate after storage at −20 °C [26]. Storage at 4 °C is therefore recommended, together with the addition of chloroform to prevent biological growth. However, chloroform should not be used in samples with high organic matter content, as the release of cellular enzymes into the samples is possible [26]. Other studies have recommended immediate analysis after sampling [47] or analysis after a short storage period at 4 °C in the dark (maximum 48 h) [48–51].

In contrast to the extensive studies on phosphate stability during storage, the stability of dissolved organic phosphorus (DOP), as operationally defined, has not been widely studied. Fig. 2b–d show the stability of DOP (strictly this includes all acid hydrolysable phosphorus because acidic digestion conditions were used) from natural water samples (salinities 0, 14 and 32, respectively) over 32 days of storage. The DRP concentration on day 0 (1.17, 1.31 and 0.54 μM for salinities 0, 14 and 32, respectively) was subtracted from all results, which were based on sampling, autoclaving of sub-samples and storage of autoclaved and non-autoclaved sub-samples for subsequent analysis. They showed that there were no significant differences in DOP concentration if the samples were stored at −20 °C, autoclaved and analysed on the same day or if they were autoclaved immediately after collection and stored until analysed. The same trend (not shown) was also observed with phytic acid spiked (1.11, 1.50 and 0.45 μM for salinities 0, 14 and 32, respectively) standards and samples. These results suggest that storage at −20 °C is suitable for DOP determination but the final result is dependent on a reliable determination of the original DRP concentration. Freezing as a method for storage of unfiltered and filtered

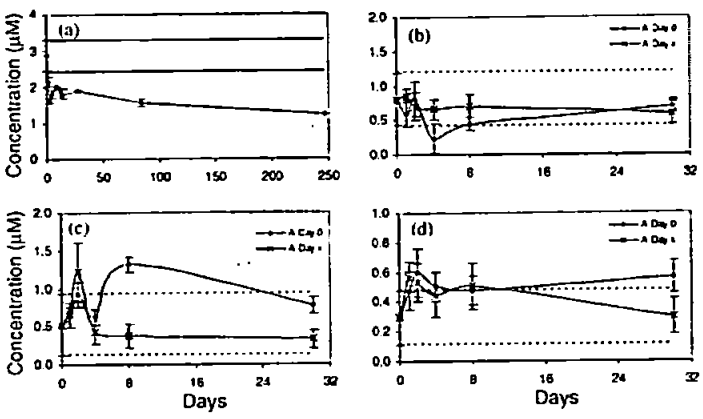


Fig. 2. Changes in the concentration of phosphorus species in natural water samples stored over time. (a) An immediate sharp decrease in DRP concentration in samples stored at −20 °C, followed by a gradual decrease over 250 days of storage. (b–d) The stability of DOP in natural water samples (salinities 0, 14 and 32, respectively) over 32 days of storage at −20 °C. A Day 0 are samples autoclaved on day 0 then stored until analysis, and a Day x are samples stored without treatment then autoclaved and analysed on day x. The dotted lines in each figure (solid lines in (a)) represent ±3 s of the measured DRP/DOP concentrations on day 0 (i.e. immediately after collection).

Table 1
Storage protocols for the determination of phosphorus species in environmental matrices (updated from [26] which was adapted from a table by Maher and Woo [75])

Phosphorus species	Matrix	Storage method	Maximum storage time	Comments	Ref.
FRP	Distilled, tap and lake water	Refrigerator (4 °C)	1 day	Polypropylene and polycarbonate containers suitable for storage. Glass containers sorbed phosphorus within 1–6 h	[32]
FRP	Standards added to rain water	Room temperature with $HgCl_2$ (0–50 mg L^{-1})	3 days	$HgCl_2$ interfered with method when ascorbic acid was used as a reducing agent	[33]
FRP	River water	−10, 4, 20 °C with/without thymol (0.01%), KF (0.01%), TBT (0.001%), H_2SO_4 (0.05 M) or $CHCl_3$ (5 mL L^{-1})	14 days	Samples showed no decrease in FRP if chloroform added and samples stored at 4 °C	[34]
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	[35]
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	[36]
FRP, TP	Tap, lake and river waters	Room temperature, 4 °C, with the addition of $HgCl_2$ (40 mg L^{-1}), H_2SO_4 (0.05 M), and chloroform	16 days	Chloroform at 4 °C was suitable for only 8 days. No significant decreases in concentration (up to day 16) were shown in samples with $HgCl_2$ stored at 4°C	[37]
FRP	Sea water	Frozen at −40 °C initially, then stored at −20 °C	147–210 days	FRP concentration decreased in samples stored longer than 4 months	[38]
TP, TDP, FRP and TRP	Lake water	Refrigerator (4 °C)	180 days	No change in TP in samples for up to 6 months	[39]
FRP	Stream water	Frozen at −16 °C	4–8 years	No significant change in FRP concentration	[40]
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 °C	[41]
FRP	Sea water	Pasteurization and stored at room temperature	18 months	FRP remained constant for 1 year. NH_4 losses after 3 days	[42]
FRP, TP	Stream water	Refrigerator (4 °C), H_2SO_4 (0.05 M), freezing with dry ice and subsequent analysis	8 days	Minimal change observed in highly concentrated (FRP > 1 mg L^{-1}) samples (1–3% loss after 8 days). 47% loss in FRP in lower concentrated samples	[43]
FRP	River water (chalk-based catchment), estuarine water (salinities of 0.5, 10 and 35)	Refrigerator (4 °C) with/without 0.1% (v/v) chloroform, −20 °C with/without 0.1% (v/v) chloroform, −80 °C without chloroform	247 days	For chalk-based samples, 4 °C with 0.1% (v/v) chloroform was the best treatment. Freezing is not recommended due to coprecipitation of inorganic phosphate with calcite	[26]
TP	River and canal water	Room temperature, refrigerator (4 °C) treatment to a pH of < 2 with H_2SO_4	28 days	No significant losses in TP concentration over the 28 day period for treated samples at 4 °C. No losses up to 7 days for room temperature (acidified) samples	[44]
FRP	Water extracts of poultry litter	Room temperature, freezing (−16 to −15 °C)	8 days	No significant losses in FRP concentration in samples stored at room temperature (up to 8 days). Freezing samples lowered concentration (up to 46%) for the 8 day period	[45]

samples for the determination of total and dissolved organic phosphorus has also been recommended by other workers [39,52–53].

3. Soils

Soil pre-treatment and storage can induce marked changes in the solubility of chemicals and therefore presents a critical control on subsequent analysis. This section focuses on phosphorus but it also has wider relevance for other elements. For example, water-extractable phosphorus is markedly influenced by even mild drying of soil. It has been known for some time that soil drying can render considerable concentrations of organic carbon soluble in water [54] and a similar effect was recently reported for phosphorus in a wide range of pasture soils from England and Wales [55]. In the latter study, 7 days air drying from approximate field moisture capacity at 30 °C increased concentrations of water-extractable organic phosphorus by up to 1900%. Organic phosphorus accounted for up to 100% of the solubilized phosphorus. This was at least partly derived from microbial cells, because a strong correlation existed between solubilized organic phosphorus and microbial phosphorus (Fig. 3). It has been reported that rapid rehydration can kill between 17 and 58% of soil microbes through osmotic shock and cell rupture [56] and the contribution of microbial lysis has been subsequently confirmed by direct bacterial cell counting in rewetted Australian pasture soils [57].

In addition to microbial lysis, the physical stresses induced by soil drying also disrupt organic matter coatings on clay and mineral surfaces [58], which may contribute to the solubilisation of both inorganic and organic phosphorus. Indeed, functional classification of water-extractable organic phosphorus from dry Australian pasture soils revealed similar proportions of microbially derived phosphate diesters and phytic acid from the non-biomass soil organic matter [59]. A similar mechanism probably occurs following freezing and thawing [60]. Such processes probably explain the increases in phosphorus extractable in bicarbonate following soil drying [61] because the high ionic strength of bicarbonate solution may reduce the degree of osmotic stress and associated lysis of viable cells compared to extraction with water [62]. The hypothesis that non-biomass organic phosphorus dominates in bicarbonate extracts is supported by the speciation of phosphorus in such extracts, which is dominated by phosphate monoesters and is, therefore, similar to the whole-soil organic phosphorus extracted in strong alkaline solution [63,64].

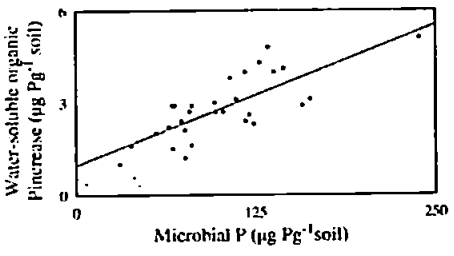


Fig. 3. The increase in water-soluble organic phosphorus after soil drying as a function of soil microbial phosphorus in a wide range of permanent pasture soils from England and Wales. Water-soluble phosphorus was determined by extracting soils at field moisture capacity with water in a 4:1 water:soil ratio for 1 h. Sub-samples were air-dried for 7 days at 30 °C and extracted in an identical manner. Adapted from [57].

The mechanisms by which soil drying could affect the solubility of non-biomass inorganic and organic phosphorus are poorly understood, but probably include both physical and chemical changes. Rapid rehydration of dry soils commonly causes aggregate breakdown [65], which increases the surface area for desorption by exposing surfaces and associated phosphorus protected within aggregates [66]. Such a process has been linked to increases in resin-extractable inorganic phosphorus following soil drying [67]. A more likely process is disruption of organic matter coatings on clay and mineral surfaces by the physical stresses induced during soil drying. This increases organic matter solubility and exposes formerly protected mineral surfaces, and has been attributed to increases in oxalate-extractable silica of up to 200% following drying of Swedish spodic B horizons [68]. Soil drying also increases the crystallinity of pure iron and aluminium oxides, which reduces the specific surface area and phosphorus sorption capacity of these minerals [69]. However, this is inconsistent with reports of increased sorption capacity of dried soils for phosphate and sulphate [70,71].

The effect of drying on phosphorus solubility does not appear to be consistent for all soils. In particular, phosphorus solubility in high organic matter soils may decrease following drying. For example, Schlichting and Leinweber [72] reported that phosphorus recovery from a German peat (pH 5.6) by a sequential fractionation procedure was markedly reduced by pre-treatment, including air-drying, freezing and lyophilization. The greatest reduction in phosphorus recovery followed lyophilization (phosphorus recovery was 75% when extracted fresh, compared with <50% from lyophilized samples) and even after storage at 4 °C for 3 weeks detectable changes were still observed.

The importance of specific artefacts that result from particular pre-treatments will vary depending on the study objectives. For example, assessment of plant-available phosphorus for fertilizer requirements is based on analysis of air-dried soils for practical reasons although field-fresh soils are needed to obtain meaningful data. This is impractical for most purposes, although refrigeration may be an acceptable alternative [58]. In this respect, there is a clear requirement for a detailed study of changes in phosphorus solubility during cold storage for several different soil types. Environmental soil phosphorus tests that involve water extraction routinely use air-dried soils and only measure inorganic phosphorus (e.g. [73,74]). The results of these tests will clearly vary depending on the moisture status of the soil prior to extraction and on the inclusion (or not) of organic phosphorus. If organic phosphorus is included in such tests,

the standardization of soil moisture prior to extraction will be necessary. For details of extraction procedures for soil organic phosphorus see Turner et al. (this issue).

4. Digestion techniques

Digestion techniques for environmental samples are necessary for the determination of total phosphorus (TP) and total dissolved phosphorus (TDP). This is because many of the phosphorus species present contain P–O–P, C–O–P and C–P bonds that need to be broken down to release phosphorus as phosphate, which can then be determined using molybdenum blue chemistry [4]. The digestion technique must also be able to release phosphorus from biological material, e.g. algal cells and plant detritus and adsorbed/occluded P from sediments [75]. Traditional methods of digestion for natural water samples include fusion, dry ashing, perchloric acid, sulphuric acid–nitric acid and boiling on a hot plate, with more recent methods generally using autoclaving, UV photo-oxidation and microwave heating [75]. UV photo-oxidation can be used for organic phosphorus compounds in marine and freshwaters [47,76,77] but condensed polyphosphates present in the sample will not be broken down by UV photo-oxidation alone [2,3,78,79] and also need to be heated to 90–120 °C in the presence of acid [75]. To ensure that all polyphosphates present in the sample are decomposed, either boiling with HCl or potassium peroxydisulfate after UV irradiation is therefore recommended [80]. McKelvie et al. used an on-line UV photo-oxidation flow injection (FI) technique and found that results were comparable with a batch peroxydisulfate method [81].

Autoclaving methods are generally straightforward, give reproducible results and use sealed vessels that are less prone to contamination [75,82–84]. The following section is therefore a summary of different autoclaving techniques, combined with peroxydisulfate in either an acidic or alkaline media, for the determination of phosphorus in natural waters, soil solutions and sediments (see Table 2). Most methods described in Table 2 are based on spectrophotometric detection but ICP-MS and ICP-AES have, in recent years, been used to determine phosphorus in agricultural runoff waters and soils and results were comparable with spectrophotometric methods [128,129]. In addition, microwave digestion combined with ICP-MS detection has been used to determine phosphorus in marine environmental samples and plant leaves with good recoveries [130–132]. However microwave heating for batch sample digestion and in FI systems with spectrophotometric detection for on-line TDP and TP digestion [3] is less widely used than UV photo-oxidation or autoclaving.

4.1. Autoclaving

4.1.1. Alkaline peroxydisulfate

Menzel and Corwin first used autoclaving with peroxydisulfate in 1965 for the digestion of seawater samples [88]. Koroleff developed an alkaline peroxydisulfate alternative in 1969 [102], which was then slightly modified [101] and simplified by introducing a borate buffer [85]. This enabled the simultaneous determination of TP and total nitrogen (TN), as nitrogen bonds are only hydrolysed/oxidised in alkaline media [98]. Using a borate buffer, the pH is alkaline (ca. 9.7) at the start of the digestion process and becomes acidic (pH 4–5) as the sodium hydroxide decomposes [78,90,98]. Hosomi and Sudo also reported that pH change was important and in their method the pH decreased from 12.8 to 2.0–2.1 to ensure that even condensed polyphosphates were digested [92].

The alkaline method has also been used for particulate material but with relatively poor recoveries [133]. For example orchard leaves gave recoveries of 80–90% for TP and TN [91]. Higher recoveries can be obtained by decreasing the ratio of sample to peroxydisulfate [92]. Alkaline digestion of model phosphorus compounds has been found to be efficient for turbid water samples [125–127] although the concentration of suspended particulate material needs to be diluted to $<150\ mg\ L^{-1}$ and difficulties can arise when this material is of soil origin rather than biological origin, e.g. algal cells and plant detritus. The alkaline method has therefore been used to determine TP in turbid lake waters and suspensions of particulate material [127].

Alkaline peroxydisulfate autoclaving, rather than acid peroxydisulfate, is recommended for the digestion of marine waters. This is because in the acid method, peroxydisulfate oxidises the chloride in seawater to free chlorine, thus reducing the oxidising power of the peroxydisulfate [104]. It is also recommended for the simultaneous determination of TP and TN.

4.1.2. Acid peroxydisulfate

An acid peroxydisulfate method developed by Gales et al. [134] has been adopted by the US Environmental Protection Agency [135]. Eisenreich et al. simplified the method [96] and various modifications of this approach are now used to digest different types of samples such as soil solutions, natural waters and river water [18,97,121]. The alkaline peroxydisulfate method for soil extracts is only appropriate if the total organic carbon concentration is $<100\ mg\ L^{-1}$ and manganese is $<1\ mg\ L^{-1}$. Above this manganese concentration, coloured solutions or precipitates are formed, which interfere with the digestion step [111]. This interference is avoided when using acid peroxydisulfate and solutions are colourless after digestion [84].

Pote et al. described standard methods for the determination of TP and TDP using sulphuric acid–nitric acid and peroxydisulfate digestions [136] and recommended the use of sulphuric acid–nitric acid digestion to achieve good recoveries for most samples. However this digestion method can be potentially dangerous if salts precipitate during digestion [89] and is less easy to control than the peroxydisulfate method [84,122]. Rowland and Haygarth compared a mild peroxydisulfate method to the more rigorous sulphuric acid–nitric

Table 2
Acidic and alkaline peroxydisulfate autoclave digestion methods

Matrix	Digestion reactant	Digestion time	Digestion temperature (°C)	pH	Model compounds[a]	Comments	Ref.
Drainage waters	Digestion reagent: 5 g $K_2S_2O_8$ and 5 mL 4.5 M H_2SO_4 in 100 mL distilled deionised water. 4 mL reagent added to 50 mL sample	30 min	115	Not reported	Not reported	Same method as [85]	[86]
Drainage waters	0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4 added to 20 mL sample	1 h	120	Not reported	Not reported	Same method as [84]	[87]
Estuarine waters	8 mL of 5% $K_2S_2O_8$ added to 50 mL seawater	1 h	120	Final pH 1.5–1.8	Orthophosphate, phenylphosphoric acid, phenylphosphorous acid	Same method as [88], but autoclaving time was increased from 30 min to 1 h. Quantitative recovery for model compounds at the 50 μg P level	[89]
Fresh and seawater	Acidic peroxydisulfate digestion reagent: 5 g $K_2S_2O_8$ and 5 mL 4.5 M H_2SO_4 in 100 mL distilled deionised water. 4 mL reagent added to 50 mL sample. Alkaline peroxydisulfate digestion reagent: 5 g $K_2S_2O_8$ and 3 g H_3BO_3 in 100 mL 0.375 M NaOH. 5 mL reagent added to 50 mL sample	30 min	115	For alkaline method, initial pH ca. 9.7, final pH 4–5	Model compounds added to demineralised water and seawater:2-AEP (108, 77, 108, 88%), PTA (100, 70, 101, 95%), 5′-GMP-Na_2 (99, 93, 100, 94%), PC (98, 37, 99, 96%), FMN (99, 99, 100, 97%), G-6-P-Na (100, 95, 101, 92%), AMP (99, 94, 100, 93%), RP (100, 94, 103, 95%), PEP-3CHA (100, 100, 101, 101%), β-GLY (99, 100, 100, 96%)	Recoveries in parentheses are in the order: acidic demineralised water, acidic seawater, alkaline demineralised water, alkaline seawater. Acidic and alkaline peroxydisulfate methods [85] compared to continuous flow UV irradiation and high temperature combustion. Alkaline peroxydisulfate method recommended for marine waters	[90]
Fresh waters	Digestion reagent: 40 g $K_2S_2O_8$ and 9 g NaOH in 1 L distilled water. 5 mL reagent added to 10 mL sample	1 h	120	Initial pH 12.8, final pH 2.0–2.1	National Bureau of Standard Reference Material 1571 orchard leaves (98%), National Institute of Environmental Studies (NIES) Reference Material No. 1 pepper bush (96%), NIES Reference Material No. 2 pond sediment (100%), NIES Reference Material No. 3 chlorella (100%) all of concentration 50 mg L^{-1}. Model compounds:5′-ATP-Na_2 (99–100%), 5′-ADP-Na_2 (98%), TSPP (99–100%), SHMP (94–97%), STP (96–97%), G-6-P-K_2 (99–102%)	Analysed for TN and TP. Obtained higher recoveries for orchard leaves than [91]	[92]
Fresh waters	1 g $K_2S_2O_8$ and sufficient H_2SO_4 to make the sample 0.15 M acid	2 h	120	Not reported	Not reported		[93]
Lake waters	'Strong' acid: 25 mL 18 M H_2SO_4 and 1 mL 18 M HNO_3 in 1 L deionised water. 1 mL 'strong' acid and 2.5 mL aqueous 4% (w/v) $K_2S_2O_8$ added to 25 mL sample	30 min	Not reported, however in the UV digestion, sample maintained at 85 °C in the silica coil	Not reported	Dipotassium hydrogenphosphate (100%), STP (100%), AMP (100%)	Compared UV digestion to autoclaving. Recoveries for lake water samples were 100% for the peroxydisulfate digestion and 97% for the UV digestion	[94]

Table 2 (*Continued*)

Matrix	Digestion reactant	Digestion time	Digestion temperature (°C)	pH	Model compounds[a]	Comments	Ref.
Lake, river and pond waters, raw sewage	Digestion reagent: 55 mL H_2SO_4 and 60 g $K_2S_2O_8$ in 1 L solution. 2.5 mL reagent added to 35 mL sample	1 h	Not reported	Not reported	G-1-P-K_2 (97.5%), G-6-P-K_2 (105%), DNA (sodium salt) (115%), AMP (95%), 5′-ADP-Na_2 (102.5%), SOP (100%), β-GLY (107.5%), TSPP (62.5%), STP (110%), SHMP (100%), disodium hydrogen orthophosphate (97.5%)	Autoclave method was compared to the hot-plate $H_2SO_4/K_2S_2O_8$ digestion. Autoclave method gave more precise values for model compounds than the hot plate procedure	[95]
Natural waters	Digestion reagent: 0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4. 1 mL reagent added to 20 mL sample	45 min	121 °C	Not reported	G-1-P (101.0%), G-6-P (103.1%), ATP (101.6%), NPP (101.9%), cAMP (101.8%), α-GLY (102.3%), myo-inositol 2-monophosphate (97.4%), PTA (85.6%), 2-AEP (99.2%), TSPP (99.5%), STP (97.7%), trisodium trimetaphosphate (98.8%), KHP (99.1%)	Method modified from [96]	[97]
Natural waters	Acidic peroxydisulfate digestion reagent: 5 g $K_2S_2O_8$ and 5 mL 4.5 M H_2SO_4 in 100 mL distilled deionised water. 0.8 mL digestion reagent added to 10 mL sample. Alkaline peroxydisulfate digestion reagent: 50 g $K_2S_2O_8$, 30 g H_3BO_3 and 350 mL NaOH in 1 L distilled deionised water. 1.3 mL digestion reagent added to 10 mL sample	30 min	120 °C	For alkaline method, initial pH ca. 9.7, final pH 4-5	NPP, α-GLY, G-6-P, tripolyphosphate, trimetaphosphate, ATP, 5′-GDP, 2-AEP. Recoveries shown in a figure, so precise values cannot be given. In general, recoveries ca. >58% for acidic method and ca. >26% for alkaline method	Compared acidic peroxydisulfate [85] and alkaline peroxydisulfate [98] autoclaving methods with magnesium nitrate high-temperature oxidation, magnesium peroxydisulfate high-temperature oxidation, and UV oxidation. Magnesium nitrate high-temperature oxidation was found to be the best method	[78]
Orchard leaves and aufwuchs	Digestion reagent: 13.4 g $K_2S_2O_8$ and 6 g NaOH in 1 L to give 200 mg peroxydisulfate per 15 mL aliquot. Other levels of peroxydisulfate also used (300, 400 and 500 mg)	1 h	100–110	Initial pH 12.00 for orchard leaf samples, final pH 2.5. Initial pH 12.8 for aufwuchs samples, final pH 3.7	National Bureau of Standards reference material 1571 (orchard leaf) (86.9–88.7% using 500 mg peroxydisulfate), and aufwuchs (93.6% using 300 mg peroxydisulfate, and 101.4% using 400 mg peroxydisulfate)	Analysed for TN and TP. Maximum recovery for orchard leaf when 500 mg peroxydisulfate was used, and 300 or 400 mg peroxydisulfate for aufwuchs	[91]
Pond water	Acidic peroxydisulfate digestion: 0.5 g $K_2S_2O_8$ and 1 mL H_2SO_4 solution (300 mL conc. H_2SO_4 in 1 L distilled water) added to 50 mL sample. Alkaline peroxydisulfate digestion: 5 mL 0.075 N NaOH and 0.1 mg $K_2S_2O_8$ added to 10 mL sample. After digestion, 1 mL borate buffer (61.8 g H_3BO_3 and 8 g NaOH in 1 L distilled water) added	30 min	110	Not reported	Water samples spiked with 0.2 mg L^{-1} KHP. Recoveries for acidic method were 88–113%, and for the alkaline method 85–112%	Acidic and alkaline peroxydisulfate methods same as [99]	[100]

Table 2 (*Continued*)

Matrix	Digestion reactant	Digestion time	Digestion temperature (°C)	pH	Model compounds[a]	Comments	Ref.
River water	Digestion reagent: 0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4. 1 mL added to 20 mL sample	45 min	121 °C	Not reported	Not reported	Method modified from [96]	[18]
River water	Digestion reagent: 20 g $K_2S_2O_8$ and 3 g NaOH in 1 L distilled deionised water. 5 mL reagent added to 5 mL sample	30 min	120 °C	Initial pH 12.57, final pH 2.0	KHP (99.6%), TSPP (97.2%), STP (99.2%), β-GLY (96.5%), SHMP (97.6%), G-1-P (99.5%), AMP (100.8%), ADP (98.9%), ATP (98.1%)	Results from this method were an improvement on the alkaline oxidation method for TN and TP of [101], which was in turn a modified method from [102]	[103]
Seawater	Two concentrations of $K_2S_2O_8$ added (4 and 40 mg mL^{-1}) to 10 mL sample acidified with sulphuric acid to pH 3	90 min	125	pH 3	Not reported	Three methods compared: autoclaving (acidic peroxydisulfate method based on [85]), UV irradiation and sequential use of both. The latter method gave the best recoveries	[104]
Seawater	8 mL of 5% $K_2S_2O_8$ added to 50 mL seawater	30 min	120	Final pH 1.5–1.8	PFA (96.5%), 1-AEP (85.5%), 2-AEP (81.2%)	Compared their nitrate oxidation method with peroxydisulfate oxidation method from [88]	[105]
Seawater	Digestion reagent: 50g $K_2S_2O_8$, 30 g H_3BO_3. 350 mL 1 M NaOH in 1 L deionised water. 4 mL reagent added to 30 mL sample	30 min	110–115	Initial pH 9.7, final pH 5–6	KHP (0.25–7 μM)	Alkaline peroxydisulfate method for TP and TN based on [85]	[98]
Seawater	8 mL of 5% $K_2S_2O_8$ added to 50 mL seawater	30 min	120	Final pH 1.5–1.8	lecithin (101%), PC (98%), AMP (99%), zooplankton (100%)	Recoveries of model compounds relative to sulphuric acid-hydrogen peroxide digestion [106]	[88]
Sediments and soils	1 mL 5.5 M H_2SO_4, 0.4 g $K_2S_2O_8$ and 1 mL distilled deionised water added to 10–50 mg sample	1 h	130	Not reported	Not reported	Acid peroxydisulfate digestion compared to perchloric acid digestion	[107]
Sewage	Digestion reagent: 9 g NaOH and 40 g $K_2S_2O_8$ in 1 L distilled deionised water. 2 mL digestion reagent added to 10 mL sample	90 min	120	Not reported, however KCl/acetate buffer pH 4.5	Sodium dihydrogen phosphate (93% using 0.15 M KCl/acetate), STP (85% using 0.4 M KCl/acetate), TSPP (96% using 0.4 M KCl/acetate)	Anion exchange chromatography used to separate ortho- and poly-phosphates using either 0.15 or 0.4 M KCl/acetate as the eluting buffer. No polyphosphates detected in raw sewage samples	[108]
Soil extracts	Digestion reagent: 0.39 M $K_2S_2O_8$ and 0.6 M NaOH. 2 mL reagent added to 8 mL sample	1 h	120	Not reported	Not reported	Same method (La Chat method 30-115-001-1-B) as [109]	[110]
Soil extracts	Digestion reagent: 13.4 g $K_2S_2O_8$ dissolved in 1 L 0.3 M NaOH. 15 mL reagent added to 10 mL sample. Added 1.5 mL 0.3 M HCl and made up to 50 mL after autoclaving	30 min	110	pH 2	KHP, PTA dodeca sodium salt (99% for 0.1 mg L^{-1}, and 106% for 1.0 mg L^{-1})	Analysed for TN and TP. PTA dissolved in different extractants: water, 0.1 M $CaCl_2$, and 0.2 M H_2SO_4, and recoveries were comparable. Alkaline peroxydisulfate method appropriate for soil extracts when concentration of total organic carbon <100 mg L^{-1}	[111]

Table 2 (*Continued*)

Matrix	Digestion reactant	Digestion time	Digestion temperature (°C)	pH	Model compounds[a]	Comments	Ref.
Soil leachate	0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4 added to 20 mL sample	1 h	120	Not reported	Not reported	Same method as [84]	[112–116]
Soil leachate	8 mg $K_2S_2O_8$ and 50 μL 0.5 M H_2SO_4 added to 1 mL sample	1 h	120	Not reported	KHP (101%), PTA (76%), TSPP (95%), STP, 1-AEP (86%), G-6-P-Na (84%), 5′-ATP-Na_2 (69%)	Preconcentration and separation method for trace P compounds using a scaled down version of [84]	[117]
Soil solutions	Digestion reagent: 0.05 M H_2SO_4 and 16 g L^{-1} $K_2S_2O_8$. 1 mL reagent added to 1 mL sample	30 min	110	Not reported	Not reported		[118]
Soil solutions	Digestion reagent: 50 mg $K_2S_2O_8$ and 0.1 mL 5.5 M H_2SO_4 added to 1 mL sample. After digestion, solutions diluted to 10 mL with deionised water	1 h	120	Not reported	KHP, PTA (93.2–95.0% in concentration range 3.23–32.26 μM)	Acid peroxydisulfate digestion compared to sulphuric–perchloric acid, nitric acid, and nitric–perchloric acid digestion. Better recoveries were found for PTA using sulphuric-perchloric acid and acid peroxydisulfate digestion methods	[119]
Soil solutions	Digestion reagent: 13.4 g $K_2S_2O_8$ dissolved in 1 L 0.3 M NaOH. 15 mL reagent added to 10 mL sample. Added 1.5 mL 0.3 M HCl and made up to 50 mL after autoclaving	30 min	110	pH 2	Not reported	Same method as [111]	[120]
Soil solutions	0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4 added to 20 mL sample	45 min	121	Not reported	Not reported	Method modified from [96]	[121]
Soil solutions	0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4 added to 20 mL sample	1 h	120	Not reported	PTA (89%), G-6-P-Na (89%), tetra-potassium pyrophosphate (102%), 5′-ATP-Na_2 (96%), AMP (96%), KHP	Acidic method compared to peroxide-Kjeldahl, and nitric acid-sulphuric acid digestions [122]. Acidic peroxydisulfate method found to be the best method	[84]
Surface runoff	0.5 g $K_2S_2O_8$ and 1 mL H_2SO_4 solution (300 mL conc. H_2SO_4 in 1 L distilled water) added to 50 mL sample	30 min	110	Not reported	Not reported	Same method as peroxydisulfate method in [99]	[123]
Surface runoff	$K_2S_2O_8$ and H_2SO_4	30 min	120	Not reported	Not reported		[124]
Turbid lake and river waters	Optimum digestion reagent: 0.27 M $K_2S_2O_8$ and 0.24 M NaOH. 2 mL reagent added to 10 mL sample	1 h	120	Final pH 2	NIES No 3 Chlorella (99–101% up to 100 μg PL^{-1}) and No 2 Pond sediment (98–104% up to 60 μg PL^{-1}, and 88% at 100 μg PL^{-1}). Model compounds added to distilled and lake water: KHP, G-6-P (113%), PTA (101%), α-GLY (108%), PEP (103%), 2-AEP (104%), PFA (106%), ω-phosphonyl ethanolamine (109%), SHMP (114%), aluminium phosphate (23%)	Compared alkaline peroxydisulfate autoclaving method to microwave and hot-plate digestion and Kjeldahl digestion for TN and TP. Results showed that all methods used were suitable for turbid lake samples when suspended material is of biological origin	[125]

Table 2 (*Continued*)

Matrix	Digestion reactant	Digestion time	Digestion temperature (°C)	pH	Model compounds[a]	Comments	Ref.
Turbid lake and river waters	Optimum digestion reagent: 0.27 M $K_2S_2O_8$ and 0.24 M NaOH, 2 mL reagent added to 10 mL sample	1 h	120 °C	Final pH 2	NIES No 3 Chlorella (99–101% up to 100 μg PL^{-1}) and No 2 Pond sediment (98–104% up to 60 μg PL^{-1}, and 88% at 100 μg PL^{-1}). Model compounds added to distilled and lake water: KHP (93–99%), PTA (93–106%), 2-AEP (93–101%), α-GLY (94–102%), PFA (93–105%), *O*-phosphonylethanol (91–106%), PEP (93–117%)	Compared alkaline peroxydisulfate autoclave method to microwave digestion, and similar results were found	[126]
Turbid lake waters	Digestion reagent: 9 g NaOH, and 40 g $K_2S_2O_8$ in 1 L water, 2 mL reagent added to 10 mL sample	1 h	120 °C	Not reported	NIES No. 3 Chlorella (94–107% up to 100 μg PL^{-1}, and 90% at 250 μg PL^{-1}) and No 2 Pond sediment (92–109% up to 100 μg PL^{-1}, and 88% at 250 μg PL^{-1}). Model compounds added to lake water: KHP (99%), STP (96%), AMP (94%), β-GLY (103%)	Compared alkaline peroxydisulfate method to nitric acid–sulphuric acid digestion method [99]. Results showed no significant difference between the two methods	[127]
Water (overland flow)	Digestion reagent: 0.39 M $K_2S_2O_8$ and 0.6 M NaOH, 2 mL reagent added to 8 mL sample	1 h	120 °C	Not reported	Not reported		[109]

[a] With recoveries given in parentheses when reported.

acid method [122] for soil solutions and leachates. The latter method gave erratic recoveries and was more prone to contamination due to the open digestion vessels used [84]. Peroxydisulfate autoclaving is also safer than perchloric acid digestion [107,137]. The acid peroxydisulfate method generally gives good recoveries for model compounds and is simple and easy to use and is therefore recommended for TP and TDP determinations in natural waters and, particularly, soil solutions.

4.2. Model compounds

It is advisable to test the efficiency of any digestion method using a range of model phosphorus containing compounds that reflect different chemical bonds and stabilities and are representative of naturally occurring compounds (see Table 3). The majority of relevant compounds contain C–O–P and/or P–O–P bonds. Few compounds reported in the literature contain C–P bonds, which are very resistant to oxidation and hydrolysis [138].

Phosphonates are refractory organic phosphorus compounds and can be released into seawater from biological sources [78,90,139], and have been detected in soils [140] and soil leachate [117]. As phosphonates contain a strong C–P bond that is resistant to acid hydrolysis [139], they are useful compounds for recovery studies [78,90,97,125,126,139]. Condensed inorganic (e.g. sodium tripolyphosphate) and organic (e.g. adenosine-5′-triphosphate) phosphates and cocarboxylase [141] have also been shown to be resistant to UV irradiation alone [79]. With acid or alkaline peroxydisulfate autoclaving, however, these compounds have been successfully broken down [97,103,125,126].

Inositol phosphates are an important class of naturally occurring organic phosphorus compounds [142]. Phytic acid, for example, is one of the more resistant compounds to hydrolysis and is also one of the most refractory organic phosphorus compounds found in soils [75,119,141]. Other organic phosphorus compounds found in soil leachate and runoff are the sugar phosphorus compounds, e.g. D-glucose-1-phosphate and D-glucose-6-phosphate, which are labile [117]. Organic condensed phosphates, e.g. adenosine-5′-triphosphate and adenosine-5′-diphosphate are also important as they originate from all living systems, e.g. algae, bacteria, fungi, insects, plant and animal tissues [117].

It is therefore recommended that model compounds selected for digestion studies should include one with a P–O–P bond (e.g. sodium tripolyphosphate), a refractory C–O–P compound (e.g. phytic acid), a labile C–O–P compound (e.g. D-glucose-1-phosphate or D-glucose-6-phosphate), a refractory C–P compound (e.g. 2-aminoethylphosphonate), and a compound containing C–O–P and P–O–P bonds (e.g. adenosine-5′-triphosphate). Orthophosphate (e.g. as potassium dihydrogen orthophosphate) should also be used in all recovery studies as a method control [90]. One should also be aware that specific matrices may require additional model compounds. For example, acid soils and sediments may well contain phosphorus associated with iron or alu-

Table 3
Model compounds used in autoclave based digestion methods

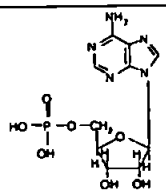
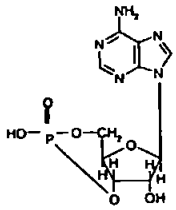
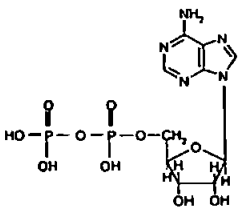
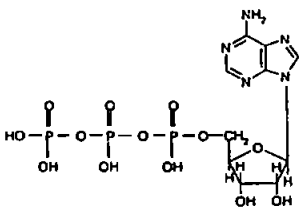
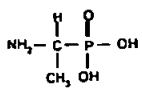
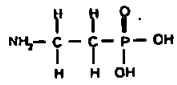
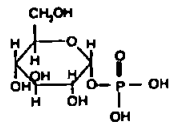
Model compound	Synonyms	Abbreviation used in text	Chemical formula	Structural formula
Adenosine-5′-monophosphate	Adenosine-5′-monophosphoric acid; 5-adenylic acid; adenosine phosphate; tert-adenylic acid; ergadenylic acid	AMP	$C_{10}H_{14}N_5O_7P$	
Adenosine-3′,5′-cyclic monophosphate	Adenosine-3′,5′-cyclophosphoric acid; cyclic AMP; 3′,5′-cyclic AMP	cAMP	$C_{10}H_{12}N_5O_6P$	
Adenosine-diphosphate		ADP	$C_{10}H_{15}N_5O_{10}P_2$	
Adenosine-5′-diphosphate (sodium salt)		5′-ADP-Na_2	$C_{10}H_{13}N_5O_{10}P_2Na_2$	Similar to ADP
Adenosine-5′-triphosphate		ATP	$C_{10}H_{16}N_5O_{13}P_3$	
Adenosine triphosphate disodium	Adenosine 5′-(tetrahydrogen triphosphate) disodium salt; adenosine 5′-triphosphate, disodium salt; adenosine 5′-triphosphate, disodium salt hydrate	5′-ATP-Na_2	$C_{10}H_{14}N_5O_{13}P_3Na_2$	Similar to ATP
1-Aminoethylphosphonate	1-Aminoethylphosphonic acid	1-AEP	$C_2H_8NO_3P$	
2-Aminoethylphosphonate	2-Aminoethylphosphonic acid	2-AEP	$C_2H_8NO_3P$	
Glucose-1-phosphate	Glucose-1-phosphoric acid	G-1-P	$C_6H_{13}O_9P$	

Table 3 (*Continued*)

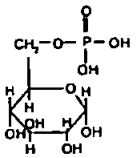
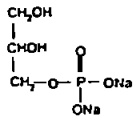
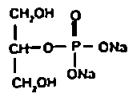
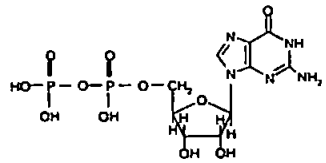
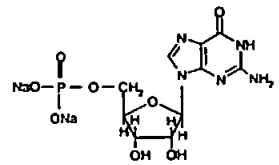
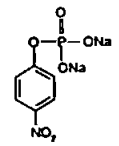
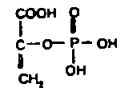
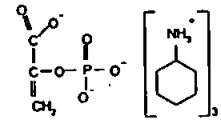
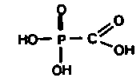
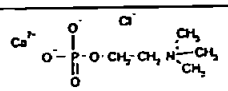
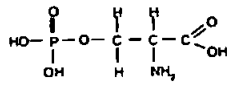
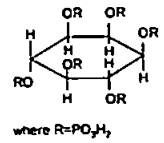
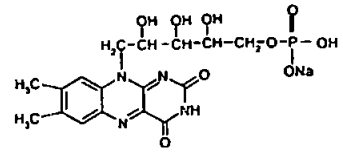
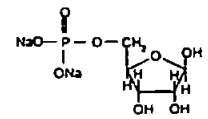
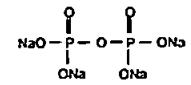
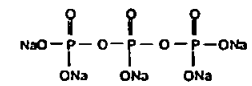
Model compound	Synonyms	Abbreviation used in text	Chemical formula	Structural formula
Glucose-1-phosphate dipotassium salt	Glucose-1-phosphoric acid (dipotassium salt)	G-1-P-K_2	$C_6H_{11}O_9PK_2$	Similar to G-1-P
Glucose-6-phosphate	Glucose-6-phosphoric acid	G-6-P	$C_6H_{13}O_9P$	
Glucose-6-phosphoric acid (dipotassium salt)	α-D-Glucose-6-phosphoric acid dipotassium salt	G-6-P-K_2	$C_6H_{11}O_9PK_2$	Similar to G-6-P
Glucose-6′-phosphate sodium salt		G-6-P-Na	$C_6H_{12}O_9PNa$	Similar to G-6-P
DL-α-Glycerophosphate disodium salt	rac-Glycerol 1-phosphate disodium salt; DL-α-glycerophosphate	α-GLY	$C_3H_7O_6PNa_2$	
β-Glycerophosphate disodium salt hydrate	Glycerol 2-phosphate disodium salt hydrate; sodium β-glycerophosphate	β-GLY	$C_3H_7O_6PNa_2$	
Guanosine 5′-diphosphate		5′-GDP	$C_{10}H_{15}N_5O_{11}P_2$	
Guanosine-5′-monophosphate disodium hydrate		5′-GMP-Na_2	$C_{10}H_{12}N_5O_8PNa_2$	
4-Nitrophenyl phosphate	*p*-Nitrophenyl phosphate	NPP	$C_6H_4NO_6PNa_2$	
Phospho(enol) pyruvate		PEP	$C_3H_5O_6P$	
phosphoenolpyruvic acid tri(cyclohexylamine) salt		PEP-3CHA	$C_3H_2O_6P(C_6H_{11}NH_3)_3$	
Phosphonoformate	Phosphonoformic acid	PFA	CH_3O_5P	

Table 3 (*Continued*)

Model compound	Synonyms	Abbreviation used in text	Chemical formula	Structural formula
Phosphoryl choline chloride calcium salt tetrahydrate	Phosphocholine chloride calcium salt tetrahydrate; calcium phosphorylcholine chloride	PC	$C_5H_{13}NO_4PCaCl{\cdot}4H_2O$	
Phosphoserine		SOP	$C_3H_8NO_6P$	
Phytic acid	Myo-inositol hexakis (dihydrogen phosphate); inositol hexaphosphoric acid	PTA	$C_6H_{18}O_{24}P_6$	 where R=PO_3H_2
Riboflavine-5′-monophosphate sodium salt	Riboflavin 5′-phosphate; FMN-Na	FMN	$C_{17}H_{20}N_4O_9PNa$	
Ribose-5-phosphate disodium salt dihydrate	D-Ribofuranose 5-phosphate	RP	$C_5H_9O_8PNa_2$	
Tetrasodium pyrophosphate	Sodium pyrophosphate; pyrophosphoric acid tetrasodium salt; diphosphoric acid, tetrasodium salt	TSPP	$Na_4O_7P_2$	
Sodium tripolyphosphate	Pentasodium tripolyphosphate dihydrate; sodium triphosphate; sodium polyphosphate; triphosphoric acid pentasodium anhydrous	STP	$Na_5P_3O_{10}$	
Sodium hexametaphosphate	Sodium metaphosphate; metaphosphoric acid, hexasodium salt; sodium polymetaphosphate	SHMP	$(NaPO_3)_n$	

minium phases, which are relatively resistant to oxidative dissolution [75]. . .

4.3. Recovery studies using alkaline and acidic peroxydisulfate autoclaving

Typical phosphorus recoveries for a range of model compounds, digested using alkaline and acid peroxydisulfate autoclaving, are shown in Fig. 4. The alkaline peroxydisulfate digestion method can be used for the simultaneous determination of TP and TN [85]. This was chosen because the borate buffer ensures that the pH is initially alkaline, to break down nitrogen containing bonds, and becomes acidic during the digestion process to break down phosphorus containing bonds. An amount of 5 mL of digestion reagent (5 g potassium peroxydisulfate and 3 g boric acid dissolved in 100 mL 0.375 M sodium hydroxide) was added to 50 mL sample. The samples were then autoclaved for 30 min a 121 °C. Model compounds chosen were phytic acid, sodium tripolyphosphate and adenosine-5′-triphosphate, and were therefore representative of a refractory C–O–P compound, a P–O–P compound and a C–O–P and P–O–P bond containing compound, respectively. Recoveries were 89 ± 13% for phytic acid, 100 ± 13%

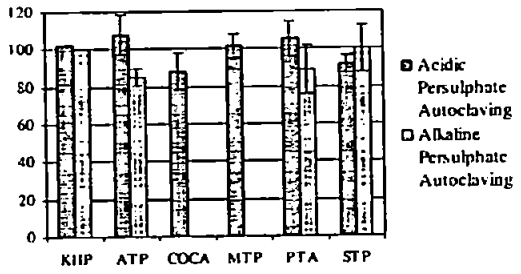


Fig. 4. Comparison of recoveries for a selection of model compounds using acidic and alkaline peroxydisulfate (40 g L^{-1}) autoclave digestions. KHP: potassium dihydrogen orthophosphate; ATP: adenosine-5′-triphosphate; COCA: cocarboxylase; MTP: methyltriphenylphosphonium bromide; PTA: phytic acid; STP: sodium tripolyphosphate. Error bars show ±3 standard deviations.

for sodium tripolyphosphate and 85 ± 4% for adenosine-5′-triphosphate.

The acid peroxydisulfate digestion method used was based on the method of Haygarth et al. [121]. One mL of 0.5 M sulphuric acid and 0.15 g potassium peroxydisulfate was added to 20 mL sample, and autoclaved for 45 min at 121 °C. The same compounds were used, plus two additional compounds that were not used in any of the autoclave methods listed in Table 2, but have been used in UV digestion studies, namely cocarboxylase containing C–O–P and P–O–P bonds [141] and methyltriphenylphosphonium bromide containing C–P bonds [143]. Recoveries were relatively low: adenosine-5′-triphosphate (74 ± 7%), cocarboxylase (68 ± 17%), methyltriphenylphosphonium bromide (93 ± 6%), phytic acid (60 ± 32%) and sodium tripolyphosphate (95 ± 4%). When the concentration of peroxydisulfate was increased from 8 to 40 g L^{-1} [81] however recoveries were greatly improved for adenosine-5′-triphosphate (108 ± 11%), cocarboxylase (88 ± 10%), methyltriphenylphosphonium bromide (102 ± 6%), phytic acid (105 ± 10%), and sodium tripolyphosphate (92 ± 5%). Peroxydisulfate concentration is the most important parameter, rather than digestion time or temperature, for improving recoveries, particularly for seawater samples [104].

4.4. Enzymatic degradation

Fig. 1 shows that DOP and total organic phosphorus (TOP) can be determined by difference following complete digestion, e.g. by autoclaving the sample (see Section 4.1). It is however desirable to be able to quantify specific organic compounds. To do this a more selective approach to digestion is required, such as the use of phosphate cleaving enzymes. This section therefore considers the use of acid and alkaline phosphatases and the particular sub-class of phytases.

Phosphatases belong to the class of enzymes called hydrolases [144] and their subclasses are alkaline phosphatase (EC.3.1.3.1) and acid phosphatase (EC.3.1.3.2). They hydrolyse phosphate monoesters to produce an alcohol and orthophosphate. Phosphatases play a key role in metabolic reactions such as the synthesis of organic phosphate compounds (transphosphorylation) and transport across cell membranes [145] and they have been isolated from a variety of sources. Alkaline phosphatase is the most studied phosphomonoesterase and has been isolated from, e.g. *Escherichia coli* [146,147]. Acid phosphatases show broad selectivity towards phosphomonoesters and have also been isolated from *E. coli* [146].

Strickland and Parsons established a classical method using phosphatase for the determination of phosphate [148] but this method was susceptible to product inhibition by reactive phosphate already present in the sample. McKelvie and coworkers immobilised *E. coli* onto CNBr-activated sepharose 4B beads in a FI system with an optimum pH of 8. The recovery of alkaline phosphatase hydrolysable phosphorus was low in natural waters but good in sediments [146]. They also applied alkaline phosphatase to soils [59]. Acid and alkaline phosphatase and phytase have been used in combination to investigate organic phosphorus speciation in soils [149].

Inositol hexaphosphate forms the bulk of extractable soil organic phosphorus [146,149,50]. Phytases (EC 3.1.3.8) are members of the family of histidine acid phosphatases [150,151] that are found in plants and micro-organisms, which catalyse the hydrolysis of phytate (myo-inositol hexakis-phosphate 1, 2, 3, 4, 5, 6) to less phosphorylated myo-inositol phosphates and free orthophosphates. Phytase from plant sources, e.g. wheat, first acts on the C_6 atom while that from microbial sources acts on the C_3 atom. McKelvie et al. [59,152] used a FI system with immobilised phytase for the determination of phytic acid in soils. Adenosine-5′-triphosphate was also hydrolysed but in low yields compared with phytic acid. Phytase has also been applied to the determination of phytic acid in the marine environment, but with low recoveries [152].

Enzymatic methods are important for assessing the potential biological availability of organic phosphorus but other methods are also needed for complete identification and this remains a challenging area of analysis.

5. Quality assurance and quality control

Phosphorus is a key determinand in most environmental monitoring and research programmes [153] and only accurate analytical data permits valid conclusions to be drawn about the phosphorus status of water bodies and soils. In addition to DRP it is also important to obtain accurate total phosphorus (TP) data because this parameter is used for load calculations, e.g. to determine discharges from sewage treatment works [18]. This has important implications regarding decisions on the installation (or not) of costly phosphorus removal technology. Programmes involving multi-national participation and international databanks [76] require adequate quality assurance/quality control (QA/QC) schemes to ensure the data integrity necessary for the comparison of data from various sources. Adherence to QA guidelines, participation in inter-

laboratory studies, use of reference materials (RMs) and certified reference materials (CRMs) are all means of achieving good data quality for phosphorus determinations [154,155].

5.1. Certified reference materials

A CRM is a reference material for which component values have been certified by a technically valid procedure and is accompanied by or traceable to a certificate or other documentation issued by a certifying body [156,157]. The use of CRMs is the most efficient way to measure and control accuracy [158] and can help produce reliable calibration and validation of measurement procedures [159]. CRMs can be either calibration CRMs, which are high purity substances or synthetically prepared mixtures, or matrix-matched CRMs, which can be natural samples or artificial samples simulating the composition of natural samples [158]. Few CRMs are commercially available for the determination of phosphorus species in environmental matrices (see Table 4), despite the need for such materials [155]. CRMs are not currently available for all environmental matrices routinely analysed for phosphorus species, such as estuarine waters, nor do they adequately span the range of phosphorus concentrations characteristic of environmental matrices. The National Research Council of Canada (NRCC) recognized the urgent need for CRMs for nutrients, including orthophosphate, for use in the marine sciences. MOOS-1, a natural seawater CRM available for the determination of nutrients in seawater, was developed in direct response to this need [159]. Analysis of MOOS-1 was carried out in 2002 by 25 expert laboratories participating in the 'NOAA/NRC 2nd intercomparison study for nutrients in seawater' [160]. Laboratories were predominantly selected on the basis of their previous satisfactory performance in a NOAA 2000 intercomparison study [159]. Flow and manual methods were used, all based on the spectrophotometric procedures of Strickland and Parsons [148]. Eighteen of the 25 laboratories achieved satisfactory Z-scores (see Section 5.2) for the determination of phosphate in seawater as shown in Fig. 5.

5.2. Intercomparison exercises

Inter-laboratory comparison studies are an essential feature of method development and validation [154] and play an important role in the certification of reference materials, such as described for MOOS-1 [159]. Performance in intercomparison studies undertaken by NOAA/NRCC in 2000 and 2002 [159,160] was used to assess the capabilities of international laboratories to quantify nutrients in MOOS-1, including orthophosphate. Z-scores [162] have been widely used for the statistical assessment of data in intercomparison exercises to give a comparative indication of performance with $|Z| < 2$ indicating satisfactory performance [160,163–166].

The main objectives of interlaboratory comparison studies are to determine inter-laboratory precision and accuracy and provide an impartial view of in-house quality control procedures. Participation can also identify best practise with respect to method, sample preparation, sample storage and training needs. The QUASIMEME project (Quality Assurance of Information for Marine Environmental Monitoring in Europe), now known as QUASIMEME Laboratory Performance Studies, was established to assist European Union labs in developing their QA/QC procedures to satisfy the data quality requirements of monitoring programmes in which they participated such as the International Marine Monitoring Programmes of the Oslo and Paris Commissions (OSPARCOM), the Helsinki Commission (HELCOM) and the MEDPOL programme [163,167]. Initially funded by the EU (1992–1996), the programme still continues by subscription of participating institutes. All institutes, worldwide, involved in chemical measurements in seawater are eligible to participate. The laboratory programmes for proficiency testing of most determinands are conducted twice per year and routinely include aqueous test materials containing orthophosphate and TP at concentrations similar to those found in estuarine, coastal and open water environments [168]. Regular testing is necessary to assure the quality of environmental data submitted since the performance of many laboratories does not remain constant [163,169]. The

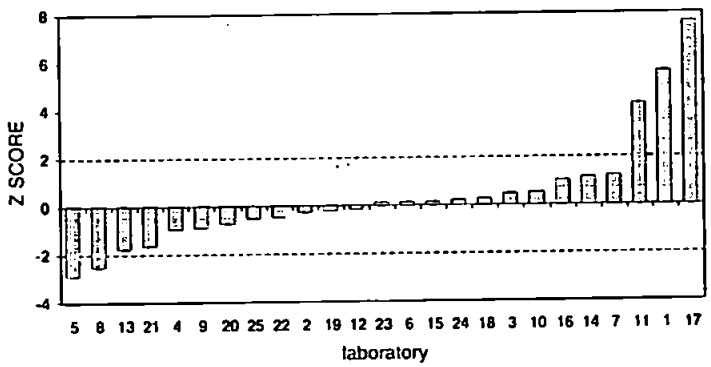


Fig. 5. Plot of Z-scores obtained by laboratories participating in the NOAA 2002 intercomparison study for the analysis of orthophosphate in MOOS-1. Z-scores calculated from the mean orthophosphate concentration, with the assigned value set at 1.6 ± 0.21 μM. $|Z| \leq 2$ represent the satisfactory Z score value for MOOS-1 [159,160].

Table 4
Commercially available CRMs for the determination of phosphorus species in environmental matrices

CRM	Matrix	Phosphorus species	Concentration	Comments	Supplier	Ref.
MOOS-1	Seawater	Orthophosphate	$1.56 \pm 0.07\ \mu mol\ L^{-1}$	Natural seawater sample, of Cape Breton Island, NS, Canada at a depth of 200 m	NRCC	[159,160]
QC RW1	Freshwater	Orthophosphate	$100\ \mu g\ L^{-1}$	Artificial sample, distributed as an ampoule to be 100 times with pure water	VKI	[161]
QC RW2	Freshwater	Total phosphorus	$200\ \mu g\ L^{-1}$	Artificial sample, distributed as an ampoule to be 100 times with pure water	VKI	[161]
Australian natural water CRM	Natural water/freshwater	Orthophosphate	$27 \pm 0.8\ \mu g\ L^{-1}$	Natural water sample obtained from Christmas Creek in the Lamington National Park, Qld., Australia	Queensland Health Scientific Services	
BCR-616	Groundwater (high carbonate content)	Total dissolved phosphorus Orthophosphate	$37 \pm 1.2\ \mu g\ L^{-1}$ $3.36 \pm 0.13\ mg\ kg^{-1}$	Artificial groundwater sample, prepared from ultrapure water, to which required salts were added; stabilized by autoclaving	BCR	www.irmm.jrc.be
SRM®-2702	Marine sediment	Total phosphorus	$0.1552 \pm 0.0.0066\%$	Material for SRM® was collected from Chesapeake Bay, USA, freeze-dried, seived at 70 μm (100% passing) and cone blended, then radiation sterilized and bottled	NIST	www.nist.gov
SRM®-1646a	Estuarine sediment	Total phosphorus	$0.027 \pm 0.0.001\%$	Material for SRM® was dredged from Chesapeake Bay, USA, freeze-dried, lightly deagglomerated and < 1 mm fraction ball milled and the < 75 μm blended and bottled	NIST	www.nist.gov
BCR-684	River sediment	NaOH-extractable P HCl-extractable Inorganic P Organic P Conc. HCL–extract P	$500 \pm 21\ mg\ kg^{-1}$ $536 \pm 28\ mg\ kg^{-1}$ $1113 \pm 24\ mg\ kg^{-1}$ $209 \pm 9\ mg\ kg^{-1}$ $1373 \pm 35\ mg\ kg^{-1}$	Material for the CRM was collected from the lower reaches of the River Po, Italy, then sieved and the <2 mm fraction was dried, lightly deagglomerated, crushed and hammer-milled and < 90 μm blended and bottled	BCR	www.irmm.jrc.be

assessment of the quality of data must be made at the time that the environmental samples are analysed. Such exercises provide vital information for improving the quality and performance of laboratories and a structure for developing robust analytical techniques. To this end, the QUASIMEME Laboratory Performance Study was designed to follow the IUPAC/ISO/AOAC international protocol for international testing [162,170]. All laboratories that submit data to the UK National Marine Monitoring Programme (NMMP) routinely participate in QUASIMEME as a means of external QA/QC of the data collected, including orthophosphate [171]. There have been several other national and international intercomparison exercises including the series of International Council for the Exploration of the Seas (ICES) exercises [164] and the Australian National Low level Nutrient (ANLLN) exercise.

5.3. Databases

Environmental monitoring and research programmes generate large amounts of information and can provide valuable databases of analytical information if appropriate QA/QC measures are used to preserve data quality. For example, databases have been generated from of the NMMP and the

'Winter Monitoring of the Western Irish Sea' programme [165] and both incorporated QA/QC schemes to ensure data integrity. Legislation such as the EU Water Framework Directive outlines an approach for managing water quality in the member states of the European Union which will require monitoring and environmental quality data (including P data) to be collected by member states and presented at the EU level. There is therefore the potential to add to the repository of data already held by the European Environmental Agency, and adherence to QA/QC practices such as intercomparison studies in conjunction with routine in-house use of RMs and CRMs is essential if such data are to be of practical use. Phosphorus data are also incorporated within larger assessment exercises dealing with broader issues such as water quality and eutrophication, e.g. the National Estuarine Eutrophication assessment in the United States [172].

6. Conclusions

Accurate determination of P species in environmental matrices is an important pre-requisite for understanding the biogeochemical cycling of the element. This in turn is essential for investigating the impact of phosphorus on ecosystem health. Key aspects of the analytical process for obtaining high quality phosphorus data are robust sampling and sample treatment protocols (see also Maher and Woo [75]). These cannot be universal due to the variability in behaviour of different matrices but nonetheless guidelines can be provided for aspects such as filtration, chemical treatment and storage conditions. For soils, wetting and drying have a considerable affect on phosphorus solubility.

In addition, for the determination of different phosphorus fractions and individual phosphorus containing compounds, particular attention needs to be given to the digestion process. Autoclaving (typically with peroxydisulfate in acid or alkaline media) is a widely used method that gives good recoveries but it is important to quantify this using a range of environmentally relevant model phosphorus containing compounds. Selective enzymatic degradation (typically using phosphatases) is a useful additional approach for the quantification of individual phosphorus containing compounds (or classes of compounds).

A critical aspect of the overall analytical process for any laboratory is participation in intercomparison exercises. This is particularly important for phosphorus determination due to the lability of the element in biologically active environmental matrices. To supplement such exercises the availability of more environmental certified reference materials is an important requirement. Finally, co-operation between analytical scientists and environmental scientists is fundamental to the generation of high quality, publicly available databases on the spatial and temporal variability of phosphorus species in aquatic and terrestrial ecosystems.

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A13.21
Integrating ecotoxicology and analytical chemistry to assess water quality status for river basin management

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The EC Water Framework Directive aims to maintain and improve the quality of aquatic ecosystems in the EU by using an integrated approach to the protection, improvement and sustainable use of the water environment. Nitrate and nitrite concentrations in natural waters rarely exceed the maximum admissible levels set by the EU for surface waters intended for abstraction for drinking water (11.3 mg L^{-1} NO_3-N) and for the protection of course freshwater fish (9.0 μg L^{-1} NO_2-N) respectively. However, in cases of extreme pollution, levels may be as high as 1 mg L^{-1} NO_2-N such as in waters strongly influenced by industrial effluent, or 200 mg L^{-1} NO_3-N in sewage polluted waters. Rapid (potentially field based) ecotoxicological tests has been used in this study to determine the effect of sub-lethal levels of nutrients on the overall health of a selected freshwater mollusc, *Anodonta cygnea*. Preliminary toxicity studies indicate that 96 hour EC_{50} values are in excess of 2 g L^{-1} nitrite and 10 g L^{-1} nitrate for this species; and the 24 and 36 hour LC_{50} values for nitrite were 2 and 5 g L^{-1}. Spectrophotometric determination of engulfed red dye particles in haemocytes (phagocytosis assay) or the uptake of the dye by lysosomes (neutral red retention assay) was used to monitor immune function and cell viability of Anodonta cygnea when exposed to sublethal concentrations of nitrite. Cardiac activity was also be monitored during these exposures, using a computer-aided physiological monitoring system (CAPMON) based on photodiode detection. Together these biomarkers allow an assessment of cell and organismal health. This integrated approach will allow the assessment of surface water quality based on the chemical and biological objectives of the Water Framework Directive.

A13.22
Low cost automatic activity monitoring of the amphipod *Gammarus pulex*, and its role in ecotoxicity testing

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Gammarid amphipods are important components of the aquatic ecosystem. They are omnivorous, utilising a wide variety of food sources including plants, dead animals and invertebrates. Gammarids in turn form a significant part of fish diet. The amphipod *Gammarus pulex* is widely distributed in standing and running fresh water habitats in Britain. It has been extensively used for ecotoxicology studies ranging from LC_{50} tests to biomonitoring applications. Current non lethal ecotoxicology tests on this organism are either laborious to perform and/or require relatively expensive equipment. We report the development of a new low cost infra red actograph system that measures relative activity of gammarids. Preliminary tests of this system demonstrate that it can readily detect very low concentrations of heavy metals. The simple design, cheap components, and high sensitivity of the equipment renders this method a useful addition to the repertoire of ecotoxicology tests.

A13.23
An evaluation of the relative sensitivity of cyto- and genotoxic biomarkers in two marine bivalve mollusc species

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In biomonitoring studies, the marine bivalve *Mytilus* sp. has been widely used as a sentinel species. For comparison, the common cockle *Cerastoderma edule* was used in this study as an alternative species for detecting biological effects of contaminants in marine and estuarine environments. In vitro validation studies were carried out on haemocytes that were collected from the posterior adductor muscle of adult *Mytilus edulis* and *Cerastoderma edule*. The samples from each species were pooled and sub-samples exposed to a range of concentrations of hydrogen peroxide (H_2O_2), a known oxidant. The level of DNA damage induced was measured with the 'comet assay', which detected a dose-dependent increase. Following these studies, haemocytes were collected from indigenous populations (in vivo) of the two species, located at six sites along the Tamar estuary (SW Devon). The 'comet' and 'neutral red lysosomal retention' assays were used in an attempt to determine dif-