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The Development and Evaluation of New Technologies for the Study of Eutrophication in Coastal Waters

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ABSTRACT

FACULTY OF SCIENCE
SCHOOL OF OCEAN AND EARTH SCIENCE

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Increased anthropogenic input of nutrients into the marine system can lead to eutrophication and can have a significant negative impact on some coastal marine ecosystems. The complicated biogeochemical and physical processes involved in the marine system make it difficult to monitor and predict future eutrophication events. In order to understand the processes which cause these events, we need to be able to measure key *in-situ* biogeochemical parameters relevant to the phytoplankton such as nutrients, at a high temporal and spatial resolution. Traditional techniques only allow limited monitoring of nutrients and are therefore likely to miss short-term transients. The NAS-2EN nitrate analyser was field tested during the PROVESS experiment and the results demonstrate that the performance of the cadmium reduction column is erratic and full data sets were not collected. However, although there were stability problems with the cadmium reduction column, the nitrate results obtained when the column was stable was within the expected range of nitrate concentrations for the two sites studied in the North Sea. Also, the data sets showed that the instrument was capable of recording short term transient events. Bench experiments investigating the reduction capability of the wire cadmium reduction column used by the instrument, found that the expected reduction efficiencies of above 97% was not being achieved and demonstrated that the column was not only reducing the nitrate to nitrite but was further reducing the nitrite. A cadmium granular column and control macro were developed and the concentration of the chemical reagents adjusted. A stable reduction efficiency of almost 100% was achieved. Therefore, the new cadmium granular reduction column, control macro and chemicals were deployed in Loch Etive as part of the REES project carried out by Dunstaffnage Marine Laboratory. The on-board standard of the surface instrument was stable and measured over 900 samples with a coefficient of variation of 3.6%. Water ingress into a seabed instrument deployed at the seabed caused it to shut down after 16 days. With these instruments it was possible to monitor the nutrient fluxes caused by the renewal events in the Loch and estimate rates of denitrification in the bottom water. The new cadmium granular column and control macro and chemicals represent a significant improvement of the instrument's performance and it has been demonstrated that the NAS-2EN used with this new method is capable of acquiring large, reliable data sets which was not previously possible.

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DECLARATION OF AUTHORSHIP

I, Joyce Boyd,

declare that the thesis entitled:

The Development and Evaluation of New Technologies for the Study of Eutrophication in Coastal Waters.

and the work presented in it are my own. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- none of this work has been published before submission.

Signed: .....[redacted].....

Date:.....12/05/04.....

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1.0 Introduction

Anthropogenic impact on the environment and particularly the release of contaminants is an increasing concern of the developing world (UNFCCC Kyoto Protocol, 1992). In order to respond to these environmental changes, it is important that we understand and monitor the sources and effects of such contaminants on the environment. This monitoring has to be coupled with studies that attempt to link the behaviour of the contaminant in the environment with changes in the ecosystem. Understanding of the ecosystem will be improved by the development of conceptual and mathematical models that can attempt to predict and manage future changes (Cloern, 2001). However, any such model will only be successful if it is based on accurate data gathered from the system.

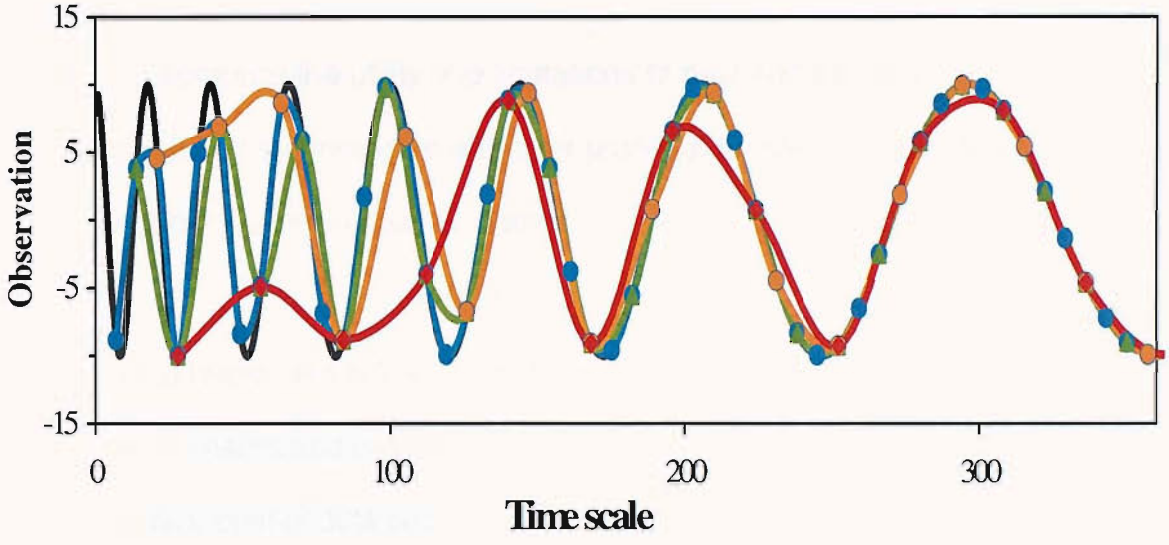
Within the marine environment the importance of nutrients, and in particular dissolved inorganic nitrogen, phosphorus and silicon, has long been known (Dugdale, 1983; Chester, 1990; Libes, 1992; Grasshoff *et al.*, 1999). Nutrients are a key factor in the production of organic phytoplanktonic matter. Although nutrients are a natural part of the marine ecosystem they have become "pollutants" owing to the increased quantities that are now entering the sea. This increase is caused by changes in land usage and systematic sewage collection, which lead to concentrations several folds higher than in pristine environments. This type of pollution is known to lead to eutrophication, the definition of which is not simple. It has been defined in several ways by different authors and will be discussed in more detail in Chapter 3. However,

since the marine environment is dynamic, especially in coastal waters, it has not been possible to gain a full understanding of how the supply and removal of nutrients affect marine systems. Computer based models that attempt to simulate these environments will assist in the understanding of the eutrophication process. This greater understanding will be realised by combining our knowledge of individual processes and observing how they interact through time and space within conceptual and numerical models (Cloern, 2001).

A key factor in the development and validation of conceptual and numerical models is the requirement and ability to gather large volume nutrient data sets of a high quality. Traditional methods of nutrient data collection are expensive, labour intensive, time consuming and only provide limited data sets (Hydes *et al.*, 2000) that may not fully represent the system. When the sample resolution is low, a distorted representation of the system, known as aliasing (Figure 1.1) can occur.

Figure 1.1 compares sample observations that are collected at four differing time intervals with the real signal. It can be seen from the diagram that less distortion of the real signal occurs at higher frequency sampling rates or if the frequency of the real signal is reduced. This agrees with Nyquist's Theorem, namely that the sampling rate must be a minimum of two times the maximum signal frequency in order to reduce the effects of aliasing.

Figure 1.1.
Plot Demonstrating the Relationship between Sampling Rates and Data Aliasing.
Black shows real signal, blue shows signal observed when sampling every 7 time units, green when sampling every 14 time units, orange when sampling every 21 time units and red when sampling every 28 time units.



Progress is being made in this direction and a new range of data gathering instruments have or are being developed (Jannasch *et al.*, 1994; Daniel *et al.*, 1995; Rawlinson, 1997; Boyd, 1998; David *et al.*, 1998; Finch *et al.*, 1998; Clayson, 2000; Masserini and Fanning, 2000) which are able to gather nutrient data at a high temporal and/or spatial resolution. As this thesis was funded by W.S. Ocean Systems, the purpose of the work reported here was to further develop and test the NAS-2EN and the NAS-2ES instrument for the *in-situ* determination of nitrate and dissolved silicon. The specific objectives are given below and the two field projects in which the development work was carried out are also introduced.

1.1 Objectives

- To review what is already known about the role of nutrients within the environment and the link between nutrients and eutrophication.

- To compare the utility and limitations of new and existing nutrient data collection technologies with their potential for use in the study of eutrophication in coastal waters.

- To advance the NAS-2EN (nitrate) and NAS-2ES (dissolved silicon) instruments and develop them to a stage where quality high temporal and/or spatial data resolution is obtained routinely.

- To conduct data collection and analysis with the chosen instrument.

1.2 Field Work

Fieldwork was carried out during a major European project PROVESS (**PRO**cesses of **V**ertical **E**xchange in **S**helf **S**eas) during 1998 and 1999. Additional fieldwork was also undertaken as part of the Scottish Association for Marine Science (SAMS) REES Project (Restricted Exchange Environment) during 2000.

1.3 Outline of Thesis Structure

The thesis will review nutrients in the marine environment and their interaction with phytoplankton, particularly with respect to nitrogen and investigate the nature, causes and effects of eutrophication. A brief introduction to the

traditional methods of nutrient analysis will then be presented followed by a review of the recent developments in *in-situ* nutrient analysis systems.

The thesis will present a detailed evaluation of the manufactured WS Oceans NAS-2EN and NAS-2ES instruments and will present the results obtained during their PROVESS deployments in 1998 and 1999. The instruments' performance in terms of stability and reliability is reported and the quality of the data sets acquired is assessed. The nitrate and dissolved silicon data sets are also analysed with respect to the PROVESS temperature, salinity and fluorometer data sets.

The results obtained from bench testing and experimentation, which was carried out in order to improve the stability and reliability of the NAS-2EN instrument's performance, is then reported. The dissolved silicon NAS-2ES was unavailable for bench testing.

The thesis also presents the results of further field testing to assess the performance of the modified NAS-2EN instrument. This instrument was deployed during the SAMS REES project in 2000. The quality of the data set in terms of stability and reliability is reported. In addition, the nitrate data set was analysed with respect to the temperature and salinity data collected during this project.

Finally, conclusions with subsequent recommendations for future work are presented.

2.0 The Nutrients.

According to Grasshoff *et al.*, (1999), the nutrients referred to by chemical oceanographers are traditionally almost exclusively nitrogen (N), phosphorus (P) and silicon (Si). This is because these elements were early on in the history of oceanography established as being essential for phytoplankton growth to occur and under certain circumstances in marine systems each of them can be in such short supply that growth is limited. They are termed the "limiting nutrients" in seawater. In addition, as early as 1935 Cooper, (1935), suggested that trace metals might also be limiting nutrients. Recently, following improvements in analytical methods, it has been accepted that in certain specific areas of the ocean this can be the case particularly elements such as iron (Martin and Fitzwater, 1988; Bruland *et al.*, 2001).

2.1 Nutrient Assimilation

The assimilation of the nutrient occurs principally through the active transport of the nutrient-containing ion across the cell membrane. Nitrogen is assimilated as the species nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) and phosphorus is assimilated as phosphate (HPO_4^-) (Libes, 1992).

Once nitrogen has been transported across the cell membrane it is converted, via a series of anabolic reactions, into metabolite compounds. In order for this process to take place, the nitrogen has to be reduced to its lowest oxidation state. This process is known as assimilatory nitrogen reduction. Once in its

reduced form (NH_3), transamination takes place, which converts the nitrogen into amino acids (Libes, 1992). It takes less energy to convert nitrogen in its most reduced form, ammonia into organic matter. Therefore, phytoplankton tend to assimilate ammonia in preference to nitrite and nitrite in preference to nitrate.

The biological requirements for phosphorus are less that that for nitrogen. Phosphorus has a critical role e.g. in the synthesis of ATP and cell membranes (Sumich, 1996). The average long-term atomic ratio of carbon to nitrogen to phosphorus in marine phytoplankton is approximately 106:16:1. As 106 moles of carbon, 16 moles of nitrogen and 1 mole of phosphate are incorporated into the marine biomass approximately 150 moles of oxygen are released. This ratio is known as the Redfield-Richards Ratio (Redfield *et al.*, 1963). The Redfield-Richards ratio defines the stoichiometry (relative abundance of atoms in a molecule or in a reaction) of the photosynthesis and remineralisation reactions. The ratio is reflected in the composition of most marine waters where the ratio of dissolved nitrate to phosphate is also about 16. Although in coastal waters and surface ocean waters this ratio may vary.

Silicon is a nutrient of which silicic acid (H_4SiO_4) is the dominant dissolved species at the pH and ionic strength of seawater (Libes, 1992). Dissolved silicon is used in the formation of biogenic opal, which, is used to construct shell material in organisms such as diatoms and chrosophyceas (Billen *et al.*, 1991). The atomic silicon to carbon ratio varies from 0.1 in marine phytoplankton species to 0.8 in freshwater diatoms.

2.2 Nutrient Uptake by Planktonic Cells

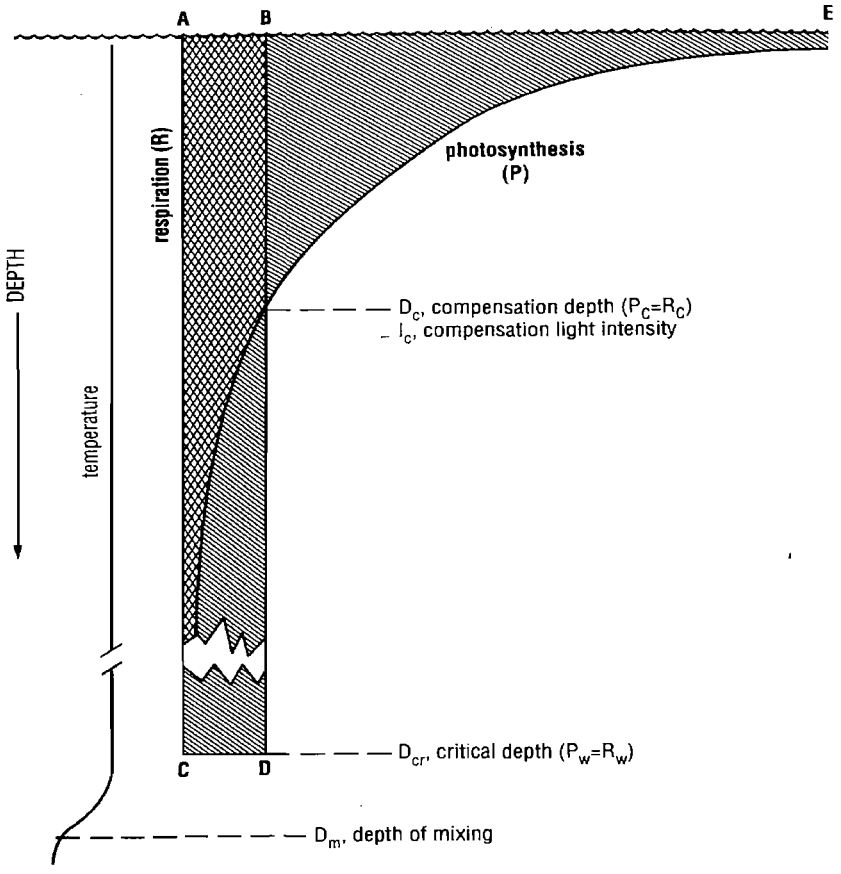
There are a number of controlling factors, which affect nutrient uptake by planktonic cells namely, light availability and advective transport of the nutrients. Predation also indirectly controls the uptake of nutrients.

2.2.1 Light

The amount and rate of photosynthesis is affected by the level of solar radiation penetrating the water column. Photosynthesis increases with increasing light up to a maximum value after which photosynthesis is inhibited. The depth at which gross photosynthesis (total amount of photosynthetic production) and respiration are equal is known as the compensation depth. Therefore, above the compensation depth there is a net gain from photosynthesis and below it there is a net loss as respiration dominates. The compensation depth defines the lower boundary of the euphotic zone (Lalli and Parsons, 1995). As the phytoplankton cells are mixed above and below the compensation depth, they experience the average light intensity of the water column. The critical depth as defined by Sverdrup, (1953), is the depth, at which the compensation light intensity equals the average light intensity and therefore the depth-integrated photosynthesis throughout the water column equals phytoplankton depth-integrated respiration (area defined by ABCD = area defined by ACE in Figure 2.1). When the critical depth is less than the depth of mixing, no net production takes place because the photosynthesis taking place throughout the mixed layer is less than that of the respiration. Therefore net photosynthetic production (amount of

photosynthetic production in excess of respiration losses) of phytoplankton only occurs when the critical depth lies below the depth of mixing.

Figure 2.1
Compensation, Critical Depth and Depth of Mixing
(Lalli and Parsons, 1995)



I_C = Compensation light intensity
R_C = Respiration of a single cell
P_C = Photosynthesis of a single cell
D_C = Compensation depth
I_D = Average light intensity
R_W = Respiration throughout water column
P_W = Photosynthesis thought water column
D_{CR}= Critical depth

2.2.2 Nutrient Uptake Efficiency of Phytoplankton Cells

The size of organism that can grow most efficiently and dominate the autotrophic plankton population tends to be determined by the concentration of available nutrients. When nutrient concentrations are low, smaller cells, which have greater nutrient uptake efficiency than larger cells tend to

dominate. Nanophytoplankton (2.0 - 20μm) and picophytoplankton (0.2 – 2.0μm) do much better in the competition for inorganic nutrients than microphytoplankton (>20μm) (Kiorboe, 1996). According to Kiorboe, (1993), empirical evidence shows that microphytoplankton dominate in localised or episodic, nutrient-rich, turbulent environments whereas the smaller motile forms dominate in oligotrophic waters.

A number of factors may account for this. If the phytoplankton cell is assumed to be a spherical cell the uptake rate of nutrients is dependant on the concentration of the nutrients in the water (C), the cell size (characterized by its radius, r), molecular diffusivity of the cell (quantified by its diffusion coefficient, D) and by the advective transport of the nutrients to the plankton cell (Sherwood number, Sh) (Kiorboe, 1996; Berg and Purcell, 1977) suggested therefore that the uptake rate of nutrients (U) is given by:

$$U = 4\pi rDShC \qquad (2.1)$$

and the volume specific uptake rate is calculated as:

$$\frac{U}{cell\ \ volume} = \frac{4\pi rDShC}{\frac{4}{3}\pi r^3} = \frac{3DShC}{r^2} \qquad (2.2)$$

From formula (2.2) it can be seen that when the Sherwood number is 1, the cell's uptake rate of nutrients decreases with the square of the cells radius. Therefore, smaller cells have a competitive advantage over the larger cells. However, the Sherwood number is a ratio of the total nutrients transported to the cell's surface (by diffusion and advection) to the nutrients transported by diffusion only (Kiorboe, 1996). If the advective transport is zero, (Sh = 1) then the uptake rate is solely determined by the rate of molecular diffusion. If the

cell is moving in the water column either due to swimming or sinking or caused by local fluid velocity gradients, then the effective advective transport and the uptake rate will be increased. The larger cells will then have the competitive advantage. For the larger cells, this increase in nutrient uptake can be significant. A cell that is swimming in the water column with a cell diameter of 1μm has a Sherwood number of 1.84 whereas a swimming cell with a diameter 100μm has a Sherwood number of 23.1 (Kiorboe, 1996).

2.2.3 Predation Control

Predation control can determine the structure of the phytoplankton populations in high-energy environments and may explain why the larger cell phytoplankton organisms overcome their competitive disadvantage and dominate in these environments (Munk and Riley, 1952; Geider *et al.*, 1986; Kiorboe, 1991; Kiorboe, 1996). Growth rate of phytoplankton cells is only weakly related to cell size (Banse, 1982; Blasco *et al.*, 1982; Nielsen and Sandjensen, 1990). The predators of picophytoplankton cells such as heterotrophic nanoflagellates have similar growth rates (in the order of hours) as the small phytoplankton cells. In this case, their predators control the smaller cells. The predators of the larger phytoplankton cells which are themselves larger more complex organisms have longer generation times which may be up to several orders of magnitude longer. This may delay the response of a predator's population to an increase in prey population size. If there is an episodic injection of nutrients into the euphotic zone there will be an increase of both the small and larger cell size phytoplankton. The increase in population of the small phytoplankton cells will tend to be controlled by their small predators but the population of the larger phytoplankton cells will

increase unchecked by their large predators because of the predators' slower generation rate. Consequently the larger phytoplankton will bloom until the nutrients have been depleted or until the predators' population catches up (Kiorboe, 1996).

In oligotrophic waters, small cell phytoplankton out-competes the larger cells for nutrients. Their predators then control this population. Here the rate of nutrient regeneration and the rate of nutrient uptake tend to be in balance. This process is not fully efficient and nutrients are lost from the euphotic zone via downward particle transfer and cross pycnal mixing. Turnover of phytoplankton will continue until it is limited by light or the supply of inorganic nutrients has been exhausted (Kiorboe, 1996).

2.3 Nutrient Re-cycling

Billen *et al.*, (1991), suggests that different processes regenerate each of the dissolved nutrients from phytoplanktonic biomass or detritus back to the water column.

Nitrogen

The organic nitrogen synthesised by the phytoplankton is recycled to dissolved inorganic nitrogen (DIN) via a number to processes. The phytoplankton cells may die and lyse dissolved organic nitrogen (DON). Predators such as protozoa or zooplankton may consume the cells. Predators will excrete and exude DON, $NH4^+$ and urea and after their death DON will be released by the lyses of their cell (Libes, 1992). Rapid degradation of the DON

by heterotrophic bacteria forms ammonia (NH_3). This process in known as ammonification (Libes, 1992). In oxic seawater nitrification takes place where the ammonium is oxidised to nitrite by the bacteria *Nitrosomonas* and the resulting nitrite is oxidised to nitrate by the bacteria *Nitrobactor* (Libes, 1992). The oxidation process requires an electron acceptor. The highest free energy yield is obtained when oxygen is used as the terminal electron acceptor. In the absence of oxygen other electron acceptors may be used which will give a different energy yield to the oxidising bacteria (Froelich *et al.*, 1979).

It is possible for more than one type of oxidation reaction to appear to occur simultaneously. However, they tend to occur in the order shown in Table 2.1. Froelich, (1979) drew up this table to help explain the stratification of the redox zones that was being formed in near surface sediments. From the surface downwards aerobic respiration continues until oxygen levels are depleted. Denitrification follows, during which some of the nitrate is reduced to nitrite and then to N_2, which is not incorporated into bacterial biomass resulting in the loss of fixed nitrogen. In the presence of small amounts of O_2, denitrification causes some of the nitrite to be reduced to N_2O. In anoxic conditions, denitrification takes place until the nitrate is depleted.

Following the reduction of nitrate in sediment pore waters, manganese and iron oxides are used as electron acceptors then sulphate is reduced to sulphides. Subsequently methane can be formed by fermentation.

Table 2.1.
Organic Matter Oxidation Pathways and Free Energy Yields
(Froelich *et al.*, 1979)

Reaction.	**$\Delta G°$ (KJ mol^{-1} of CH_2O)**
Oxic Respiration $CH_2O + O_2 \rightarrow CO_2 + H_2O$	-475
Denitrification $5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O$	-448
Mn-Oxide Reduction $CH_2O + 3CO_2 + H_2O + 2MnO_2 \rightarrow 2Mn^{2+} + 4HCO_3^-$	-349
Fe-Oxide Reduction $CH_2O + 7CO_2 + 4Fe(OH)_3 \rightarrow 4Fe^{2+} + 8HCO_3^- + 3H_2O$	-114
Sulphate Reduction $2CH_20 + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$	-77
Methane Production $2CH_2O + 2H_2O \rightarrow 2CO_2 + 4H_2$ and $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-58

Phosphorus

In the phosphorus cycle, bacteria utilise organic phosphorus compounds via extracellular hydrolysis, which results in the direct release of the phosphate ion from decaying cells. The phosphate released in sediments is readily adsorbed by iron (III) compounds, which are present in most oxic marine sediments (Billen *et al.*, 1991). This tends to prevent the escape of P and leads to the burial of P in the sediments. In anoxic conditions the iron compounds that bind the phosphate, are reduced during anoxic respiration and dissolve so that phosphate is released into the pore waters. The presence of oxygen and/or nitrate prevents the significant re-cycling of P from the sediments (Billen *et al.*, 1991). In exceptional circumstances, substances such as sulphate interfere with the adsorption of phosphate. In hyper saline waters with high sulphate concentrations, the adsorption of phosphate is limited (Caraco *et al.*, 1989).

Silicon

Silicon is less affected by the redox conditions of the sediments and dissolved silicon is readily released into the pore waters of the sediment from where it diffuses into the water column. According to Wollast, (1991), dissolved silicon in pore waters may react with terrigenous clay minerals to form siliceous marine clays in marine or estuarine waters. This process is also known as "reverse weathering". According to Billen *et. al.,* (1991), the preservation of a small fraction of biogenic opal can occur either by secondary organic coating or by diagenetic transformation into cristobalite or quartz. The dissolution of silicon in the form of biogenic opal is a slow process, which can only begin when the outer protective organic coating in the cell has been degraded after the death of the diatom (Billen *et al.*, 1991).

Due to these different processes, organic phosphorus is recycled faster than nitrogen, which in turn is recycled faster than silicon (Billen *et al.*, 1991). As a result of their faster rates of regeneration, nitrogen and phosphorus can be recycled within the euphotic zone but due to the slow dissolution rate of silicon it tends to be regenerated below the euphotic zone.

The re-cycling of organic matter in the water column is not wholly efficient and a fraction of the particulate organic matter (POM) will reach the sediments. The fraction that reaches the sediments in coastal waters is mostly in the range of 25–50% of the primary productivity (Nixon, 1981; Wollast, 1991; Jorgensen and Richardson, 1996). However, typically only ~1% of the carbon leaving surface water is actually buried in the sediments. In oxic conditions the

nutrients are continuously re-cycled in the sediments and are released into the water column via advection and diffusion processes throughout the year. The majority is released during the phytoplankton growth season following major deposition events after the spring bloom at temperate latitudes (Jorgensen and Richardson, 1996).

The PON that reaches the sediment will continue to be oxidised. During periods of low organic input to the marine sediments, the amount of nitrogen released, (mainly in the form of nitrate) is proportional to the input (Billen *et al.*, 1991). No denitrification takes place as long as the sediments are oxic. However, if during periods of high production the increased organic material that reaches the sediments exceeds a critical level, an anaerobic layer is formed in the sediment. According to Billen, (1991), once this critical level has been exceeded, denitrification takes place in the anaerobic layer. If the input of organic material to the sediment surface continues, the upper aerobic layer may become so reduced that nitrate production is limited, which in turn limits denitrification. However, if the overlying waters have a high nitrate concentration i.e. a riverine or estuarine system, the nitrate will diffuse across the sediment-water interface and denitrification will continue.

2.4 Seasonal Patterns of Marine Phytoplankton Blooms in Temperate Latitudes.

In temperate latitudes primary production tends to follow a distinct seasonal pattern (Prandle *et. al.,* 1997). During the spring, light irradiance increases and as there is a large pool of inorganic nutrients present, a phytoplankton

bloom occurs. A bloom is where dense concentrations of phytoplankton colours the sea, (Purdie, 1996). The phytoplankton bloom in estuarine or coastal waters may only be a small-localised feature. However, in open ocean and shelf waters blooms may cover hundreds of square kilometres.

Diatoms (relatively large cells) tend to be the first to develop and dominate during the spring bloom because they can grow rapidly in relatively low light conditions, where a large nutrient pool is available. They are able to grow and accumulate more rapidly than they are dispersed by the turbulent conditions prevalent during relatively early spring (Purdie, 1996).

The increase in phytoplankton biomass during the bloom may be limited by;

1. An increase in predators (i.e. protozoa and zooplankton), which limits the increase in phytoplankton biomass, and causes the bloom to reach its peak (Libes, 1992; Kiorboe, 1996).

2. The rapid growth rates during the bloom and losses of organic material from the euphotic zone cause the nutrient pool to be diminished. This is especially so for dissolved silicon, which has a slower re-cycling efficiency than nitrogen and phosphorus. The loss of dissolved silicon from the euphotic zone may cause the diatom population to be replaced with another phytoplankton population such as a dinoflagellate population, which do not require the dissolved silicon nutrient (Officer and Ryther, 1980).

3. Surface heating during the spring and summer induces a thermocline, which, blocks the return of dissolved inorganic nutrients to the euphotic zone causing a decline of the standing crop of phytoplankton during the summer.

This latter process can be reversed in the autumn leading to an "autumn bloom". In the autumn the decreasing temperature and the onset of unstable weather patterns erode the thermocline, which results in the mixing of nutrients into the euphotic zone. As light levels can still be sufficiently high for the euphotic zone to be deeper than the critical depth (Lockwood *et al.*, 1996) a phytoplankton bloom may occur. This bloom is then halted by decreasing light and increasing turbulence (Lockwood *et al.*, 1996).

During the winter period light is limiting and there is low phytoplankton production and abundance but heterotrophic and dissolution activity continues increasing the pool of dissolved inorganic nutrients. The water column tends to be well mixed during the winter and therefore concentrations of nutrients tend also to be uniform through the water column to the base of the winter mixed layer.

Freshwater input and tidal mixing in coastal and estuarine systems impose a number of physical processes, which affect the return of the nutrients to the euphotic zone. These processes such as, tidal stirring, salinity stratification, the formation of fronts and the local morphology play an important role in the dynamics of nutrient flow (Lucas *et al.*, 1998).

2.5 Nutrient Sources.

The main biogenic nutrients (N, P and Si) are transported to the ocean via atmospheric deposition (mostly N) or via riverine systems from soil leaching, erosion (N, P and Si) and the direct discharge of wastewater by human activity (N and P). Atmospheric deposition (either wet or dry) transports the nutrients directly from land and may deposit it many miles offshore into the oceans (Jickells, 1998). Riverine input is a slower and a less direct method of nutrient input than atmospheric input and the nutrients may pass through areas of high biogeochemical activity such as estuaries and coastal zones on their journey to the oceans (Jickells, 1998). Table 2.2 summarises nutrient inputs from terrestrial sources.

Soil leaching and erosion result in the input of N, P and Si into surface waters. Input is controlled by the interaction between water, soil minerals and biota. Agriculture and the systematic collection of sewage will enhance N and P input but will not significantly affect the input of dissolved silicon into surface waters. N and P remain in approximately the same ratio as in forested systems (atom ratio of 25 – 200).

In industrialised and densely populated areas, the wastewater discharge of the N and P load from industrial sources is of the same order of magnitude as that from domestic wastewater inputs (Billen *et al.*, 1991). Domestic wastewater input of N and P is attributed to excrement, household wastewater and input from washing powders. P is released in relatively higher amounts so that the N/P atomic ratio drops close to 10 except in areas where sewage

treatment results in the removal of P. Industrial discharges are mainly due to the food processing and chemical industries. Table 2.3 summarises input of nutrients to the North Atlantic from both land and sewage including wastewater.

Table 2.2.
Representative values of the input of nutrients from terrestrial sources to continental surface water per unit watershed areas (Adapted from Billen *et.al.*, 1991).
(in kg N, P, Si km^2 yr^{-1}.)

	NO_3	NH_3	DON	TDN	PO_4	TDP	Si	N:P:Si atomic ratio
Atmospheric Deposition								
Mean (exoreic) continents	80	225	225	630	5	10	200	143:1:23
Industrialised regions	1,250	225	225	1,700	20	60	200	64:1:4
Soil Leaching								
Non-agricultural watersheds								
Temperate	75	7	55	135	4.6	12	3,400	25:1:323
Tropical: -dry				127		5	2,000	57:1:456
-humid	60	5	168	228	36	65	10,300	8:1:180
Temperature agricultural watersheds						60	3,400	38:1:65
Grassland: -loam/clay						60	3,400	133:1:65
-sand								
						60	3,400	95:1:65
Arable land: -loam/clay						60	3,400	273:1:65
-sand								
Wastewater from domestic discharges in industrialised countries								
				3-4.6		0.4		
Excrements				0.4		0.1		
Housework wastewater						0.7-		
Washing powders						1.1		
				3.4-4			0.5-1	3.4-4:1.3-1.7:0.5-1
Total						1.3-1.7		

DON = dissolved organic nitrogen

TDN = total dissolved nitrogen

TDP= total dissolved phosphorus

Billen *et al.*, (1991), observed that the overall inputs of nutrients from watersheds show that inputs from forested watersheds are lower in P than in both N and Si, with respect to the requirements of phytoplankton (N/P ratio =

100-1000). Deforestation leads to enhancement of N and to reduced P leaching.

According to Paerl and Fogel, (1994), atmospheric nitrogen input is significant. In coastal Atlantic Ocean waters atmospheric nitrogen accounts for 20 – 50% of external nitrogen input to estuarine and coastal waters.

Table 2.3.
Input of N and P from land, sewage and wastewater into North Atlantic
(Adapted from tables in Howarth, 1996)

	TN Tg yr^{-1}	Gmol yr^{-1}	TP Tg yr^{-1}	Gmol yr^{-1}	N:P (molar)	Sewage N Input Tg yr^{-1}	% of Total N Input
North Canadian Rivers	0.30	22	0.018	0.57	37	0.022	7%
St. Lawrence basin	0.66	47	0.02	0.63	73	0.072	11%
NE coast of US	0.51	37	0.067	2.1	17	0.13	26%
SE Coast of US	0.24	17	0.011	0.35	47	0.034	14%
Eastern Gulf of Mexico	0.23	17	0.011	0.35	46	0.049	21%
Mississippi basin	1.82	130	0.107	3.4	38	0.16	9%
Western Gulf of Mexico	0.85	61	0.007	0.22	271	0.082	10%
North American total	4.61	331	0.241	7.65	42	0.55	12%
Caribbean Islands and Central America (incl. Orinoco)	1.09	78	0.141	4.5	17	0.140	13%
Amazon &Tocantins basin	3.28	234	1.53	49	4.8	0.0003	0.01%
Central & South American Total	4.37	312	1.67	53	5.8	0.14	3%
Baltic Sea	0.74	53	0.072	2.3	23	0.10	14%
North sea	1.22	87	0.099	3.1	28	0.42	34%
NW European coast	0.44	32	0.028	0.89	36	0.073	16%
SW European Coast	0.20	14	0.054	1.7	8.3	0.047	23%
Western Europe total*	2.60	186	0.253	8.0	23	0.64	25%
NW Africa	1.48	106	0.087	2.8	38	0.096	6%
Total *	**13.1**	**935**	**2.25**	**71**	**13**	**1.43**	**11%**
*Not including Mediterranean Sea							

The atmospheric deposition of phosphorus to estuaries and coastal waters is negligible when compared to the contribution from other sources (Asman and Larsen, 1996). There are two processes that account for the input of atmospheric nitrogen to surface waters namely wet and dry deposition. Wet deposition, the removal from the atmosphere by precipitation takes place over

the whole atmospheric layer but it occurs only during precipitation. Dry deposition, the removal by atmospheric turbulence at the sea or land interface takes place very near the land or sea surface. Dry deposition occurs constantly whereas wet deposition only occurs during precipitation (5 - 10% of the time in Western Europe) (Asman and Larsen, 1996).

2.6 Summary

In summary, it is clear that phytoplankton abundance is intimately linked with nutrients. When the light conditions are correct and nutrients are present, blooms occur. In temperate systems undisturbed by man, there is a natural periodicity to phytoplankton growth and decline in respect to light and available nutrients. There is evidence of increased anthropogenic input of nutrients into the marine system and the effects of such increases are discussed in Chapter 3.

3.0 Eutrophication

3.1 Definition of Eutrophication

The term eutrophication is commonly used when describing environmental issues, which affect marine coastal waters. According to Richardson and Jorgensen, (1996), a number of definitions of the term are commonly, and in some cases incorrectly, used. Richardson and Jorgensen (1996), state that the Greek analysis of the term suggests that eutrophication could be defined as the process of changing the nutritional status of a given water body by increasing the nutrient resources. Nixon, (1995), suggested that eutrophication could be defined as an increase in the rate of supply of organic material to an ecosystem. He suggested a four tier trophic classification (Oligotrophic, Mesotrophic, Eutrophic and Hypertrophic) for primary production in marine coastal and estuarine ecosystems. This system is similar to a classification developed for fresh water by Rodhe, (1969), (Table 3.1). Nixon, (1995), states that an increase in organic material is caused by a number of factors of which nutrient enrichment is clearly the most common and significant. Also the (1991) UWWTD defines eutrophication as:

"*the enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned*".

In all these definitions, the importance of the increase in nutrients into the marine system is highlighted. A key feature associated with the increased rate of supply of nutrients is an increase in the production of organic carbon, the degradation of which can lead to major reduction in water quality e.g. reduced oxygen levels and toxic blooms.

Natural physical processes such as up-welling in which nutrient-rich deep water is transported to surface waters can provide an input of nutrients to a marine system. However, a major source of nutrients to marine coastal systems is from anthropogenic sources i.e. the land run off of nutrients into river systems from intensively farmed land, drainage from urbanised conurbations and from atmospheric deposition (Nixon, 1995). Jaworski *et al.*, (1997), estimated that there has been an increase of biologically available nitrogen fluxes to coastal rivers of the North-Eastern United States of between 5 to 14 times above natural rates. According to Nixon (1995), the spread of the increase in anthropogenic input of nutrients is a global phenomenon. The affects on marine eutrophication are a major environmental issue, which affects coastal waters world-wide (Table 3.2).

Table 3.1.
Trophic Classification of Primary Production
(Nixon, 1995; Rodhe, 1969)

Nixon's (1995) Classification for Marine Waters	**Organic Carbon Supply** $g\ C\ m^{-2}\ y^{-1}$
Oligotrophic	<100
Mesotrophic	100-300
Eutrophic	301-500
Hypertrophic	>500
Rodhe's (1969) Classification for Lakes	
Oligotrophic	7-25
Eutrophic (natural)	75-250
Eutrophic (polluted)	350-700

Although nutrient enrichment is one of the most important stressor that leads to eutrophication, there are a number of other important stressors. Cloern, (2001), showed a conceptual model of the coastal eutrophication problem which illustrates these additional stressors. The model also demonstrates that the system-specific attributes of a marine environment, determines the response of that environment to either a single or multiple stressors.

Table 3.2.
Examples of Eutrophic Regions

Regions	References
Black Sea	Mee, (1986)
Baltic Sea	Nehring, (1992)
Archipelago Sea (Northern Baltic)	Bonsdorff *et al.*, (1997)
Irish Sea	Allen *et. al.*, (1998)
North Sea	Richardson & Jorgensen, (1996)
Wadden Sea	Cadee, (1984)
Adriatic	Purdie, (1996)
China (Data from 8 river/estuarine systems)	Zhang, (1996)
Gulf of Mexico	Vollenweider, (1990)
Chesapeake Bay	Cloern, (2001)
Mississippi River	Rabalais *et al.*, (1996)
San Joaquin River	Kratzer and Shelton, (1998)
Vilaine River	Moreau *et al.*, (1998)
Peel-Harvey Estuary (western Australia)	Mc Comb *et al.*, (1981)

This model is based on five fundamental questions: -

1. How do system-specific attributes constrain or amplify the responses of coastal ecosystems to nutrient enrichment?

2. How does nutrient enrichment interact with other stressors (toxic contaminants, fishing harvest, aquaculture, non-indigenous species, habitat loss, climate change and hydrological manipulations) to change coastal ecosystems?

3. How are responses to multiple stressors linked?

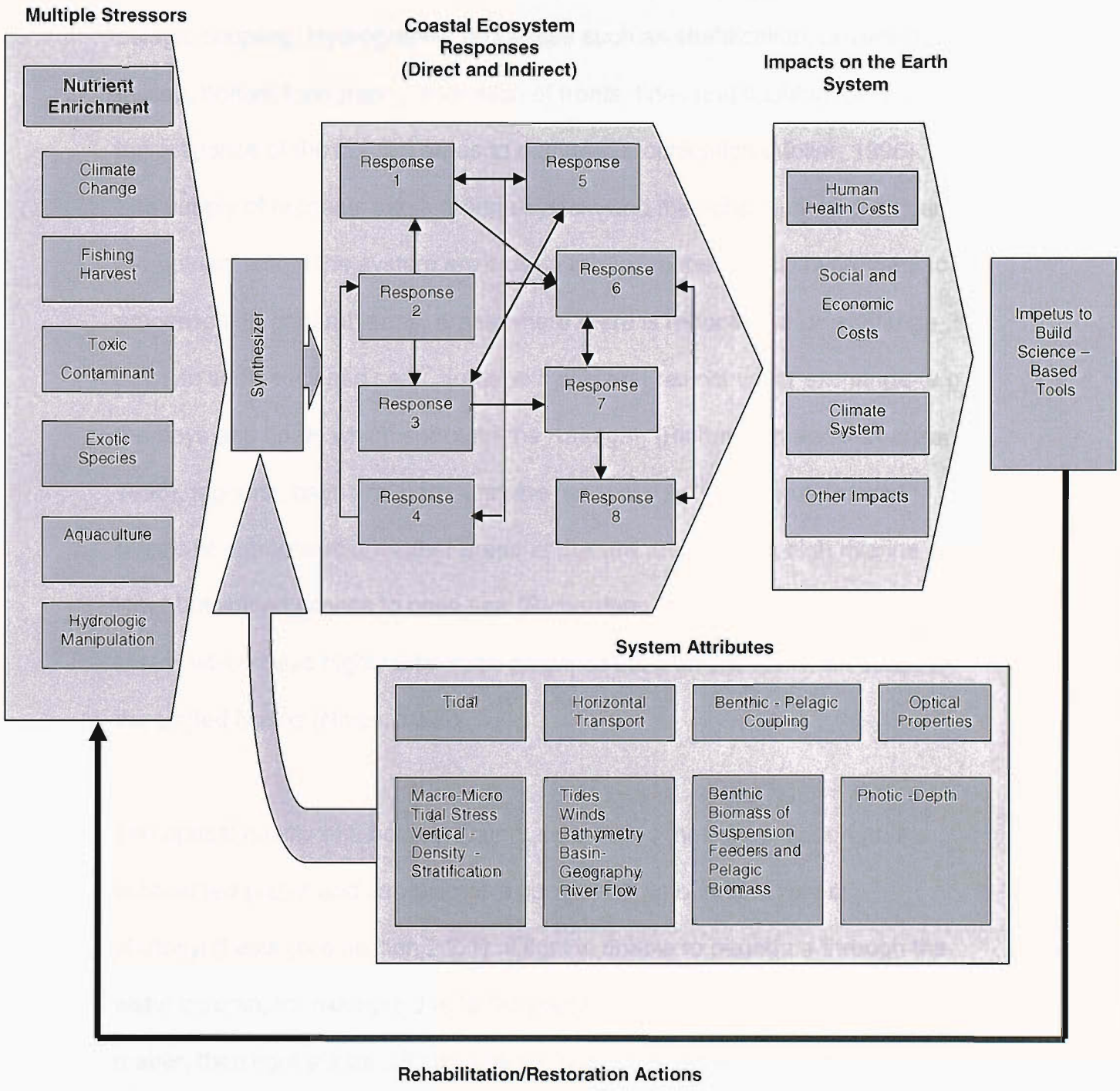
4. How does human-induced change in the coastal zone impact the Earth systems as habitat for humanity and other species?

5. How can a deeper understanding of the coastal eutrophication problem be applied to develop tools for building strategies for ecosystem restoration or rehabilitation?

In this model Cloern, (2001), refers to the system specific attributes as a filter. The presentation of the model here (Figure 3.1) has been adapted by replacing the "filter" box with a "synthesizer" box and by defining the system specific attributes. This adaptation of the model provides a clearer definition of the system specific attributes. In addition, it illustrates how these system attributes, when synthesized with either single or multiple stressors produce a set of responses that will either intensify or weaken their effect on the marine environment.

Cloern's, (2001), use of the term "Filter" is misleading, as it implies that the system attributes are in themselves the filter which may prohibit some of the stressors from causing a response. This is untrue as there will always be a response, either positive or negative, in the marine system due to one or more stressors regardless of the system attributes. It is the magnitude and nature of response that will be controlled by the system attributes.

Figure 3.1.
Conceptual Model of Coastal Eutrophication Demonstrating the Relationship between the Multiple Stressors and their Impact on the Environment.
(Adapted from Cloern, 2001)



3.2 System Attributes

Important factors, which affect marine eutrophication, are water circulation and stratification, optical properties and the biological component of benthic-pelagic coupling. Hydrographic processes such as stratification, advection, mixing, bottom topography, formation of fronts, tides and flushing rates affect the response of the coastal areas to marine eutrophication (Moller, 1996). The supply of nutrients into a marine system and the subsequent behaviour of the nutrient within the system are closely related to these local hydrographic processes. In general terms, areas where there is reduced water exchange such as semi-enclosed seas, fjords with sills that restrict water exchange (e.g. the bays and fjords which surround the Kattegat, (Richardson and Jorgensen, 1996), lagoons, bays and harbours) are more at risk from the undesirable effects of eutrophication. Other areas at risk are areas with a high riverine input but limited access to open sea (Richardson and Jorgensen, 1996) or areas, which have highly urbanised estuaries such as the northeast coast of the United States (Nixon, 1995).

The optical quality of a body of waters determines the light exposure to the submerged plants and can therefore control the amount and rate of photosynthesis (see section 2.2.1). If light is unable to penetrate through the water column, for example due to the presence of high loads of suspended matter, then light will be a limiting factor in the production of new biomass regardless of the amount of nutrients present.

Benthic-pelagic coupling is another important system attribute that can determine the system's response to single or multiple stressors. The rate of consumption by suspension feeders (Cloern, 2001) can have a major impact in marine systems. Herman *et al.*, (1996), carried out simulation experimentation that suggested that a doubling of nutrient loading would only produce small increases in phytoplankton biomass. This is due to the resulting increase in the consumption of phytoplankton biomass by suspension feeders. Cloern, (2001), also cited an example where the colonization of non-indigenous suspension feeders caused a 5-fold decline in primary production in San-Francisco Bay in 1987.

3.3 Direct and Indirect Responses of Eutrophication.

Gray, (1992), defines a model of the effects of eutrophication. The model consists of the following levels: Enrichment phase, Initial effect, Secondary effect, Extreme effect and Ultimate effect.

3.3.1 The Enrichment Phase.

In this phase macroalgae biomass increases (Twilley *et al.*, 1985; Cambridge *et al.*, 1986; Borum, 1996; Kinney and Roman, 1998) and phytoplankton biomass is increased (Boynton *et al.*, 1982; Nixon and Pilson, 1983; Monbet, 1992; Borum, 1996). The increase of biomass results in an increase of organic sedimentation, which in turn causes an increase of benthic biomass. The increase of both the benthic and pelagic biomass with the resulting increase of organic material in the marine system increases the food source

for fish, which may also result in an increase of fish biomass. Richardson and Jorgensen, (1996), cite suggestions in Tatara, (1991), and Larsson *et al.*, (1985), that the increases in fisheries' yields recorded in recent centuries are directly attributable to anthropogenic eutrophication.

3.3.2 Initial and Secondary Effects.

In the initial phase, a change of species composition may take place. Heterotrophic bacteria production will increase and will quickly recycle the dissolved inorganic nitrogen and phosphorus. Due to the lower recycling efficiency of silicon, the surface waters may become depleted of dissolved inorganic silicon and thus initiate a change of species composition. Any induced change in species composition may affect the energy flow within the ecosystem (Richardson and Jorgensen, 1996). A secondary effect is the decrease in the depth of light penetration in the water column. This is caused by the increase in plant biomass and may subsequently alter the depth distribution of benthic plants (Borum, 1996). The formation of a rapid bloom of single species phytoplankton is another secondary effect (Richardson and Jorgensen, 1996). These blooms, which may also be toxic, cause visual pollution for recreational users (Officer and Ryther, 1980). Beaches may be covered with foam and noxious slime deposits (Purdie, 1996). This has been experienced on beaches in North Wales, the Netherlands and Belgium and also in many coastal regions of the Adriatic Sea (Purdie, 1996). Hypoxia is another secondary effect. The increased biomass caused by eutrophication as already stated causes an increase of organic material, which sinks to the bottom. Heterotrophic bacteria will decompose the organic bacteria resulting

in the recycling of dissolved inorganic nutrients. However, during this process the oxygen in the surrounding waters will be consumed (Richardson and Jorgensen, 1996).

3.3.3 Extreme and Ultimate Effects.

In the extreme phase, the effect of oxygen depletion and in some cases hydrogen sulfide (H_2S) production can cause species mortality. In severe cases, hypoxia may lead to anoxia (zero oxygen concentration) and the enhanced production of H_2S causing the formation of mats of sulphur reducing bacteria on the sea floor (Richardson and Jorgensen, 1996). These conditions may ultimately result in mass mortality.

The repeated exposure to hypoxia over a number of years hinders the ability of the benthic fauna to re-establish itself thus impacting the marine ecosystem even further (Richardson and Jorgensen, 1996).

3.4 Monitoring Coastal Waters

It has thus been shown that the effects of eutrophication can have a significant negative impact on some coastal marine ecosystems. The many biogeochemical and physical processes, which result in these effects, make it difficult to monitor and predict future events. Models, such as that shown in Figure 3.2, need to be generated in order to predict future eutrophication events and help to manage systems, thus minimising the impact of the eutrophication event. In order to understand the processes which cause these

events we need to be able to measure key *in-situ* biogeochemical parameters at a high temporal and spatial resolution in addition to the physical parameters which are already routinely measured at these resolutions. This information would not only improve our conceptual model of the effect of nutrient dynamics on marine phytoplankton communities but would supply the high quality data required for creating and validating the relevant numerical models with a prognostic capability. Nutrients are thus a particularly important target for high resolution monitoring because of their fundamental importance to the ecosystems and their potential to be modified by Man.

Traditional techniques for monitoring nutrients typically involve the collection of daily, weekly or monthly samples taken from a set of estuarine, coastal or shelf sea stations. Samples are then preserved and analysed later in the laboratory. Other strategies involve cruises where samples are collected from either water bottles or via an on-board flow-through system and the samples are either analysed in the ship's laboratory or preserved and analysed later on-shore. These methods are expensive, labour intensive and by their very nature, can only supply limited data sets. Sampling regimes which operate continuously over a long period of time at a high resolution give rise to logistical difficulties and can also place a great deal of strain upon the analytical facilities. It is also likely that the traditional monitoring techniques will miss short-term transients such as rainfall and storm effects which can impact phytoplankton blooms (Gallegos *et al.*, 1992; Balls *et al.*, 1997). There is therefore a clear requirement for *in-situ* nutrient analysers capable of measuring at a high temporal resolution.

There are now a number of instruments available that claim to be able to reliably measure *in-situ* nutrients. Chapter 4 gives a brief overview of the traditional laboratory methods of nutrient analysis and investigates a sample of the available *in-situ* instruments. Bench and field-testing of one of the instruments is also described.

4.0 Nutrient Analysis

Nutrients have been measured routinely for over seventy years and a wide range of approaches are taken. Examples of some of the different approaches used for the determination of nutrients is shown in Table 4.1.

4.1 Colorimetric Nutrient Analysis.

In colorimetric analysis the chemical component of interest is engaged in a chemical reaction, which converts it in to a coloured compound. Development of the method requires finding conditions, which generate the coloured compound exclusively or with minimum interference from other likely components of the original sample or in the reaction mixture. Conditions are further adjusted so the amount of colour produced can be directly related to the original concentration of the analyte.

A light, of appropriate wavelength corresponding to the absorbance spectrum of the compound formed, is then passed through the coloured solution. The logarithm of the ratio of light intensities before and after passage through the solution is called the absorbance. The absorbance is a linear function of the concentration of the absorbing component (for optically thin solutions) in accordance to Beer-Lambert's law: -

$$\text{Log } \frac{Io}{I} = \varepsilon.c.d \qquad (4.1)$$

Where *Io* is the initial light intensity, *I* is the intensity after passing through the solution, ε is the molar absorptivity, *c* is concentration of absorbing compound and *d* is the optical path length (Grasshoff *et al.*, 1999).

Table 4.1
Important Examples of Automated Methods Used for the Determination of Nutrients in Seawater

Colorimetric					
Method		**Nutrient**	**Advantages**	**Limitations**	**Reference**
Laboratory	Segmented Flow Analysis	Nitrogen, Phosphorus and Silicon	Introduction of automated sampling. Could handle large number of samples in exactly the same way.	Samples need to be carefully handled.	Skeggs, (1957)
Laboratory	Flow Injection Analysis	Nitrogen, Phosphorus and Silicon	Faster than Segmented Flow Analysis.	Reduced Sensitivity as absorbance measured before completion of colour reaction.	Ruzicka and Hansen, (1975); Beecher *et. al.*, (1975)
Laboratory	Reverse Flow Injection Analysis	Nitrogen, Phosphorus and Silicon	Used if large volume of sample are available.	Requires large volume of sample.	Johnson and Petty, (1982)
In-Situ	Osmotic Continuous Flow Analysis	Nitrogen	Uses on-board standard to correct base line drift. Mechanically simple and has low power budget.	Slow response time and sensitive to temperature changes.	Jannasch *et. al.*, (1994)
In-Situ	Flow Injection Analysis	Nitrogen	Accurate and precise.	Uses large volume of chemicals. Needs further field-testing to determine durability and stability during long-term deployments.	Daniel *et.al.,*(1995); David *et.al.,* (1998.)
In-Situ	NAS-2EN	Nitrogen	Autonomous can be deployed in the field for up to 3 months.	Needs to improve stability of nitrate reduction column.	Rawlinson, (1997); Boyd, (1998)
In-Situ	Ultra Violet Absorbance (SUV-6)	Nitrogen	Requires no chemicals. Deploys easily for long periods of time.	Unstable. Interference for organic material.	Finch *et.al.* (1998)
Fluorometric					
Laboratory	Segmented Flow Analysis	Ammonia	Very Sensitive. Easy method with only one reagent.	Not autonomous and requires large volumes of chemicals.	Kerouel and Aminot, (1997)
In-Situ	Reverse Flow Injection Analysis	Nitrogen	Very Sensitive. Easy method with only one reagent.	Not autonomous and requires large volumes of chemicals.	Masserini and Fanning, (2000)
Other					
Laboratory	Biosensor	Nitrogen	Simple to use.	Need to improve stability and durability.	Larsen *et. al.*, (1997)

Originally many of the methods developed (Strickland and Parsons, 1968; Grasshoff *et al.*, 1999) were carried out manually and this is still the case in numerous laboratories today. However, for ease of use many methods have been automated and Grasshoff, (1969), pioneered the use of the Technicon Auto Analyser for use on oceanographic cruises.

4.1.1 Automated Colorimetric Nutrient Analysis

The main automatic colorimetric methods for laboratory analysis of nutrients uses segmented Continuous Flow Analysis. The continuous flow analysis technique, based on manual colorimetric methods (Strickland and Parsons, 1968), consists of a sample dispenser, pump, mixing unit and a detector linked by tubing. The sample and reagents are sequentially mixed along the flow path before entering the detector. Up to 1957 this technique was not viable due to excessive dispersion and tailing (Mee, 1986). Skeggs, (1957), introduced a flow of regular bubbles to the fluid stream to make the first successful attempt at continuous flow analysis. The bubbles which segmented the reaction stream were removed immediately before the sample entered the detector. The Technicon Corporation commercially manufactured the technique in the early 1960s. The introduction of segmented flow analysis (SFA) for the colorimetric analysis of nutrients caused an increase of activity in marine chemistry due to the increase in sample handling capacity.

The main advantage of this method over manual methods was the ability for the SFA to handle large numbers of samples treating each in exactly the

same way within strictly prescribed and maintained operating conditions (Mee, 1986).

In an attempt to produce simpler systems which could work at higher sampling rates, Ruzicka and Hansen, (1975) and Beecher *et al.*, (1975), developed the Flow Injection (unsegmented) Analysis (FIA) technique. In this technique a plug of sample is injected into a flowing carrier stream in a narrow bore tube. The sample plug disperses and mixes with adjacent reagents as it travels along the tubing to the detector. The set-up of the system is adjusted to control this dispersion to produce as sharp a peak as possible of detectable analyte species (Mee, 1986). Johnson and Petty, (1982), developed an alternative system, known as reverse Flow Injection Analysis (rFIA) in which a reagent plug is injected into a sample stream. The FIA method is 5-10 times faster at analysing samples than corresponding SFA systems as the colour is analysed earlier in the reaction. However, this method often has reduced sensitivity since the FIA method uses an increased speed of analysis and thus colour formation is not complete prior to colour measurement (Kirkwood, 1992).

The colorimeter detectors in most SFA systems consist of a light source (tungsten or quartz- halogen lamp), collimator lenses, fixed or movable slit, low pass interference filter, continuous flow cell and a detector (photo diode, photoresistor or phototransistor). To minimise the effect of noise and drift in the light source and power supply, a second matched flow cell with associated optics and detector may be used (Mee, 1986).

4.1.2 *In-Situ* Colorimetric Nutrient Analysis.

There are presently a number of instruments which are either commercially available or in development which, are able to measure concentrations of nutrients *in-situ*. SFA systems cannot be used *in-situ* due to shrinkage of the bubbles under pressure therefore, FIA systems and systems which do not rely on the formation of bubbles to separate samples have been developed.

4.1.3 *In-Situ* Osmotic System.

A low power FIA system was developed by Jannasch *et al.*, (1994), for long-term deployments on moorings. It has been used for the continuous determination of *in-situ* nitrate. This instrument uses osmotic pumps to propel the sample and reagents through a miniature continuous flow injection type manifold and has an endurance of at least one-month. The fluid flow in an osmotic pump is driven by the osmotic pressure differential developed across the semipermeable membrane separating a saturated brine solution from a solution of lower salinity. The net flow of water diffusing across the semipermeable membrane results in reagents being propelled out of a reagent pump and liquids being drawn into a sample pump. The osmotic pump has the major advantage of not requiring an energy source. The analyser has a sample flow rate of 12μL h^{-1} with a reagent flow of 1μL h^{-1}. The system has a linear response up to 20μM nitrate with a detection limit of 0.1 μM and a 90% response time of 30 minutes. The instrument uses an on-board standard to correct for baseline shift and loss of sensitivity due to the deterioration of the cadmium reduction column. The chemical method uses sulphanilamide and napthylethelymedihydrochloride (NED) reagents to

produce an azo dye (method adapted from Bendschneider and Robinson, 1952). This reaction takes place after the cadmium reduction of the nitrate to nitrite on a copperized cadmium surface.

Temperature change is a possible source of error for this type of instrument and rapid temperature changes may cause reverse flow resulting in the acidic sulphanilamide reagent to backwash over the cadmium-reducing column thereby desorbing the Cu from the cadmium surface. The system is also vulnerable to internal and external bio-fouling and has a slow response time. The main advantages for this technique are that it:

1. Uses on-board standards to calibrate the system *in-situ.*

2. Is mechanically simple which increases reliability.

3. Has a low power budget.

4. Is relatively small and cheap to manufacture.

4.1.4 In-Situ FIA Systems

Daniel *et al.*, (1995), describes a submersible flow-injection analyser system which, was developed for marine use *in-situ.* The instrument is deployed from a ship on an umbilical connecting cable. The umbilical delivers two power supplies, one for the electronics and one for the pump and valve system,

control instructions, data transfer and mechanical support. The instrument uses the standard azo dye technique (Bendschneider and Robinson, 1952). The cadmium reduction column is constructed of a 10cm glass tube (id 0.2cm) packed with copperized cadmium granules. The tube is plugged at each end with glass wool and has a reduction efficiency of 100%. The instrument was tested in the Bay of Brest and the results show that the instrument is capable of measuring nitrate and nitrite with an inter-calibration precision of 1.3% compared with the ship AutoAnlyser. The instrument used a sampling rate of 40 samples per hour up to a depth of 300m. The instrument has the potential of delivering data-sets with high temporal and spatial resolution. However it's main disadvantage is that presently it must be deployed from a ship with scientific support.

David *et al.*, (1998), published the results of an *in-situ* nutrient analyser, which is also based on the flow injection principle with solid-state spectrophotometer detection. This instrument was developed to remotely monitor nitrate in coastal and estuarine waters at a high spatial and temporal resolution in order to understand the link between nitrate and primary production. It uses the same chemical method as the FIA system described by Daniel *et al.*, (1995). The cadmium reduction however is via an in-line copperized cadmium powder column. The instrument has a detection limit of 0.1µM with a linear range of 0.1µM – 55µM using a 20mm flow cell path-length and a 260µL flow loop. The range can be altered by adjustment of the path-length and flow loop. The instrument is pressure tested up to 45m and during trials was deployed at 4m from the bow of the ship in the Tamar estuary and also in the North Sea. The

calibration data for the North Sea show a CV of less than 5%. The results obtained from the instrument were compared with the results obtained by the ship on-board Auto Analyser and show that the instrument over-estimated nitrate concentration at the lower ranges <0.05mg/l and consistently under-estimate at levels >0.05mg/l. The reasons given to explain these differences were that: -

1. The two reagents were mixed together in the instrument which would give a higher background level.

2. An artefact between the timing of the Auto Analyser and instrument.

This instrument has the potential was gathering nitrate data *in-situ* with a high temporal resolution. However, the results published are not as precise as the results published by Daniel *et al.*, (1995), but the main advantage of this instrument compared to the instrument described by Daniel *et al.*, (1995), is that it could be deployed autonomously. The instrument uses a large amount of chemicals and as stated in the paper, a 30-day deployment would require 20l of carrier solution and 12l of reagent. Also, no data was presented on the efficiency and durability of the cadmium reduction column. These factors could be important if the instrument is to be deployed autonomously for extended periods.

4.1.5 *In-Situ* Programmable Syringe Colorimetric System

W.S. Ocean Systems Ltd manufactures the NAS-2E instrument. The instrument is an *in-situ* autonomous nutrient analyser and uses conventional

wet chemistry and colorimetric analysis to determine nutrient concentrations. The instrument evolved from an original design developed at the Scottish Office Marine Laboratory, Aberdeen and has been commercially available for over 10 years. The instrument uses the standard azo dye technique to measure nitrate and the reduction column consists of a cadmium wire coated in copper.

The instrument has been deployed to determine nitrate concentrations (Rawlinson, 1997) and is presently being adapted to measure dissolved silicon (Boyd, 1998), ammonia and phosphate. A full evaluation of this instrument is found in Chapter 5.

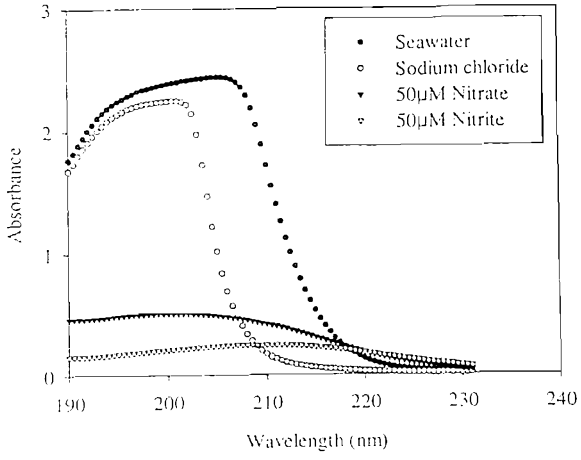
A version of the instrument is presently being developed as a shipboard flow through analyser. This instrument is called ANA, is being designed to connect to a ship's non-toxic seawater supply.

4.1.6 Ultra Violet Absorbance System

The SUV-6 is an UV nitrate detector, which is under development at the Southampton Oceanography Centre and licensed to Valeport. Finch *et al.*, (1998), reports that the instrument rapidly measures (sampling rate of 1 Hz) a number of wavelengths in the region of 220nm in order to determine concentrations of nitrate and sea salt. A channel also measures at 280nm, which, is used as a reference to correct for changes in light output and scattering by seawater particles. The instrument is comprised of a xenon flash lamp light source, fused silica windows and lenses, a sample cavity, a grating

spectrometer and UV enhanced silicon photodiode detectors. The instrument has a power consumption typically between 3 to 4W, which is dependant on the selected repetition rate.

Figure 4.1.
Absorption Spectrum versus Wavelength for Nitrate, Nitrite, Sodium Chloride and Seawater Measured in Distilled Water (Finch *et. al.,* 1998).



Laboratory calibration tests resulted in a second order linear fit (r^2 >0.99, p<0.05) with respect to nitrate concentrations. The instruments response to changing salinity also resulted in a second order linear fit (r^2>0.99, p<0.05). The salinity calibration showed that a change in salinity of 1psu resulted in a change of absorbance equivalent to 0.52μM NO_3^-. The minimum detectable change in nitrate concentration, which is a function of the signal to noise ratio, was reported to be >0.21μM NO_3^-. The instrument was also tested at sea and the results of a comparison of nitrate concentrations measured by the instrument and an on-board auto analyser show a close correspondence. When the instrument results were plotted against the auto analyser results the r^2 correlation coefficient for the linear regression of the two data sets was 0.990.

The main disadvantage of this instrument is instability of the light source. Further development work is being carried out to rectify this problem. The other disadvantage is the possible interference of the organic and carbonate absorption spectra with the halide and nitrate absorption spectra (Clayson, 2000). This is not a problem in coastal or estuarine environments as the nitrate signal is relatively large compared to the other signals. However, it is a problem in the open ocean where the nitrate signal is low. Thomas and Gallot, (1990), proposed a possible method of isolating the nitrate signal by multi-wavelength absorptimetry. Clayson, (2000), also suggested an alternative technique whereby if the salinity was measured independently, the measured salinity could be compared to the derived salinity. One could then infer that any differences may then be due to interference by organic matter. Another possible problem for this type of instrument is from bio-fouling of the optical windows.

4.2 Fluorometric Analysis

In fluorometric analysis, a light beam of ultra violet light is passed into a solution. The molecules in the solution absorb the light causing the electrons in the molecules to change from their normal low energy state in to a high-energy state. This is known as excitation. The high-energy state is unstable and the electrons relax back to the ground state and light is emitted. The light can be detected at 90° to the excitation light beam. This emitted light (fluorescence) has a longer wavelength and therefore lower frequency than the excitation light. The intensity of the emitted light detected at the lower frequency is a function of the concentration of the fluorescent component, the strength of the incident light, the quantum efficiency of the fluorophore, the

molar absorption coefficient and the thickness and length of the incident light beam.

This method is more sensitive than colorimetry. In fluorometry the signal and the relative noise is small compared to the signal size as the signal is measured relative to zero background light. In colorimetry, the detected light is the difference between the light entering and leaving the solution. Therefore, as the concentration measured decreases the signal becomes lost in the noise of the measurement of these two large signals.

4.2.1 Segmented Flow Analysis for Determination of Ammonia

Kerouel and Aminot, (1997), described a laboratory based Fluorometric method for the determination of ammonia in seawater using segmented flow analysis. The chemical method is based on the reaction of ammonia with orthophtaldialdehyde (OPA) and sulfite. The excitation wavelength is 370nm, which produces an emission peak of 425nm. The method was reported to be highly selective and therefore not subject to interference from amino acids. The salt effect in the salinity range of 0-35% was found not to exceed 3% and interference from primary amines was found to be < 0.5%. The detection limit of the method was 1.5nM and a standard deviation of 0.01µM was determined in marine samples with a concentration range of 0.5 to 5µM. The concentration range of the method can be increased to 250µM with on-line dilution.

The main advantages of this method are that Fluorometric techniques are more sensitive than standard colorimetric techniques used to determine ammonia. The method does not use toxic chemicals such as phenol and nitroprusside as used frequently in the commonly used indophenols blue technique (Grasshoff *et al.*, 1999). Interference from primary amines, naturally occurring compounds (sulfide) and salt are low. The method is also easy to use as only one combined reagent is used. However, according to Kerouel and Aminot, (1997), contamination from sample handling and laboratory air can be critical as with other standard methods of ammonia detection, especially when detecting ammonia in the nanomolar range. They also reported that due of adsorption onto solid surfaces, freshwater samples prove to be problematic. Frequent cleaning of the glassware with 1M HCL and the preparation of standard solutions with $0.2gl^{-1}$ NaCl solution overcome this problem.

4.2.2 Fluorometric Detection of Nitrate, Nitrite and Ammonia.

Masserini and Fanning, (2000), describe a rFIA instrument, which uses fluorescence-based chemistry to detect nitrate, nitrite and ammonia in nanomolar concentrations. This system was developed to measure the low concentration of inorganic nitrogen in the oligotrophic waters in the upper 10m of the central oceanic gyres in order to investigate their role in limiting phytoplankton primary production. The ultimate goal of this work was to develop a system, which could be deployed in a autonomous underwater vehicle. The chemical method first requires that the nitrate is reduced to nitrite via a copper activated cadmium column. The resulting nitrite is analysed by a

synthetic organic chemistry method, which causes the acidification of nitrite to form the nitrosium ion. The subsequent combination of this ion with a primary aromatic amine forms a diazonium ion (Seyhan, 1989). Aniline is used as the primary aromatic amine and the fluorescence of the resulting benzenediazonium ion is a function of the nitrite present. The excitation wavelength is 220nm, which produces an emission peak of 295nm. An rFIA method is used in order to correct for background fluorescence caused by interference from dissolved organic material in the sample. The use of platinum-cured silicon manifold tubing reduced contamination from leachable fluorescence contaminates. This tubing also reduced baseline drift. The dynamic range of the nitrite chemistry is 0.25 – 25µM at the lowest gain setting and 5.0 – 1250nM at the highest gain setting.

In order to detect ammonia Masserini and Fanning (2000), modified the method described by Jones, (1991). The 12µL flow cell was replaced by a 40µL flow cell and only the OPA solution and the mixture of the H_2SO_4 carrier/sample solution with the NaOH/Na citrate solution were heated instead of the whole manifold. The detection limit of the system was reported to be approximately 1nM, which was equivalent to that reported by Jones, (1991).

The throughput of the three-channel sensor package is 18 samples per hour. An inter-laboratory comparison was made between this technique and the chemiluminescence technique of Braman and Hendrix, (1989). The results of the comparison of nitrite spiked low nutrient seawater samples analysed by both methods show a r-squared value of 0.9956.

The sensor package was also field tested by attaching the sensor package and a CTD to the seawater flow-through system of a ship. It is reported that the results obtained by the sensor package show that it functioned for many hours at the same sample frequency, accuracy and reproducibility. Actual figures were not given for the field test.

The preliminary results show that this sensor package could be a valuable tool in evaluating horizontal nutrient gradients in surface oligotrophic waters. One of the ultimate aims of this instrument is to adapt it for *in-situ* nutrient analysis by an Autonomous Underwater Vehicle (AUV). However, in order to achieve this the instrument would have to be adapted for autonomous instrument control and data acquisition and storage. Also, the instrument is limited by power consumption and reagent capacity.

4.3 Other Methods of Nutrient Analysis.

4.3.1 A Microscale NO_3 Biosensor.

This sensor reported by Larsen *et al.*, (1997), contains immobilised denitrifying bacteria and a reservoir of liquid growth medium. This sensor works by reducing NO_3 to N_2O, which is then quantified by a built-in electrochemical transducer.

Electron donors and nutrients are constantly supplied to the immobilised bacteria in the tip of the sensor by diffusion through a space between the N_2O electrode and the glass casing, which is used as the medium chamber.

Interfering agents are NO_2 and N_2O. This is not thought to be a major problem as the sensor was designed to be used in environments where concentrations of the interfering agents are very low i.e. oligotrophic environments.

The sensor exhibits a linear response to nitrate in both fresh and seawater with a detection limit of ~1μM. The 90% response time to changes in nitrate concentration was found to be between 15 and 60 seconds at room temperature. Decreases in temperature, cause a decrease in the diffusion coefficients with results in longer response times. The lowest operating temperature is 6°C.

The actual dimensions of the biosensor determine the operating range of the sensor. As it is impossible in manufacture to reproduce exact dimensions, all biosensors have to be individually calibrated. This type of sensor is a valuable tool for laboratory or short-term field studies in nitrogen metabolism especially in marine and freshwater sediments. However, this type of biosensor needs further development to increase both its stability and durability before it can compete with the other chemical methods used for nitrate analysis.

4.4 Discussion and Conclusion

In summary, in order to be commercially viable and practical for marine field work an instrument used for nutrient analysis requires the following qualities: -

1. Stable, accurate and precise with a good response time (<30 minutes for coastal work).

2. The ability to work autonomously for periods exceeding at least one month.

3. Must be durable and robust.

4. Must be mechanically simple.

The instrument described by Jannasch *et. al.,* (1994), meets most of the requirements but has a slow response time and is temperature sensitive. The Daniel *et.al.,* (1995), submersible flow-injection instrument is accurate and precise but is not autonomous and has a high manual support overhead. The David *et. al.,* (1998), nutrient analyser is not as precise as Daniel *et.al.,* (1995), analyser but is autonomous. However, this instrument needs further field-testing to determine its durability and stability in long-term deployments (at least one month). A drawback of this instrument is the large volume of reagents it requires in the set-up reported by David *et.al.,* (1998).

The SUV-6 instrument has the great advantage of being a "sensor" type instrument which requires no chemicals and can be easily deployed autonomously for long periods. However, this instrument is too unstable as yet for coastal work. This instrument will also be vulnerable to bio fouling.

The fluorometric methods described by Kerouel and Aminot, (1997), and Masserini and Fanning, (2000), are more sensitive than the colorimetric

instruments but as yet both these instruments are laboratory or ship based and require daily maintenance. The Kerouel and Aminot, (1997), method has the potential to be easily adapted for use in other platforms such as the NAS-2E.

The biosensor described by Larsen *et.al.,* (1997), requires further development in order to improve its stability and durability. This type of instrument is also not autonomous and is labour intensive.

Finally, the NAS-2EN nitrate and NAS2-ES silicate analysers are autonomous instruments with a low manual and chemical overhead. This instrument is relatively mechanically simple compared to the FIA and rFIA systems. They are self-calibrating using an on-board standard and can be deployed for up to two months. These instruments do however have stability problems but have the potential of meeting all of the requirements previously listed. A full evaluation of the nitrate NAS-2EN and Silicate NAS-2ES instrument is given in Chapter 5. It was decided to concentrate future development and field-work on the WS Oceans Ltd. NAS platform as:-

1. The NAS-2EN instrument had been commercially available for the previous 8 years.

2. WS Ocean Systems Ltd, the company who produced the instrument, were offering studentship support to further develop the NAS platform.

3. Access to instruments and technical support was available.

5.0 An Overview of the W.S. Ocean Systems Nas-2EN.

This chapter contains a review of the NAS-2EN instrument, which is manufactured by WS Ocean Systems Ltd. The instrument has evolved from an original design developed at the Scottish Office Marine Laboratory, Aberdeen (Rawlinson, 1997) to measure concentrations of nitrate *in-situ.* The chemical reagents, instrument components and the standard manufacturer's method will be presented. Published results from previous deployments will be reported and the results obtained from fieldwork carried out as part of the PROVESS project will be reported in Chapter 6.

A review of the dissolved silicon NAS-2ES instrument will also be presented along with the results obtained by this instrument during the PROVESS deployments.

5.1 The Nitrate NAS-2EN - Standard Manufacturer's Method

5.1.1 Chemical Reagents

Sulphanilamide: Dissolve 10g in 200ml of 50% HCl, dilute to 1l with distilled water, and then add 2ml BRIJ-35 wetting agent (30% solution).

Napthylethelynedihydrochloride (NED): Dissolve 1g in 1l distilled water.

Ammonium chloride: Dissolve 20g and 0.2g copper sulphate in 1l of distilled water.

Low Nutrient Seawater (LNS): aged surface seawater containing less than 0.1 µM of nitrate plus nitrite.

Refresh: An amount of a primary standard containing 10.00 mM of nitrate as potassium nitrate in distilled water is added to the LNS to give a nitrate concentration at the upper end of the range expected for the observations to be carried out. This is periodically passed over the cadmium column to help maintain columns reduction efficiency.

On-board Standard: For marine applications the On Board Nitrate Standard (OBS) is prepared in LNS. Ideally, the concentration of the 0n-board standard should be chosen to reflect the upper range of the expected concentration of nitrate in the deployment site.

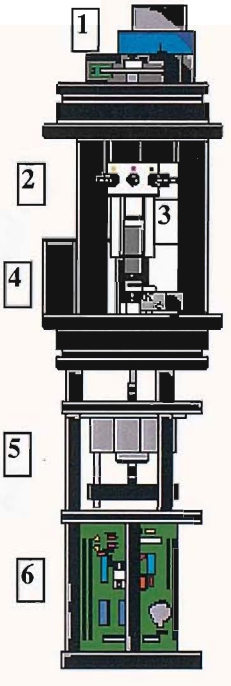
Cadmium wire column: the column is constructed by wrapping 125cm cadmium wire (1mm diameter wire) around a 27.5cm length of the same diameter cadmium wire. The wire column is then gently pushed inside a 41.6cm length of Nalgene tubing. The column is activated by passing a hydrochloric acid solution over the column to remove any grease or dust particles, which would stop the copper from coating the column. A copper sulphate solution is then slowly passed over the column followed by a seawater wash, which removes any loose copper

particles. The column is then filled with the ammonium chloride buffer solution, which also contains copper.

5.1.2 Instrument Components

The NAS-2EN instrument is a colorimetric electro-mechanical device for the measurement of discrete samples. The NAS-2EN instrument is constructed of five main components (Figure 5.1) namely an eight way rotary valve, a motor driven syringe, a colorimeter, reagent housing, and an electronic housing unit.

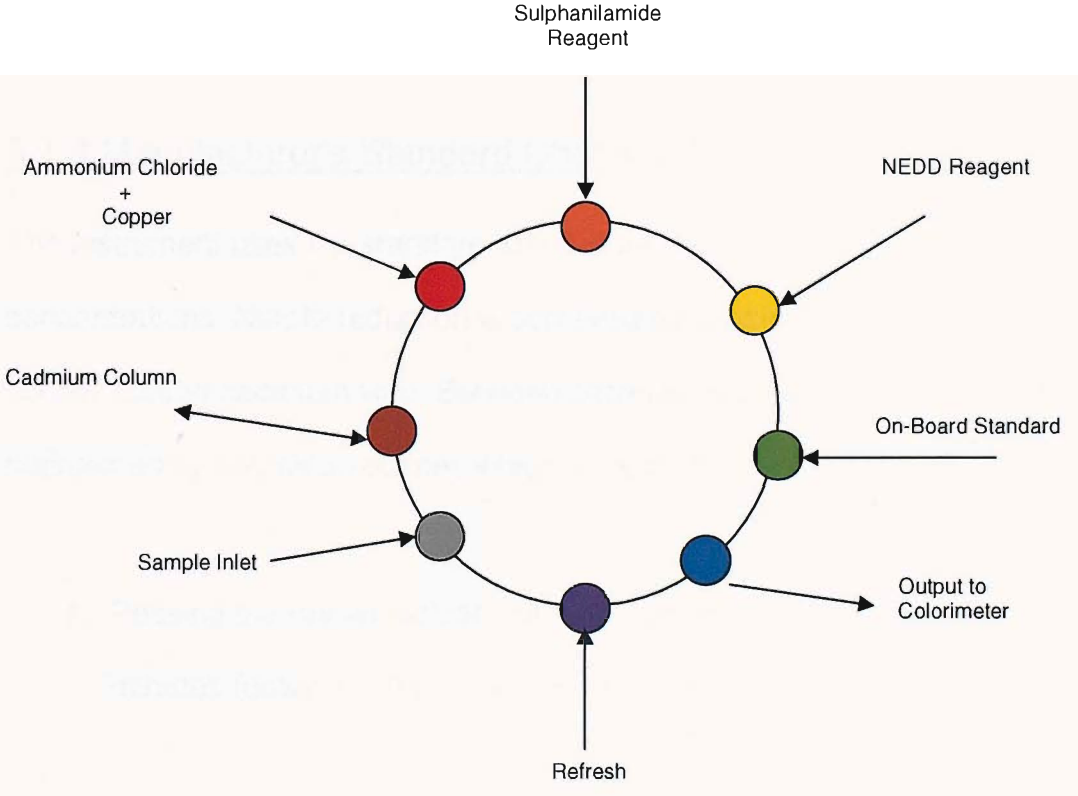
Figure 5.1.
Diagram of mechanical structure of a NAS-2E (Hydes *et.al,* 1999).
1. Stepper motor. 2. Multi-port valve 3. Syringe 4. Colorimeter 5. Syringe drive motor. 6. Control electronics



The instrument is about 80cm long, 21cm in diameter and weights ~10Kgs. The chemical system is essentially pressure balanced as all the chemical circuitry is at ambient pressure and external to the electronic instrumentation. The depth rating of the instrument is 100m, which is the pressure rating of the electronic housing.

A pressure sealed "cap" encloses the stepper motor. A reagent housing is then attached to this "cap". The reagents are stored in plastic "transfusion" type bags within this container and connected via tubing to a corresponding valve on the multi-port valve unit (Figure 5.2)

Figure 5.2.
Diagram Demonstrating the Configuration of the Multi-Port Valve for the NAS-2EN Instrument



Control and retrieval of data can be achieved by direct connection of a PC-computer through a RS232 communications port on the instrument, or remotely by means of telemetry. Instructions can be given either by a menu driven program, or by means of a command language (PROCOM).

A macro program controls the chemical routines. Each instrument has the manufacturers pre-defined macro pre-programmed into the instrument. However, the user can override this macro by installing a user defined macro routine.

The colorimeter consists of a narrow linear capillary tube with a LED source at one end and a photodiode detector at the other. The matching LED source and matching photodiode in the nitrate instrument has a peak response of 590nm. An additional detector is placed adjacent to the source to monitor and correct for changes in LED intensity.

5.1.3 Manufacturer's Standard Chemical Routine

The instrument uses the standard azo dye technique to determine nitrate concentrations. Nitrate reduction is achieved by passing the sample over a copper coated cadmium wire. Between seawater samples column stability is maintained by chemical routines which consist of:-

1. Passing the refresh solution over the column approximately every 15 minutes (determined by the sampling rate).

2. Between analyses the column is filled with an ammonium chloride buffer solution, which contains copper.

A 1ml water sample is taken by aligning the rotary valve to the inlet port then drawing the syringe plunger down. This is the blank aliquot which is then injected into the colorimeter and the value recorded. This is recorded as the sample blank and is considered to be "*Io*" in equation 4.1. After expulsion of the blank, another 1ml aliquot is taken. The sample is then pushed out of the syringe onto the cadmium reduction column. After the reduction from nitrate to nitrite has been achieved by passing the sample over the column twice, the sample is drawn back into the syringe. The valve moves around to the next ports and draws in 0.03ml of the two reagents in turn. By moving the piston up and down 4 times the sample and reagents are effectively mixed by the vortices set up in the liquid each time it re-enters the syringe cylinder. A pause of two minutes is allowed for colour formation then the solution is pushed into the colorimeter where the value is measured. The value is considered to be "*I*" in equation 4.1.

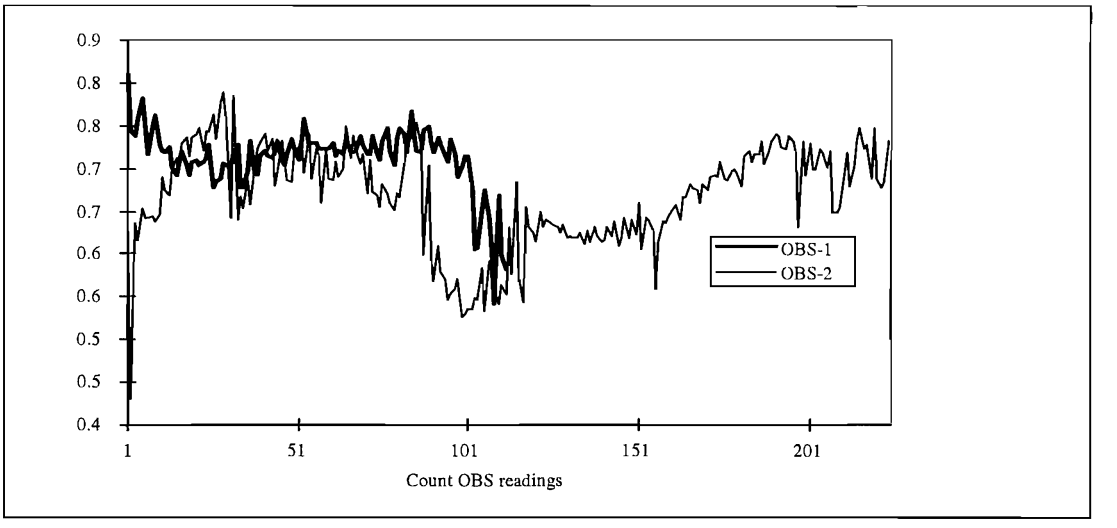
The concentration of nitrate is calculated by comparing the absorbance of the sample to the value obtained for an "on-board standard" (OBS) after correcting the measured absorbencies by the value obtained for the blank. As mentioned above, blanks are determined prior to every sample taken. OBS values are normally determined at a rate of one per six samples but this rate can be user defined. The repeated analysis of the OBS allows the user to monitor how the unit has drifted over the time. Drift can be caused by changes in the functioning of the colorimeter circuitry and because the amount of colour developed in sample will vary as the temperature of the instrument changes in response to changes in the surrounding water. In the case of the determination of nitrate this temperature drift is likely to be relatively small as the colour forming reaction is

rapid and the reaction will be near to its end point before it is introduced into the colorimeter. For the determination of nitrate the major cause of drift detected in the values obtained for the OBS samples is likely to be shifts in the efficiency of the cadmium reduction column.

5.1.4 Published Deployments of NAS-2EN Instrument

Hydes *et al.*, (2000), published results of three deployments, one off the French coast and two in Southampton water during the SONUS project (**SO**uthampton Water **NU**trient **S**tudy). During the French deployment a parallel set of samples were measured in the laboratory using standard auto-analyser methods. These samples show a coefficient of variation of 13.4% when compared to the readings taken by the NAS-2EN instrument.

Figure 5.3.
Plot of the variation with time of the absorbance measured for the On Board Standard during the deployment off the French coast - OBS-1 and the first SONUS deployment - OBS-2 (Hydes *et.al.*, 1999).



The On-Board Standard results showed high variability (Figure 5.3) with the CV equal to 5.3% for the French deployment, 8.9% for the first Southampton deployment and 13.4% for the second Southampton deployment (not graphed). This data indicates that there was a need for further developments to improve OBS stability.

5.2 The Dissolved Silicon NAS-2ES

The dissolved silicon NAS-2ES was initially developed as part of an undergraduate project (Boyd, 1998). However, vigorous field-testing and further development is still needed.

5.2.1 Chemical Reagents

Stock Molybdate: Dissolve 30g of ammonium molybdate in 1L of pure water.

Sulphuric Acid: Dilute 140ml of concentrated sulphuric acid with 900ml of pure water.

Surfactant: Dissolve 50g of Sodium Dodecyl Sulphate (SDS) in 1L of pure water.

Molybdate Reagent: Add 20ml of dilute sulphuric acid to 160ml of molybdate stock solution. Add 50ml of SDS and make up to 500ml with pure water.

Oxalic Reagent: Dissolve 50g of oxalic acid in pure water and make up to 1l.

Ascorbic Reagent: Dissolve 16g of ascorbic acid and 1ml of surfactant (SDS) in 1l of pure water.

Low Nutrient Seawater (LNS): aged surface seawater containing less than 0.1 μM of dissolved silicon.

On-board Standard: For marine applications the On Board Standard (OBS) is prepared in LNS. An amount of a primary standard containing 10.00mM of dissolved silicon as disodium hexafluorosilicate in distilled water is added to the LNS. The concentration of the standard is chosen to reflect the expected highest dissolved silicon concentration at the deployment site.

5.2.2 Main Instrument Components

The main instrument components for the dissolved silicon analyser are the same as that of the nitrate analyser. The only difference is the colorimeter and lack of reduction column. The colorimeter of the dissolved silicon analyser contains a LED source and matching photodiode detector with a peak response of 880nm. The reagents are connected to the main rotary valve as shown in Figure 5.4.

5.2.3 Manufacturer's Standard Chemical Routine

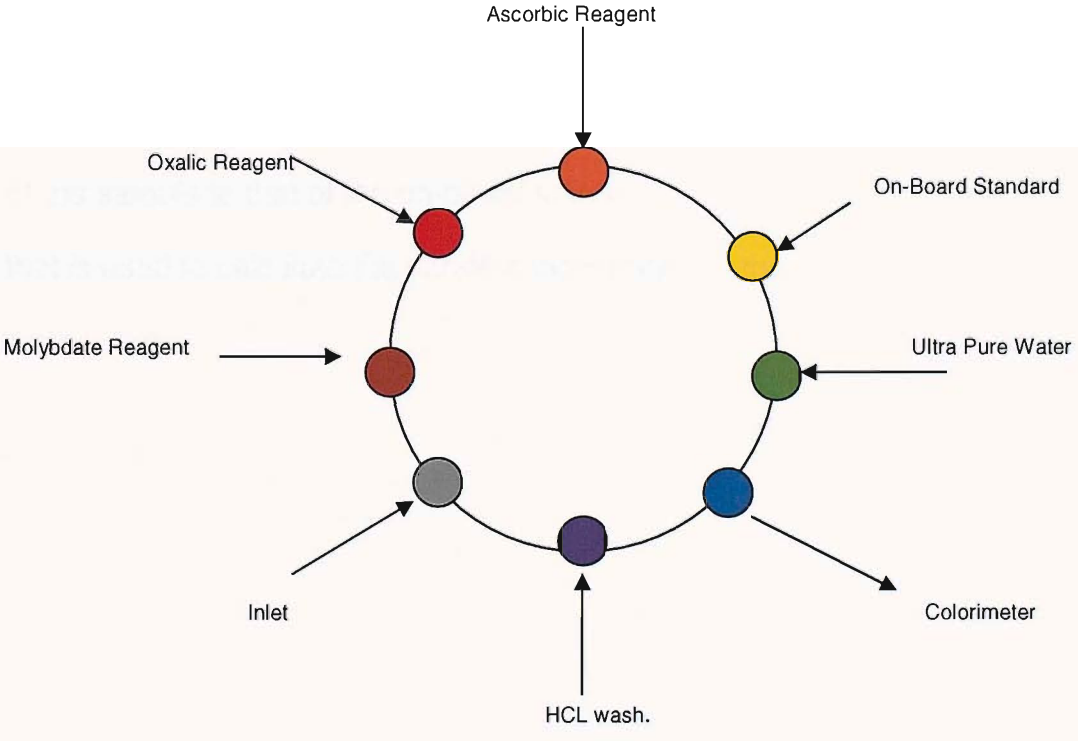
The determination of dissolved silicon is based on the formation of a yellow silicomolybdate acid when the sample is treated with a molybdate solution (Mullin and Riley, 1955). The complex is further reduced to an intensely blue complex by

the addition of the ascorbic reagent (Koroleff, 1971). The oxalic reagent is added prior to the ascorbic reagent to avoid:

1. The reduction of excess molybdate. Without the addition of the oxalic acid reagent the colour formation does not stabilize but continues to develop.

2. To eliminate the influence of phosphate present.

Figure 5.4.
Diagram Demonstrating the Configuration of the Multi-Port Valve for the NAS-2ES Instrument



A water sample is taken by aligning the rotary valve to the inlet port then drawing the syringe plunger down. This is a blank aliquot, which is then injected into the

colorimeter and the value recorded. This is recorded as the sample blank and is considered to be "*Io*" in equation 4.1. This water sample is then discarded.

0.5 ml of molybdate is drawn into the syringe to which 1ml of seawater is added. Moving the piston up and down twice mixes the molybdate and sample. After a pause of six minutes 0.5ml of oxalic reagent is added to the sample followed immediately by an addition of 0.5ml of ascorbic acid. The solution is further mixed and the procedure paused for three minutes. The solution is passed into the colorimeter and a value recorded after five seconds. This value is considered to be "*I*" in equation 4.1. The syringe and colorimeter is finally rinsed with HCL and pure water.

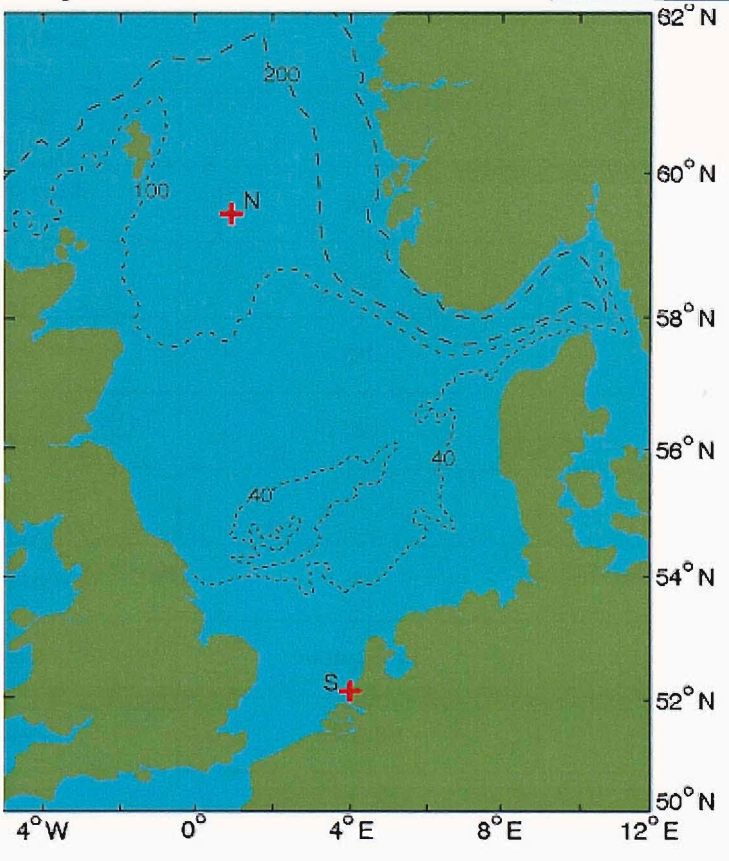
The concentration of dissolved silicon is calculated by comparing the absorbance of the sample to that of the on-board standard. This method is the same method that is used to calculate the nitrate concentration in the nitrate NAS-2EN instrument.

6.0 Field Work

6.1 PROVESS Project

The initial field-evaluation for the WS Oceans NAS-2EN instrument using the manufacturer's method was conducted during the PROVESS project (1998/99). PROVESS is a joint European funded project providing an interdisciplinary study of the vertical fluxes of properties through the water column and the surface and bottom boundaries based on the integrated application of new measuring techniques and new advances in turbulence theory and new models. The project, supported by the European Commission through the MAST III program ran from March 1998 until May 2001.

Figure 6.1.
Map Indicating the Position of the PROVESS Stations (www.pol.ac.uk/provess



Two contrasting North Sea sites (Figure 6.1) were studied as part of this project, one deep low energy site in the northern North Sea and the other a shallow high energy site in the southern North Sea (Table 6.1).

The PROVESS 1 experiment was carried out at the northern site in the North Sea (Figure 6.2) during the autumn of 1998. This is a low energy site with a depth of ≈100m. The water mass in this region of the North Sea, as identified by Laevastru, (1963), originates from the North Atlantic and flows south from the Shetland Islands down the Scottish and northern English coast.

Table 6.1
Characteristics of PROVESS Stations (www.pol.ac.uk/provess)

Characteristics	**PROVESS 1 SNS**	**PROVESS 2 NNS**
Position	52° 15.0′ N 4° 17.0′ E	59 ° 20.0′ N 1° 00.00′ E
Water depth (m)	16	100
M2 max amplitude (m)	0.75	0.15
Max current (m s-1)	1.0	0.6
Thermocline depth (m)	n/a	35-100
Halocline depth (m)	5-10	as thermocline
Max wind speed (m s-1)	20	25
Max wave height (m)	5	10
Max wave period (s)	8	10
Internal motion	No	Yes
Sediment	muddy-sand	muddy-sand
Biology	eutrophic	oligotrophic

This is a relatively oligotrophic region with nitrate concentrations ranging from a summer minimum of <1μM to a winter maximum of <10μM (Lee, 1980; Brockmann and Topcu, 2001).

The second experiment was carried out at the southern site of the North Sea during the spring of 1999. This is a high-energy area, which is shallower than the northern site with a depth of ≈16m. The water mass in this area is Continental Coastal Water (Laevastru, 1963) and flows north easterly along the Dutch Coast. The area is periodically eutrophic with nitrate concentrations ranging from a summer minimum of 10-20μM to a winter maximum of 45μM and dissolved silicon concentrations ranging from a summer minimum of 1-2 μM to a winter maximum of 20-30μM (Lee, 1980).

6.2 PROVESS 1 Deployment

6.2.1. Nitrate NAS-2EN Deployments.

The three environmental moorings which contained nitrate analysers were deployed on the 9th and 10th of September 1998. The moorings were configured such that a nitrate NAS-2EN was deployed 1m from the surface, at mid-water (52m) and 9m above the seabed. The instruments were set to sample every two hours with on-board standards (OBS) measured every 12 hours.

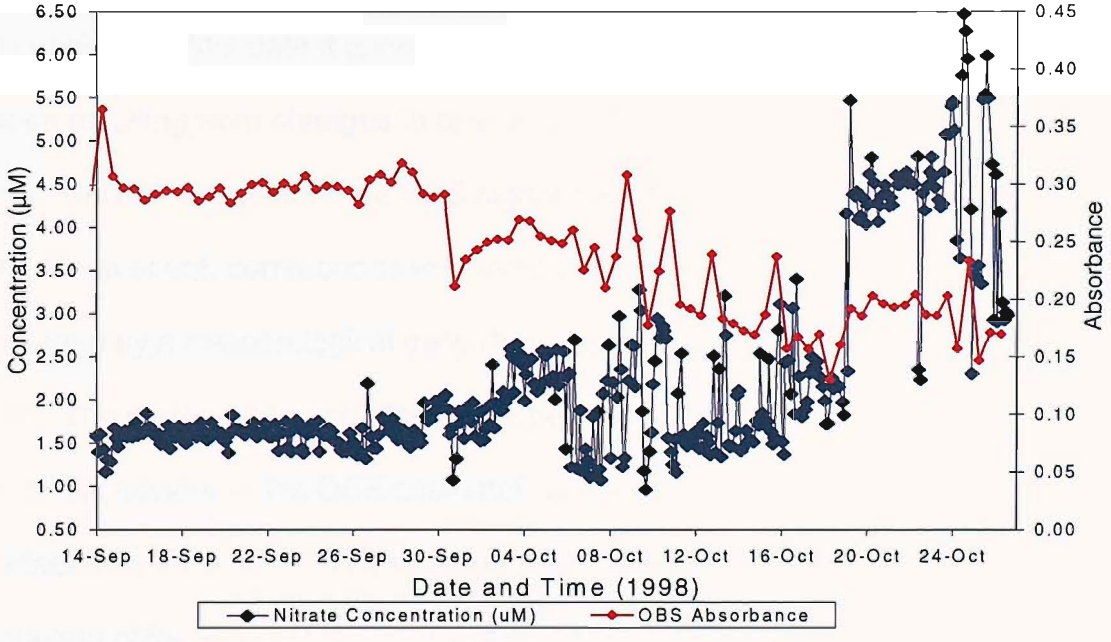
The moorings were recovered at the end of October 1998 and data from the nutrient analysers downloaded. On inspection it was discovered that the power

cable of the nitrate analyser on the seabed mooring (station J2) had been disconnected and therefore, the instrument had not started.

The data from the mid-water nitrate analyser was found to be unusable as there was no consistency in the OBS data. The cause of this problem was identified as being a faulty battery on the instruments processor board.

The nitrate analyser from the surface mooring provided a full data set from the 9th of September to 26th October (Figure 6.2).

Figure 6.2
Nitrate and OBS Data from Surface NAS-2EN, PROVESS 1.



The OBS data show that the standard was stable until the 30th of September with a CV of less than 4%. After the 30th September the OBS became less stable with

the CV increasing to above 10%. However, the OBS stabilised once again between the 19th and 23rd October with a CV of less than 4%.

The nitrate signal has a periodicity of 12 hours and the nitrate concentrations from the start of the deployment until 06:08 GMT on the 30th of September were low with an average value of 1.62µM (range 1.17µM – 2.20µM, nitrate CV of 9.21%). When the cadmium reduction column stabilised between 06:08 GMT on the 19th October and 18:08 GMT 23rd October (OBS CV 3.66%), the nitrate levels increased to an average value of 4.52µM (range 4.04µm- 5.45µM, nitrate CV 6.95%).

6.2.2. Discussion of Results for the Surface NAS-EN

In considering this data it is necessary to try to distinguish real changes from those resulting from changes in operating efficiency of the NAS-2EN instrument. From the 30th September the OBS signal becomes erratic which, except for the first storm event, corresponds with increases in the significant wave height data recorded by a meteorological buoy deployed within the PROVESS area (Figure 6.3). The blank voltages during these periods remained stable therefore the erratic behaviour of the OBS could not have been caused by air bubbles from the surface waters or any debris entering the colorimeter. A further possibility is that buffeting of the wire cadmium reduction column during storm events (Figure 6.3) may have caused some of the copper to be detached from the surface of cadmium wire thus altering the efficiency of the reduction column (see Chapter 7).

In order to try and eliminate any data which may have been adversely affected by spurious OBS the OBS data was divided into groups excluding any obvious spikes such that the CV of the OBS was less than 5% (Table 6.2). The nitrate data was only calculated if the OBS attributed to that sample was within one of the groups. Data outside these groups were discarded.

Figure 6.3.
Plot of OBS Absorbance and Wave Height Data, PROVESS 1.

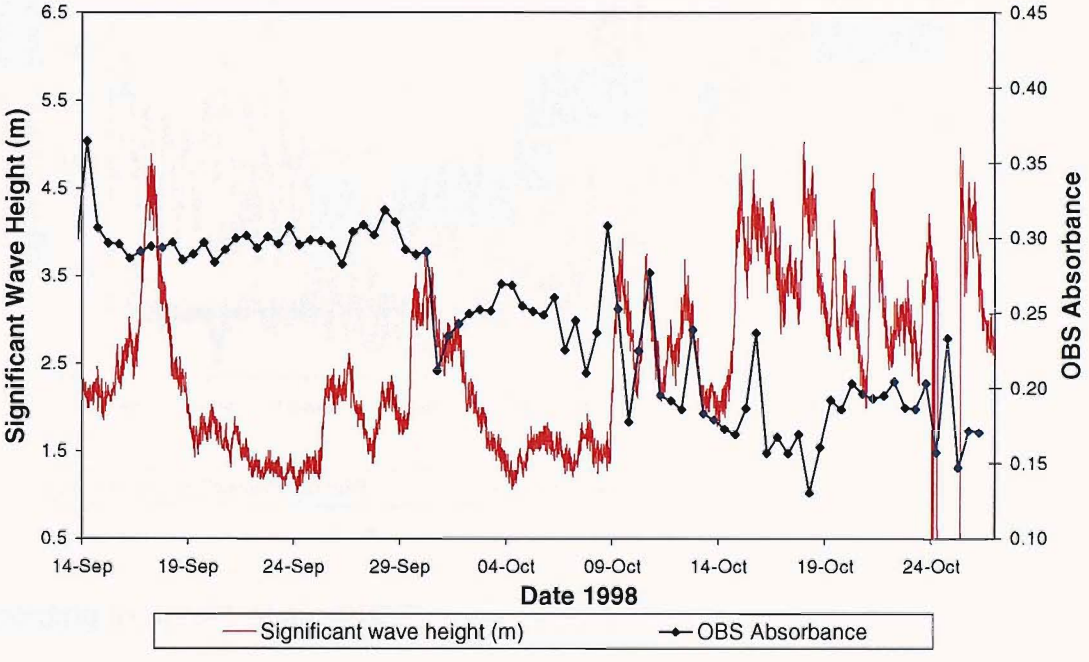


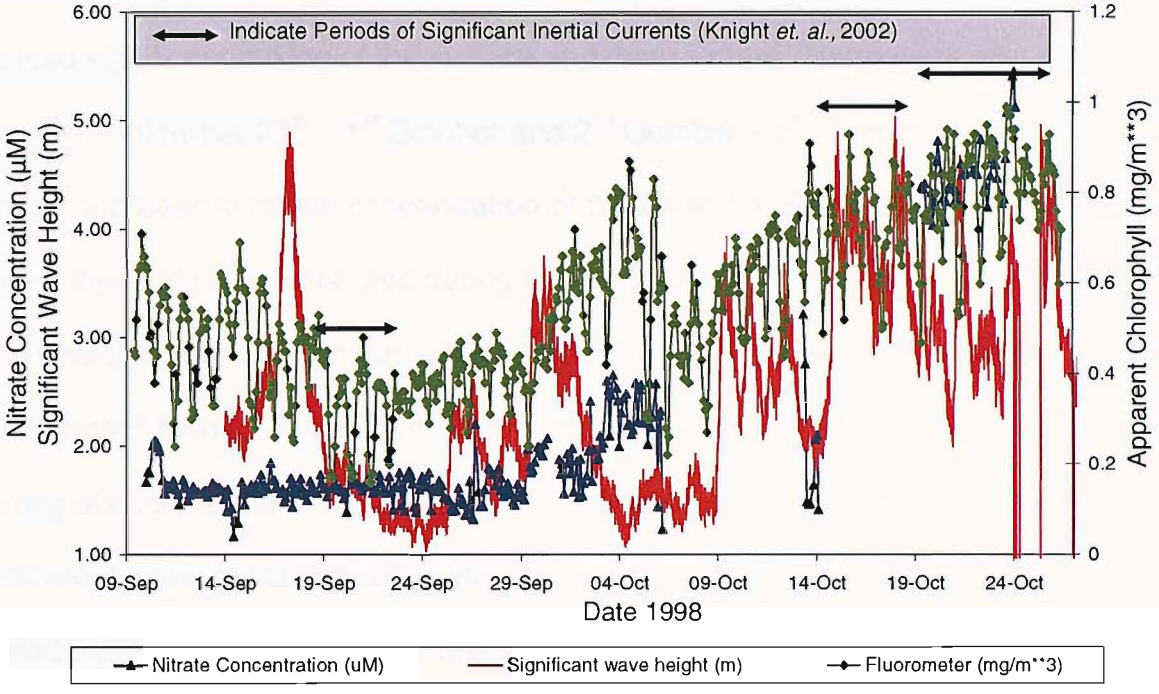
Table 6.2.
Nitrate Data with OBS CV of less than 5%

Time Period	Average Nitrate Concentration (µM)	Nitrate Range (µM)	Sample CV	OBS CV
10/09/98 – 30/9/98	1.62	1.17 – 2.20	9.21%	4.43%
1/10/98 – 6/10/98	2.11	1.23 – 2.65	16.52%	3.09%
13/10/98 – 15/10/98	1.82	1.41 – 3.21	27.03%	4.02%
19/10/98 – 24/10/98	4.52	4.04 – 5.45	6.95%	3.66%

Analysis of the subsequent data indicate that the rise in the average nitrate concentration during the deployment from 1.62µM to 4.52µM correspond with the

summer minimum levels (1 to 4µM) reported by Lee, (1980), for this water mass in the North Sea.

Figure 6.4
Nitrate, Temperature and Fluorometer Data for the Surface Waters, PROVESS1



According to Knight *et al.*, (2002), there were three episodes of large amplitude inertial currents (Figure 6.4). During these episodes the Richardson number was <1 indicating mixing across the thermocline. During the first episode (19^{th} - 21^{st} September 1998) there was no increase seen in the nitrate data. There was unfortunately no reliable nitrate data during the second episode (16^{th} – 18^{th} October) however, during the 3^{rd} episode (21^{st} – 25^{th} October), which exhibited the largest amplitude inertial currents, the nitrate data shows a significant increase in the nitrate concentration (from 1.5µM to 4.5µM). This indicates that mixing of the higher nitrate bottom waters with the surface waters occurred. The

resulting increase in nitrate in the surface waters would probably account for the increases that are evident in the apparent chlorophyll data from the fluorometer.

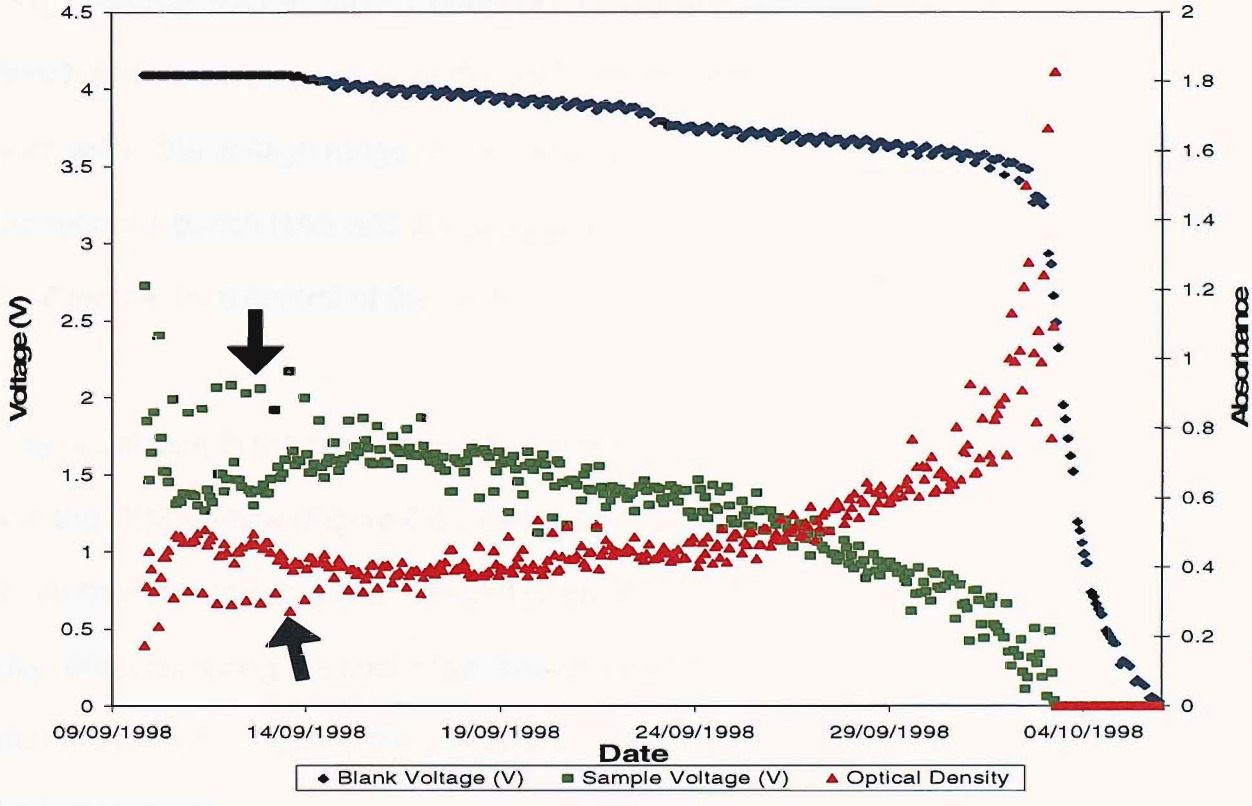
It is interesting that the first inertial current episode had no effect on the nitrate data. Possibly the duration and intensity of this episode was not sufficient to have caused significant mixing of the surface and deep waters. There were also two events (September 29th – 1st October and 2nd October – 5th October) where there was an increase in nitrate concentration of 0.5µM and 1 µM respectively. The chlorophyll data also increased during these events with the same magnitude as the nitrate data with maximum values initially increasing from ~0.48mg/m^3 to ~0.66mg/m^3 then to ~0.83mg/m^3. During these events there was no evidence of mixing across the thermocline therefore the increase was probably attributable to a different water mass with different nutrient and salinity characteristics. Unfortunately the surface conductivity meter malfunctioned so that no surface salinity data was available.

6.2.3 Dissolved Silicon NAS 2ES Deployment

The dissolved silicon NAS was deployed on a double frame along with the nitrate NAS 9m from the seabed. The instrument was deployed on the 9th September 1998 and was recovered at the end of October 1998. The instrument was set to sample every 90 minutes with on-board standards measured every 9 hours. Inspection of the dissolved silicon analyser post deployment found that the colorimeter, syringe and tubing were coated with a fine white precipitate.

The dissolved silicon analyser had started but unfortunately a clip was left on the tubing connecting the on-board standard to the rotary valve therefore, no on-board standard data was measured (Figure 6.5). The instrument measured seawater data from the 9th September up to the 3rd of October after which only zeros were recorded. The dissolved silicon concentration could not be calculated due to the absence of the on-board standard data.

Figure 6.5.
Plot of Data Downloaded from NAS2-ES, PROVESS 1.



6.2.4 Discussion of Results

The blank voltage signal (Figure 6.5) was over scale from the start of the deployment until the 13th September. After this date a slight undulation in the blank voltage is seen the blank voltage which continued to fall reaching a zero

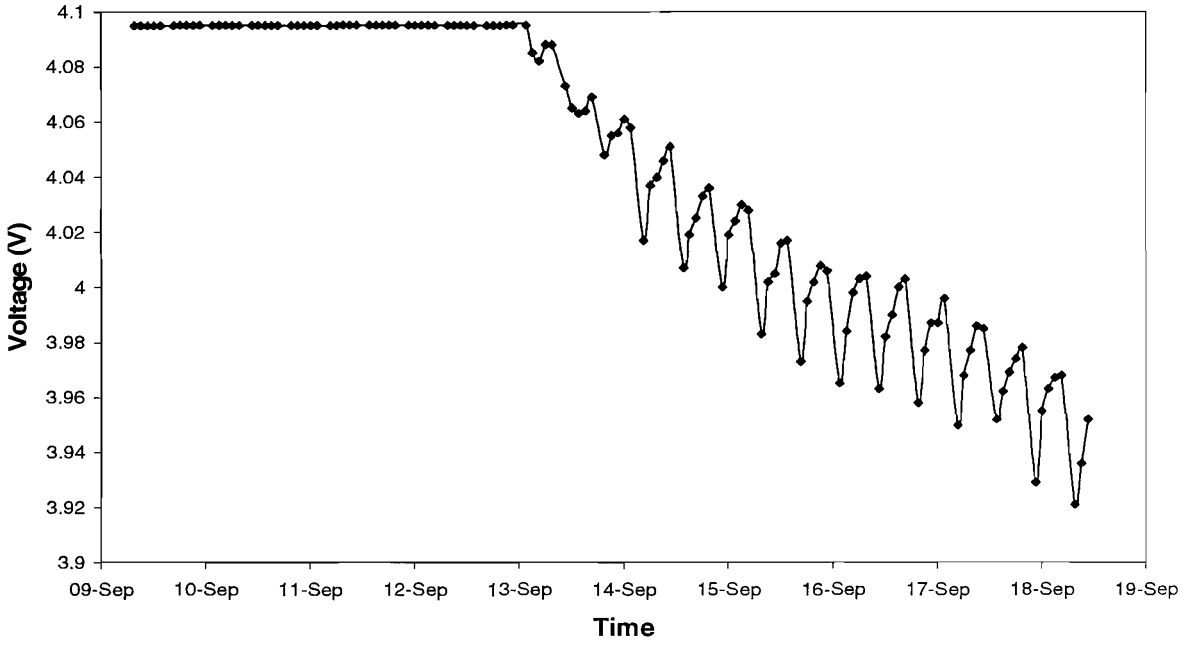
voltage by the 6th October. The instability of the blank signal is not normally observed on the NAS platform and it is suspected that this was caused by three different problems. Firstly, it is suspected that there is a problem with circuitry that controls the instrument LED drive and photo-diode detection. The circuitry that controls the colorimeter was originally designed for the nitrate NAS-2EN instrument which uses a LED with a different wavelength and intensity. Consequently, the photodiode detector which controls the LED drive current and photo diode detector sensitivity is unstable and intermittently changes the LED drive. This has the detrimental effect of causing small jumps in the blank voltage. Bench tests immediately before the deployment confirm that the blank voltages were within the voltage range of the instrument. Therefore, during the period between the bench tests and the deployment of the instrument, there was a jump in either the drive control of the LED or the sensitivity of the photo diode detector.

The undulation in the blank signal has a period of nine hours which corresponds with the OBS routine (Figure 6.6). As the OBS bag was clipped, the syringe would have contained a high concentration of reagents which may have not been fully removed during the final rinse. Therefore when the first blank is measured after the OBS routine, any residual reagent would change the optical density of the blank sample causing the blank signal to drop. The clipped OBS bag would also probably account for the spurious sample points indicated in Figure 6.5. These samples are the first sample recorded after the OBS routine.

Finally, the build-up of the white precipitate probably accounts for the large drop in the blank voltage from the start of the deployment until it finally reaches zero.

The origin and composition of the white precipitate is as yet not known. It may be precipitation from the molybdate reagent or formation of calcium oxalates when the seawater samples are mixed with the oxalic acid reagent (*pers com,* Strain). The solubility of calcium oxalate is very low.

Figure 6.6.
Blank Voltage Data on the NAS-2ES Signifying Significant Instability



In order to address these problems WS Oceans adapted the colorimeter control electronics. To try and stop the build-up of the white precipitate an acid wash was incorporated (15% HCl) into the sampling macro for the PROVESS 2 deployment and the cell was also rinsed with distilled water between samples. As an instrument was unavailable for testing, this was not trialled in the laboratory before the PROVESS 2 deployment.

The absorbance data indicates that the dissolved silicon concentration declined between the 10th and 16th September then increased rising more rapidly after the

24th September. The initial decline cannot be confirmed as the blank voltage was over scale (above 4.094) until 13th September. The dissolved silicon concentration was probably increasing over the whole deployment. This would be expected as dissolution activity from diatom skeletal material would release dissolved silicon back into the water column throughout the whole period.

6.3 PROVESS 2 Deployment

6.3.1 Nitrate NAS-2EN Deployments

The nitrate NAS-2EN instruments were deployed on 30th March 1999 and were recovered on the 18th May 1999. The moorings were configured such that one nitrate NAS-2EN was deployed 5m above the seabed and a second NAS-2EN was deployed 1m below the surface. Calibrations of the nitrate instruments were carried out immediately before deployment (Figures 6.7 and 6.8).

Figure 6.7.
Pre-Deployment Calibration of Surface NAS-2EN, PROVESS 2.

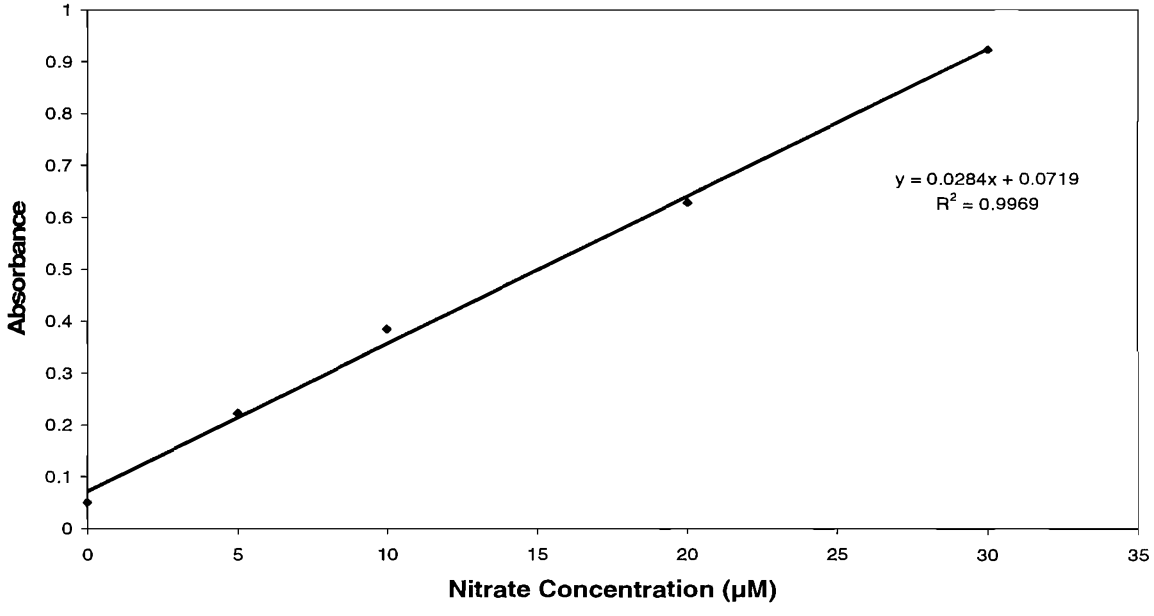
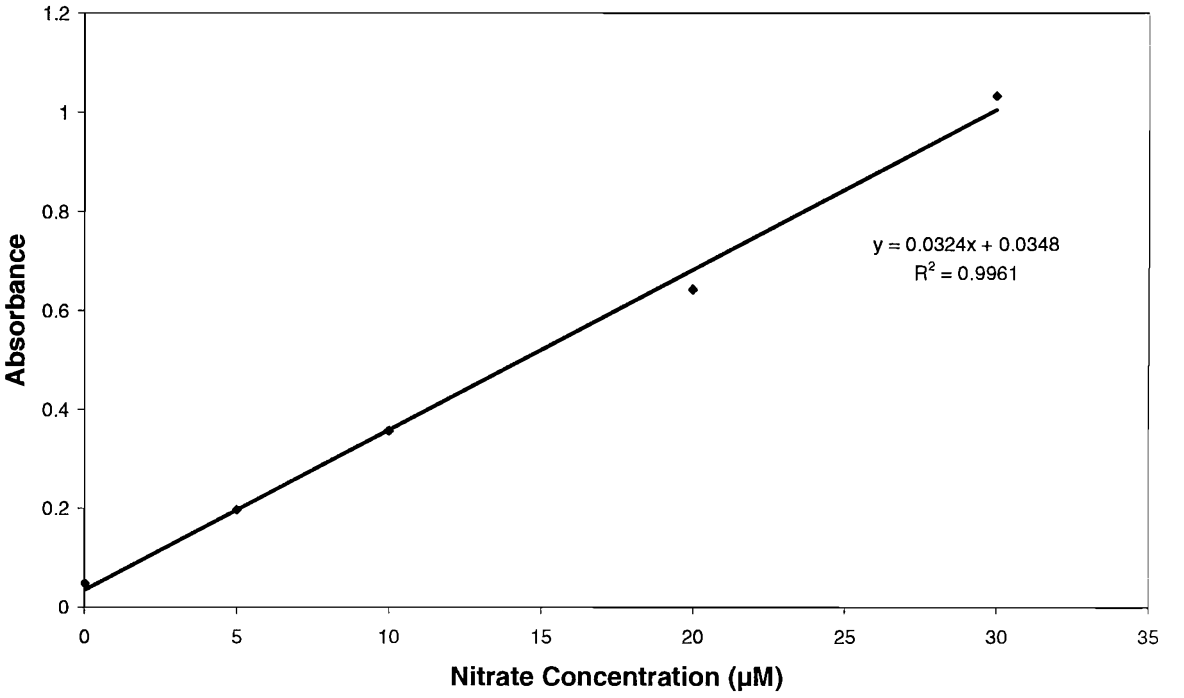


Figure 6.8.
Pre-Deployment Calibration of Seabed NAS-2EN, PROVESS 2.



The on-board standard data retrieved from the bottom nitrate NAS-2EN instrument (Figure 6.9) show that the on-board standard data was reasonably stable, with a coefficient of variation of 7.5% until 27th April. After this date the absorbance started to fall until the 17th May. This indicates that the cadmium reduction column was either becoming under-reactive and reducing less of the nitrate in the standard or was possibly becoming over reactive, further reducing the nitrite in the standard.

The on-board standard data retrieved from the surface NAS-2EN instrument (Figure 6.10) show that the absorbance of the on-board standard was unstable from the start of the deployment. The absorbance fell steadily from 0.37 on 31/03/99 to zero on the 26th of April. The cause of the poor performance of the surface instrument is as yet unknown.

Figure 6.9.
Nitrate and OBS Data from Seabed NAS-2EN, PROVESS 2

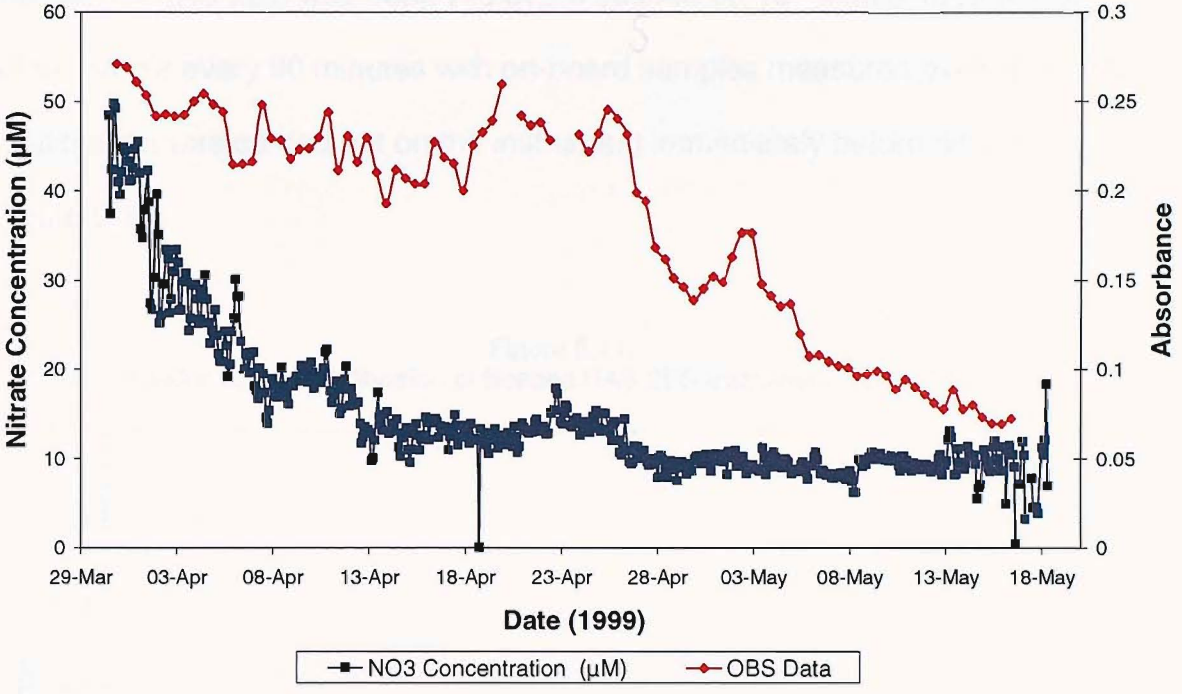
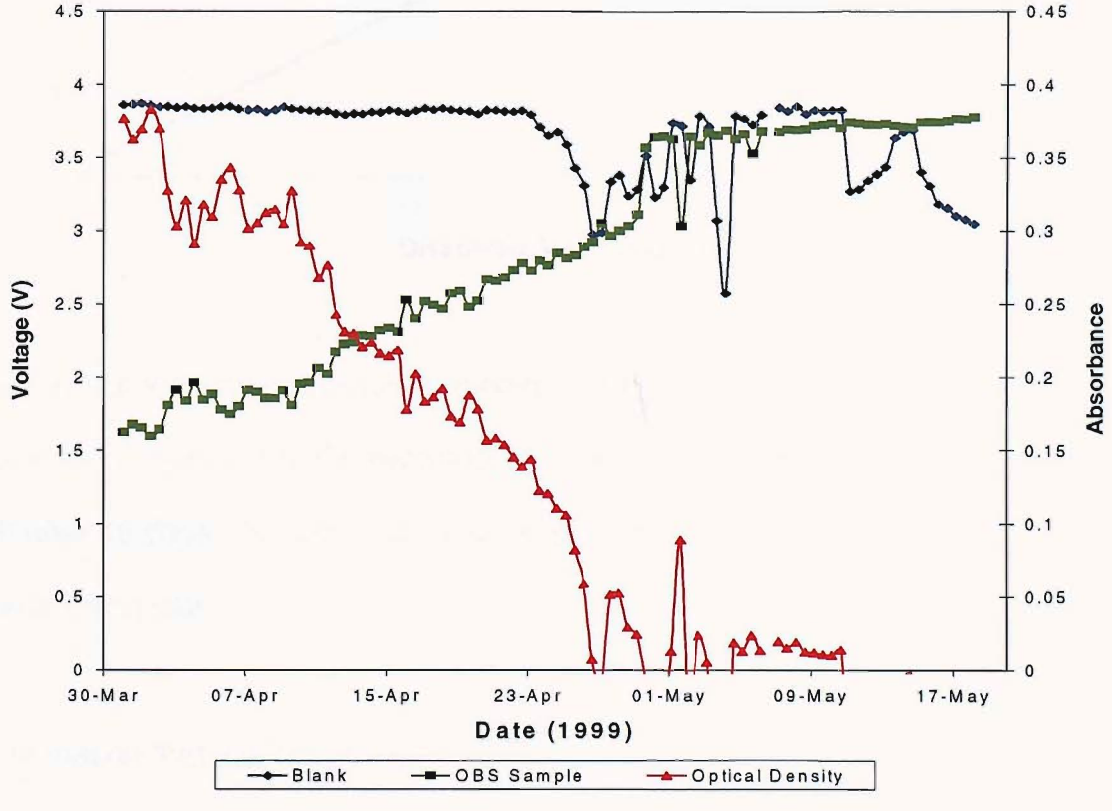


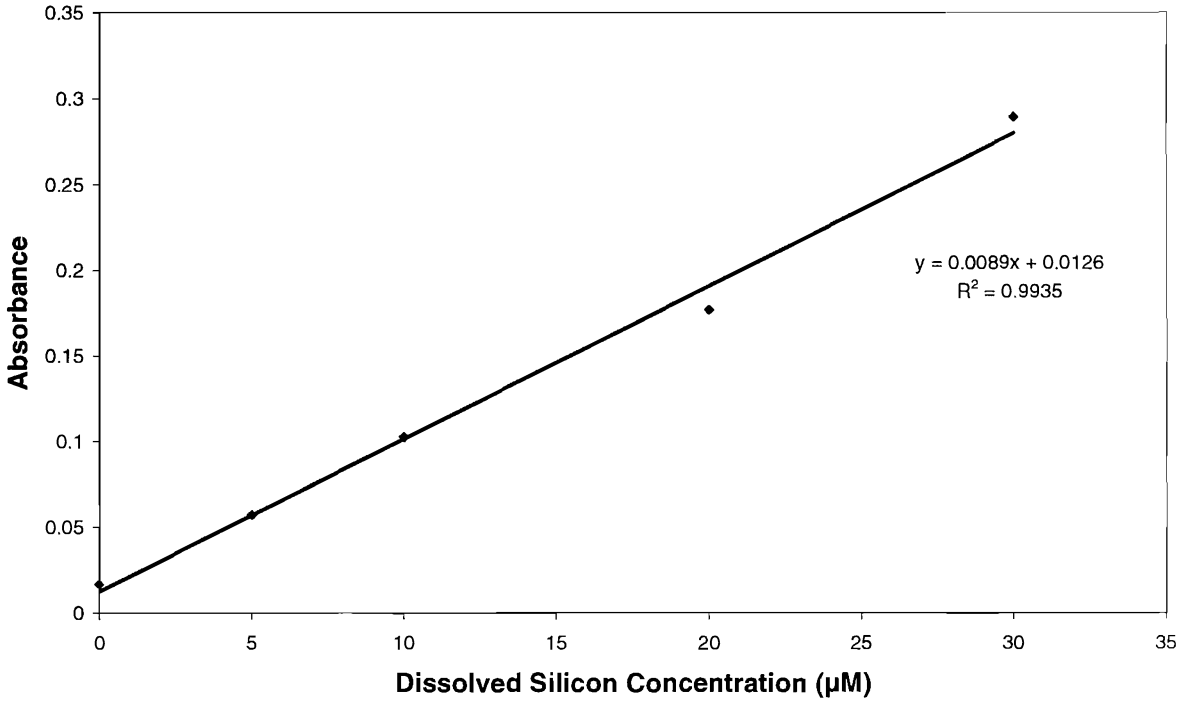
Figure 6.10.
Nitrate and OBS Data from Surface NAS-2EN, PROVESS 2



6.3.2 Dissolved Silicon NAS-2ES Deployment

The silicate NAS-2ES was deployed at the seabed on 13th March 1999 and was set to sample every 90 minutes with on-board samples measured every 9 hours. A calibration was carried out on the instrument immediately before deployment (Figure 6.11).

Figure 6.11.
Pre-Deployment Calibration of Seabed NAS-2ES Instrument, PROVESS 2.

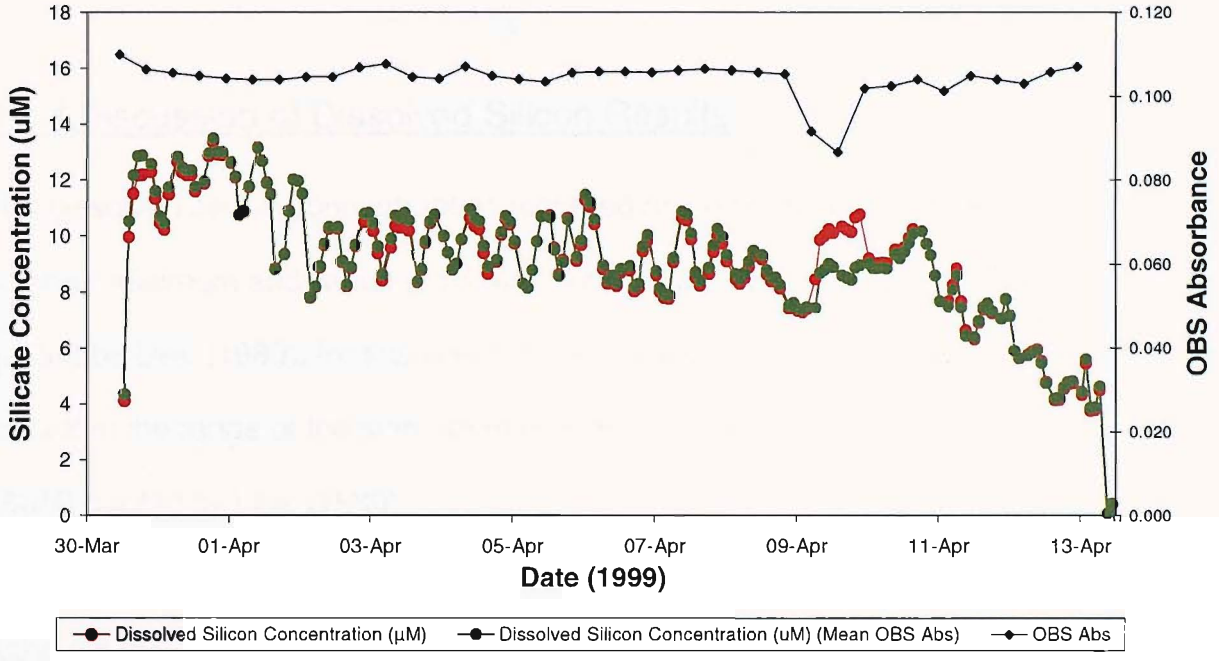


The instrument was successfully recovered on the 18th May1999 and the data set retrieved (Figure 6.12). On examination, it was found that the instrument switched off after 16 days. The tubes and colorimeter were again found to be coated in a white precipitate.

The reason that the instrument stopped working prematurely was that water leaked into the bottom casing causing the instrument to shut down. However, as

the instrument switch off after 16 days and was idle for 6 weeks, it was not known if the acid and distilled water wash had been effective in improving the instruments performance.

Figure 6.12.
Dissolved Silicon and OBS Data from NAS-2ES, PROVESS 2.



The on-board standard data from the bottom dissolved silicon instrument show that the absorbance remained constant from the beginning of the deployment until it switched off on the 13th of April except for two data points recorded on the 9th April.

The CV for the data recorded was 3.9%, which falls to 1.6% when the two lower values recorded on the 9th of April are removed. Some sort of blockage in the OBS tubing or valve may have caused the dip in OBS absorbance between 06:20 and 21:32 on 9th April.

The dissolved silicon concentration has been calculated using both the actual OBS absorbance data and an averaged OBS absorbance (Figure 6.12). This has had the effect of reducing the dissolved silicon peaks during the time of the two problem OBS readings and the resulting dissolved silicon peaks look more realistic.

6.3.3 Discussion of Dissolved Silicon Results

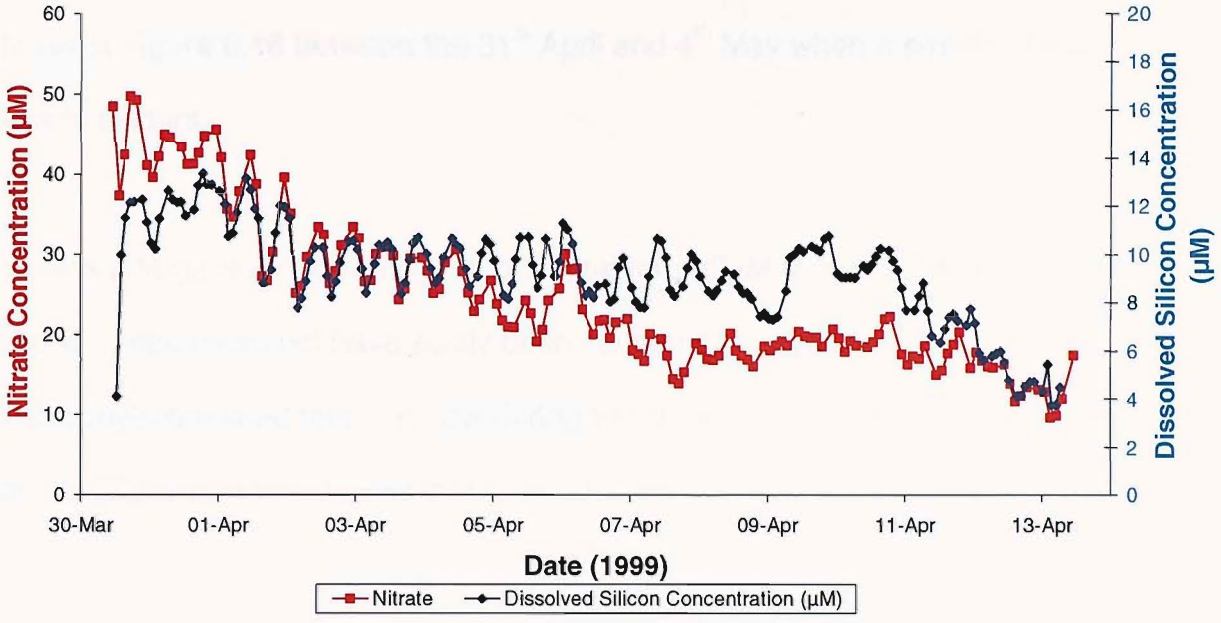
The dissolved silicon concentrations recorded are lower than the range of the summer minimum and winter maximum concentrations (1-2µM to 20-30µM) quoted by Lee, (1980), for this water mass. However, the nitrate results mainly fall within the range of the summer minimum and winter maximum (10-20µM to 45µM) quoted by Lee, (1980).

Although the dissolved silicon signal is lower than the nitrate signal (Table 6.3, Figure 6.13), both signals have the same pattern with a periodicity of 12.6 hours. This is most likely to be a tidal signal as its period closely corresponds with the M2 tidal component.

Table 6.3.
Summary of Dissolved Silicon and Nitrate Data

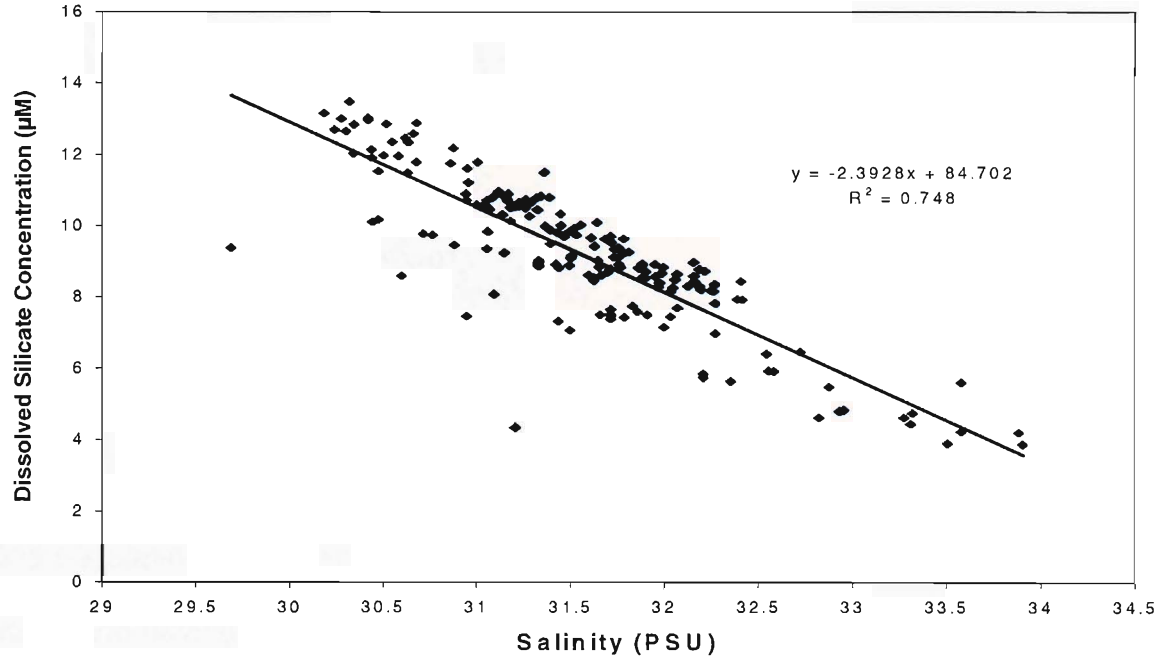
	Number of data Points	Mean Concentration (µM)	Median Concentration (µM)	Max Concentration (µM)	Min Concentration (µM)	Cadmium Column Efficiency
Dissolved Silicon	187	9.22	9.37	3.76	13.38	1.6%
Nitrate	140	24.77	21.74	9.77	49.78	7.3%

Figure 6.13.
Seabed Nitrate and Dissolved Silicon Data, PROVESS 2



The dissolved silicon signal when plotted against salinity (Figure 6.14) indicates a conservative relationship in that the concentration of the dissolved silicon is

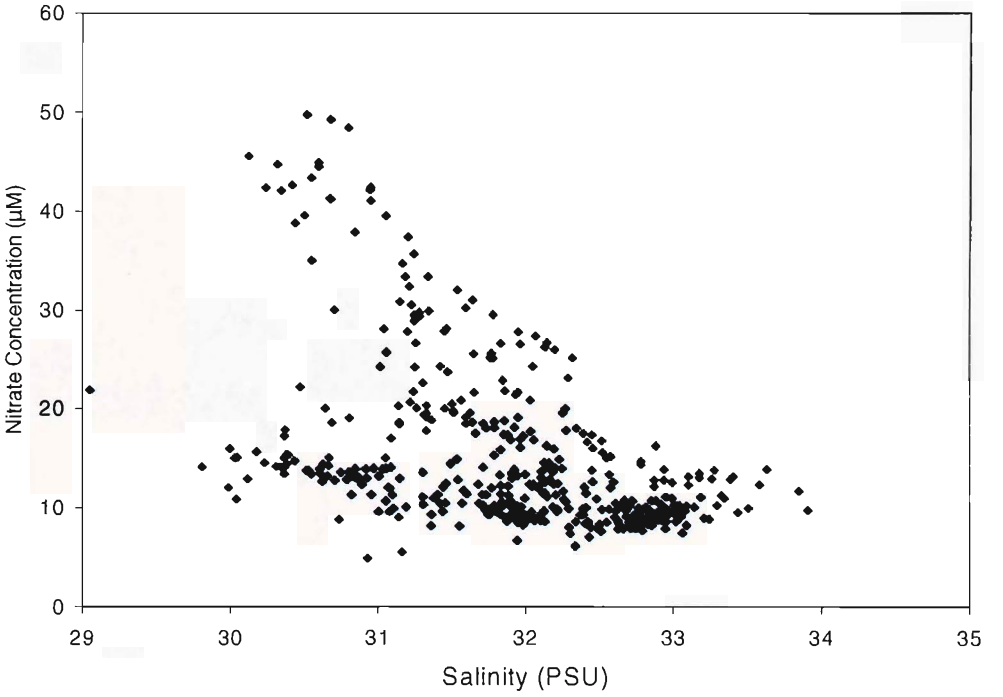
Figure 6.14.
Dissolved Silicon Data v Salinity Data Indicating a Conservative Relationship.



almost solely attributed to its salinity ($R^2 = 74.8\%$). This pattern is commonly seen in riverine systems as the dissolved silicon is diluted as it mixes with the seawater. There is some evidence of biological removal in the chlorophyll data shown in Figure 6.16 between the 31st April and 4th May when a small chlorophyll peak is evident.

There is a large drop in nitrate during this period (49µM to less than 20µM). This drop in nitrate could not have solely been caused by biological activity as the Chlorophyll remained relatively low during this time. The plot of nitrate against salinity (Figure 6.15) indicates that there are two mixing lines present in the data.

Figure 6.15.
Nitrate Data v Salinity Data Indicating the Presence of Two Mixing Lines.

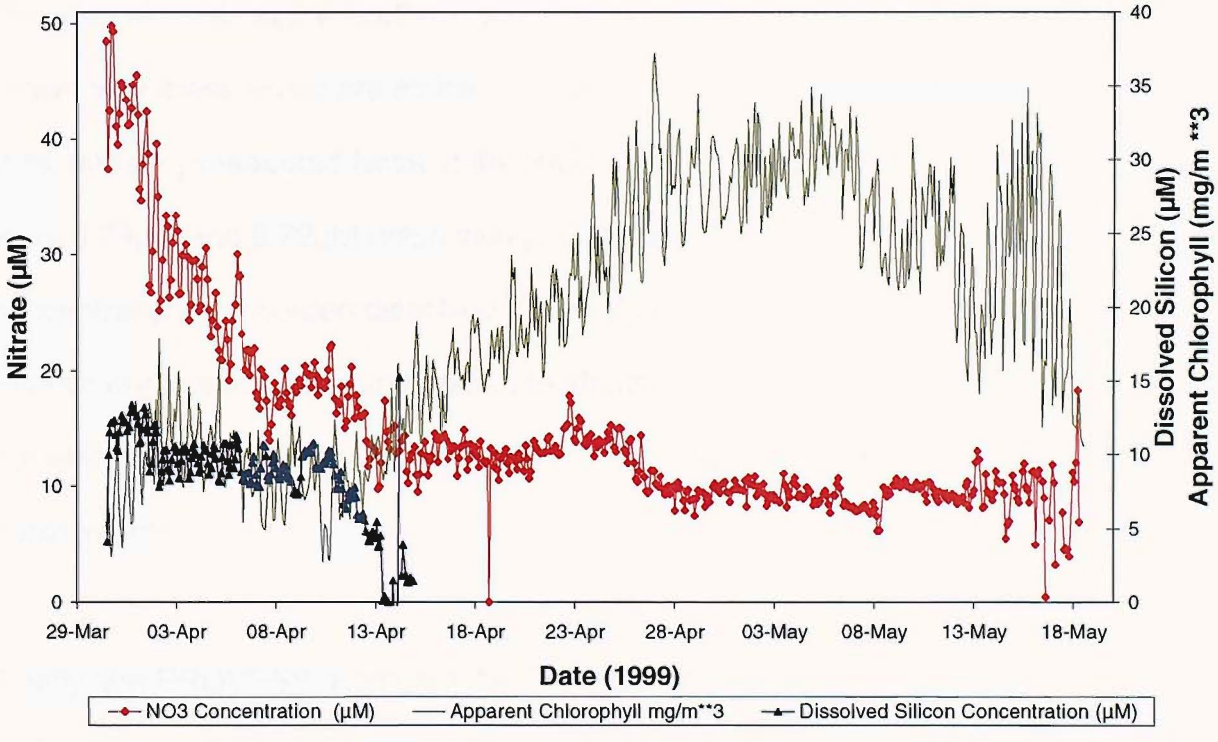


One population has an end member concentration of ≈20µM with the second population having an end member concentration of ≈90µM. It is though that there are two freshwater end members shown in the data. It may be that the higher

concentration population originates from the Rhine water plume whereas the other end member may originate from other less industrialised rivers. Figure 6.15 shows that the high nitrate plumb is diluted and hence depleted in nitrate before the phytoplankton bloom began.

There is also evidence of correlation between chlorophyll and nitrate data (Figure 6.16). A decrease in nitrate concentration from ≈20μM to ≈12μM takes place between the 9th and the 15th of April. This drop in nitrate concentration corresponds with the start of the chlorophyll bloom.

Figure 6.16.
Dissolved Silicon, Nitrate and Chlorophyll Data from Seabed NAS-2EN, PROVESS 2



Between the 5th and 25th the nitrate levels stabilise but the chlorophyll continues to rise. Another drop in nitrate is seen between the 25th and the 27th after which it remains steady at ≈10μM.

6.4 Conclusion

Analysis of the nitrate data gathered both by Hydes *et al.*, (2000), and the PROVESS experiments show that the performance of the cadmium reduction column is erratic. Therefore, work on increasing column stability and durability needs to be undertaken. However, although there are stability problems with the cadmium reduction column, the nitrate results obtained when the column was stable is within the expected range of nitrate concentrations for the two sites. This makes this instrument a valuable tool to aid the understanding of the dynamics of the nutrient cycle especially if the stability problems of the reduction column can be addressed.

The silicate levels at the southern site are lower than the expected range. It is not known why these levels are so low. The 10µM dissolved silicon standard was independently measured twice at the NIOZ nutrient laboratory in Texel and found to be 9.79µM and 9.72µM respectively. This would therefore reduce the concentration of recorded dissolved silicon data even more by on average 2.5%. Further work needs to be undertaken to identify and eradicate the formation of the white precipitate found in the Nas-2ES instrument on both PROVESS deployments.

Finally, the PROVESS deployments highlighted the need for physical parameters to be measured in conjunction with nutrient data on similar deployments. This aided the interpretation of the southern PROVESS nutrient data.

7.0 Improved Operation of the NAS-2EN Instrument

The results gained from the PROVESS experiments show that the stability and durability of the copper coated cadmium reduction column is erratic. This causes uncertainties in the data obtained by the instrument and also results in smaller data sets being acquired, thus reducing the effectiveness of the instrument. Also, the construction and preparation of the wire column is difficult. The wire columns which, have to be manually wound (takes roughly an hour to wind) are cleaned and then coated with copper by passing a copper sulphate solution over the column and then finally rinsed with artificial seawater. If the column is rinsed too vigorously, the copper can be detached or loosened from the cadmium wire which gives rise to the danger of the copper fragments breaking free and blocking the instrument valves. Therefore, the reduction efficiency of the wire column is intimately linked to its construction and preparation and a column prepared by one person may differ significantly in reduction efficiency to a column made subsequently or by a different person.

Nydahl, (1976), suggests that there are a number of factors which affect the reduction columns efficiency. He suggests that at a constant pH, the maximum yield of nitrite is obtained at a certain flow rate, which is the result of a rapid formation and simultaneously proceeding slow reduction of nitrite. When the cadmium column is treated with copper sulphate solution, the precipitated copper adheres to the cadmium forming a porous layer and thus produces a galvanic cell with the copper acting as the cathode. The method increases the standard reduction potential from 0.403V to 0.740V and increases the reduction power. However, the increase in reduction power also applies to the nitrite, which can

then be further reduced to hydroxylamine and small amounts of ammonia. Therefore, when a reduction column is designed, the surface area with which the sample comes into contact with the copperized cadmium along with the flow rate of sample over the column must be optimised to ensure maximum nitrate reduction with minimal nitrite reduction.

The reduction efficiency of the cadmium reduction columns used by Auto Analyser instruments routinely exceeds 97% and Grasshoff *et al.*, (1999), suggests that the cadmium reduction column should be discarded if this reduction efficiency falls below 92%. However, the reduction efficiency of the cadmium wire column used in the NAS-2EN is unknown. Therefore, a series of laboratory experiments were set up to:-

1. Evaluate the reduction efficiency of the cadmium wire column which is recommended by the manufacturer to be used with the NAS-2EN instrument.

2. Determine the effect of replacing the standard tubing, which houses the wire column, with a tube which was less permeable to oxygen. This was carried out to see if this tubing would better preserve the columns reactivity.

3. Evaluate the reduction efficiency of a cadmium reduction column which uses copper coated cadmium granules in place of the copper coated cadmium wire.

4. Investigate the effect of temperature on the reduction efficiency of the reduction column.

5. Field test the most efficient cadmium reduction column and macro during a six week deployment in Loch Etive during a REES project.

7.1 Cadmium Wire Reduction Columns Experiment.

Two types of cadmium column designs were simultaneously tested on two Nitrate NAS-2EN instruments. For these experiments, two nitrate NAS-2EN's (20mm path length colorimeters) from Dunstaffnage Marine Laboratory were used. Both of the instruments used the same set of chemical reagents made up in accordance to the instrument instruction manual and they both used the standard W.S. Oceans macro.

The two NAS-2EN instruments numbers 1714 and 1751 used cadmium wire columns supplied by W.S. Ocean systems. The wire cadmium column used by Instrument 1714 was contained in standard tubing while the wire cadmium column used by Instrument 1751 was contained in Tygon fep lined tubing. Both columns were activated by the method outlines in section 5.1.1. The sample was a 10μM nitrate solution made up in a 40‰ sodium chloride solution and the OBS was a 10μM nitrite solution made up in a 40‰ sodium chloride solution. The nitrite sample (OBS) was also passed over a dummy column consisting of a length of tubing which did not contain the cadmium wire reduction column. The dummy column was attached to a spare valve on the multi-port valve assembly.

The reduction efficiency of the nitrite sample was then calculated by comparing the absorbance of the nitrate sample to the absorbance of the nitrite sample which passed over the dummy column. The sampling rate was every 60 minutes to simulate sampling conditions in the field with OBS carried out every 6th sample. The ammonium chloride solution used by the instruments contained copper sulphate.

7.1.1. Results of Cadmium Wire Reduction Column Experiment.

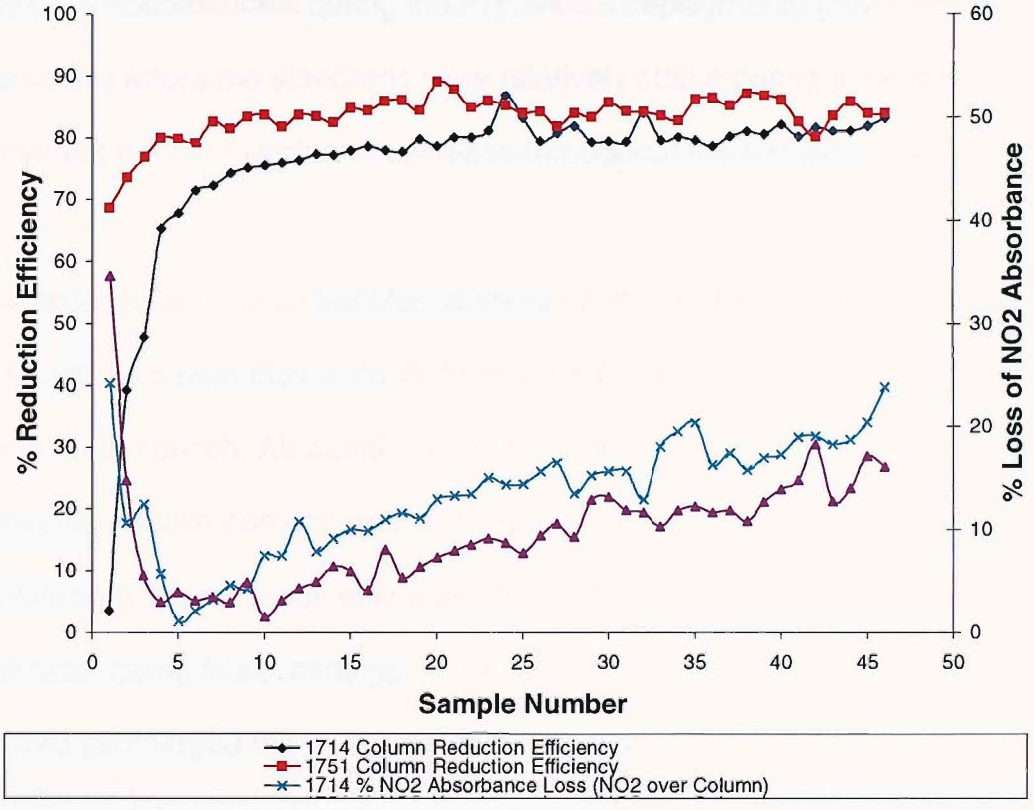
Table 7.1
Summary of the Cadmium Wire Reduction Column Results

	Instrument 1714			Instrument 1751 (Fep tubing)		
	Average	**Standard Deviation**	**CV**	**Average**	**Standard Deviation**	**CV**
10 µM Nitrite Absorbance	0.306	0.018	5.86%	0.305	0.006	1.86%
10µM Nitrate Absorbance	0.233	0.048	20.38%	0.255	0.013	4.95%
Column Efficiency	75.6%			83.43%		

The results show that a greater mean reduction efficiency of 83.4% was achieved by the column contained within the Tygon fep lined tubing (instrument 1751) compared to that of 75.6% achieved by the column in the standard tubing (instrument 1714)[Table 7.1, Figure 7.1]. The results obtained by instrument 1751 also have a lower CV (4.9%) for nitrate reduction than that of instrument 1714 (20.3%). Instrument 1751 reaches a stable level of reduction efficiency after approximately 15 samples and instrument 1714 achieves this after 20 samples. Initially both instruments show a loss in nitrite however, by the 5th sample this trend is reversed and nitrite is lost by both instruments at a similar rate. Overall instrument 1714 lost more nitrite (mean loss of 13.3%) than instrument 1751 (mean loss of 9.6%). It was also noted that by the end of the experiment both

columns had blackened in appearance and that there were less air bubbles in the Tygon Fep tubing (instrument 1751).

Figure 7.1.
Cadmium Wire Column Reduction Efficiency and NO2 Loss for Each Instrument.



7.1.2. Discussion of Results

The results from the above experiments suggest that the copper contained in the ammonium chloride buffer solution increased, rather than maintained, the reduction power of the cadmium column. The additional copper from the buffer solution probably adhered to the reduction column causing an overall increase in the nitrate reduction (Figure 7.1). This would account for the observed blackening of the columns. The slow rise in the loss of nitrite after the 5th sample indicates that the reduction column was becoming more reactive, reducing not only the

nitrate to nitrite but further reducing the nitrite to hydroxylamine and small amounts of ammonia as described by Nydahl, (1976). If the experiment had continued, the reduction efficiency of the column would have probably started to decrease as the nitrite was increasingly reduced. This may explain the behaviour of the OBS absorbance's during the PROVESS deployments (Figures 6.2, 6.3, 6.4 and 6.5) where the standards were relatively stable during the initial deployment but then started to decrease throughout the remaining deployment.

The smaller number of air bubbles observed in the Tygon tubing which contained the reduction column may account for the more stable performance of this column on the bench. Air bubbles can get trapped on the reduction column altering the surface contact area of the sample and the wire reduction column, thus reducing the reduction efficiency. Air bubbles can also get trapped in the colorimeter giving false readings. However, as this instrument is normally deployed submerged this is unlikely to be a problem. Also, the internal diameter of the Tygon tubing was less than the standard tubing, therefore the column would have a different bed-volume which would affect the contact time of the sample on the reduction column. According to Nydahl, (1976), reduction efficiency is closely related to bed volume and flow rate. Therefore, the results suggest that the bed-volume in the Tygon tubing probably provided better conditions for nitrate reduction.

The rationale for replacing the standard tubing with the Tygon fep lined tubing was that Wood *et al.,* (1967), suggested that if the cadmium reduction column was exposed to air then the reduction potential of the column would be damaged.

However, Nydahl, (1975), disputed this, suggesting that the hypothesis was unsupported by experimental evidence. The results from the experiments reported here tend to agree with Nydahl's, (1975), supposition, as the difference in performance between the two columns is marginal. Therefore there is little benefit in replacing the standard tubing with the Tygon tubing.

7.2 Granular Cadmium Reduction Column.

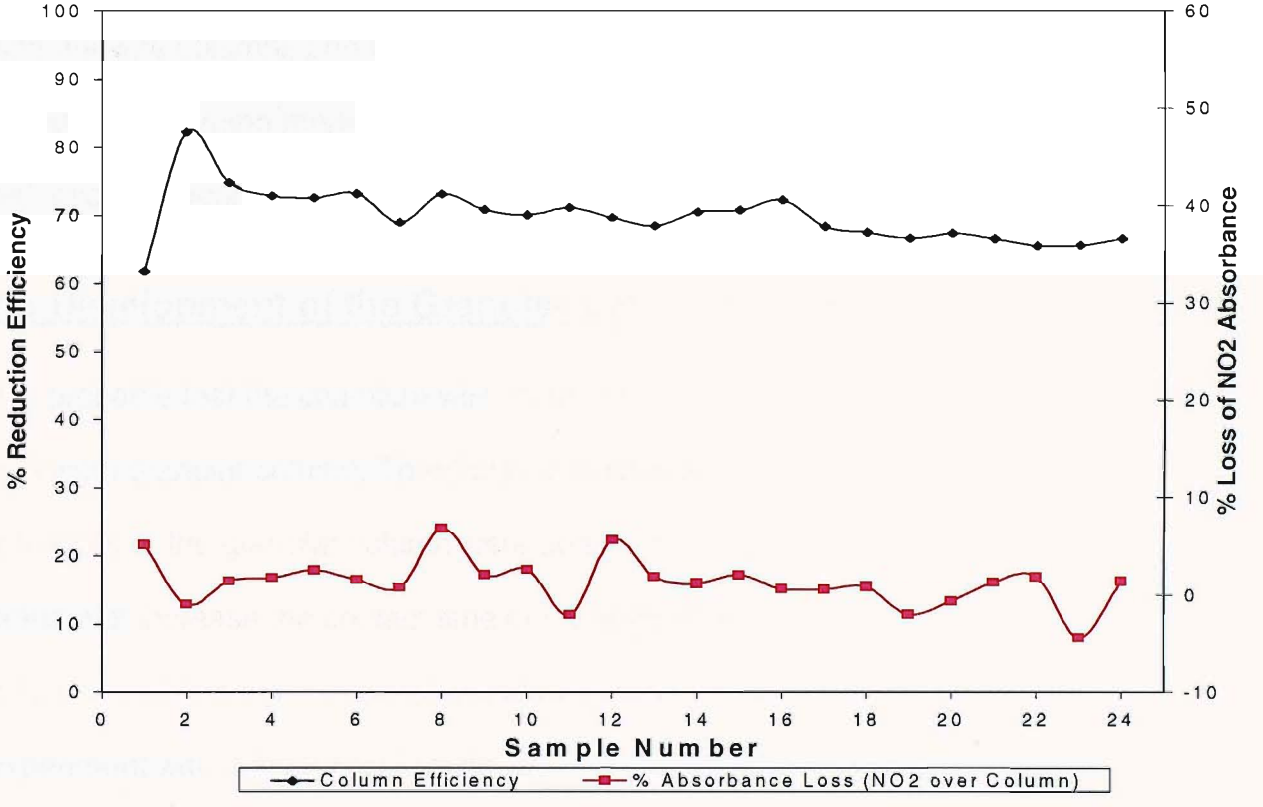
A cadmium granular column was constructed and tested on a NAS-2EN Instrument with a 5mm path length colorimeter. The column consisted of a glass tube containing 3.2g of Skalar copper coated cadmium granules which are mixed with copper flecks. As the Skalar cadmium reduction columns used by Auto-Analysers contain 3.2g of the cadmium granules this was chosen as the starting weight. Glass wool plugs were inserted into each end of the glass tube to keep the granules in place. The control macro was the same as the manufacturer's macro used by the wire column experiment except that the sample was mixed with the buffer solution with a dilution of 1:1 before being passed over the reduction column. This was carried out to make the chemical routine follow as closely as possible the routines carried out by the Auto Analysers, which routinely achieve a reduction efficiency of above 97% for this type of column. The sampling rate for all the experiments was hourly. Also the ammonia chloride buffer solution did not contain copper sulphate as the Skalar granules are interspersed with flecks of copper and the addition of copper is not required to maintain efficiency.

7.2.1. Results of Cadmium Granular Reduction Column.

Table 7.2
Summary of Cadmium Granular Reduction Column Results

	Granular Column		
	Average	**Standard Deviation**	**CV**
10 µM Nitrite Absorbance	0.0750	0.0009	1.19%
10µM Nitrate Absorbance	0.0525	0.0030	5.7%
Column Efficiency	69.92%		

Figure 7.2
Plot of the Granular Column Efficiency and NO2 Loss



Although the mean reduction efficiency of the granular column, 69.92% was less than that of the wire columns, the mean loss of nitrite was 2.4%, which is significantly less that that of the wire columns (1714 – 13.3% and 1751-9.6%).

Also the granular column settled after only two samples, which was significantly faster than the 5-20 samples taken by the wire columns to settle.

7.2.2. Summary of the Cadmium Wire and Granular Reduction Column Experiments.

The wire columns had greater reduction efficiency than the granular column but the granular column did not lose nitrite, was stable and took less time to settle down. The construction of the granular column was quick and easy compared with the wire columns and was more reproducible. It was therefore decided that the granular column merited further experimentation in order to investigate if the reduction efficiency could be improved.

7.3 Development of the Granular Cadmium Reduction Column.

It is probable that the cadmium wire column had a greater surface area than the cadmium granular column. Therefore, two options to improve the reduction efficiency of the granular column were considered; either use a larger granular column or increase the contact time of the sample within the column. As a larger column would be more expensive and awkward to manage, it was decided to experiment with different contact times.

7.3.1. Optimal Contact Time of Sample and Column.

A second cadmium granular column was constructed by inserting 3.2g of Skalar cadmium granules into standard plastic tubing. Plastic was used instead of glass to make the column less fragile. Glass wool was placed at each end of the

column to stop the granules escaping from the tube. A number of macros were written in order to:-

1. Adjust how the sample was passed over the column (i.e. all at once or stepped on and off the column).

2. Change the number of times that the sample was passed over the column.

3. Adjust the time with which the sample was "paused" on the column.

These experiments and the results are summarised in table 7.3.

7.3.2. Results of Optimal Contact Time of Sample and Column

The macro used in experiment 2 (Table 7.3), where the sample was moved on and off the column 10 times in 60 steps with a 2 second residence time, gave the highest column efficiency of 80.72% with the least loss of nitrite, 0.97%. It was therefore, decided to use this macro (now known as the "master nitrate macro" listed in Appendix 1) with a new column.

Table 7.3.
Summary of the Results from the Experimental Cadmium Reduction Routines

Test Macro	NO3			NO2 Column			NO2 Dummy Column			% Efficiency Column.	% Efficiency Dummy Column	% NO2 Loss
	n	Abs	%SD	n	Abs	%SD	n	Abs	%SD			
Experiment 1. 30 steps on and off with 2 sec residence. 5 Repeats	54	0.050	5.53	12	0.074	2.07	12	0.075	1.44	67.5	66.6	0.9
Experiment 2. 60 steps on and off with 2 sec residence. 10 Repeats	5	0.054	0.71	5	0.0661	1.90	5	0.0669	1.47	81.69	80.72	0.97
Experiment 3. 10 steps on x 6 with 60 steps off, 1 sec pause. 1 Repeat	40	0.039	2.45	21	0.068	3.03	21	0.074	2.36	57.35	52.7	4.65
Experiment 4. 10 steps on x 6 with 60 steps off, 2 sec pause. 1 Repeat	42	0.040	3.02	22	0.067	4.52	22	0.074	2.28	59.7	54.05	5.65
Experiment 5. 10 steps on x 6 with 60 steps off, 20 sec pause. 1 Repeat	12	0.0406	6.18	6	0.0703	1.59	6	0.0738	4.66	57	55	2
Experiment 6. 10 steps on x 6 with 60 steps off, 1 sec pause between steps. Mixing on Dummy Column	4	0.0373		0			0					
Experiment 7. 10 steps on x 6 with 60 steps off, 1 sec pause between steps. 2 Repeats	27	0.042	6.27	14	0.0692	2.96	14	0.0746	4.67	60.69	56.3	4.39
Experiment 8. 10 steps on x 6 with 60 steps off, 1 sec pause between steps. 6 Repeats	15	0.0459	3.04	5	0.069	1.75	5	0.0749	0.55	66.52	61.28	5.2
Experiment 9. 10 steps on x 6 with 60 steps off, 1 sec pause between steps. 10 Repeats	3	0.0438	3.41	2	0.0668	0.31	2	0.0729	2.88	65.56	60.08	5.48
Experiment 10. 60 steps on and off with 2 sec residence. 20 Repeats	20	0.0442	2.75	10	0.0622	2.83	10	0.0716	1.66	71.06	61.73	9.33

7.3.3. Granular Cadmium Column with New Chemicals.

A second Skalar cadmium granular reduction column was constructed using 3.0g of cadmium granules. The concentrations of chemical reagents were made in accordance to the method published by Grasshoff *et al.*, (1999), for the determination of nitrate by segmented flow Auto Analysers as these differed from those suggested by the NAS-2EN manufacturer [Section 5.1.1]:-

Sulphanilamide: Dissolve 5g in 100ml of 50% HCl, dilute to 1l with distilled water, and then add 2ml BRIJ-35 wetting agent (30% solution).

Napthylethelynedihydrochloride: Dissolve 0.5g in 1l distilled water.

Ammonium chloride: Dissolve 25g in 1l of distilled water (no copper is added to the ammonium chloride solution).

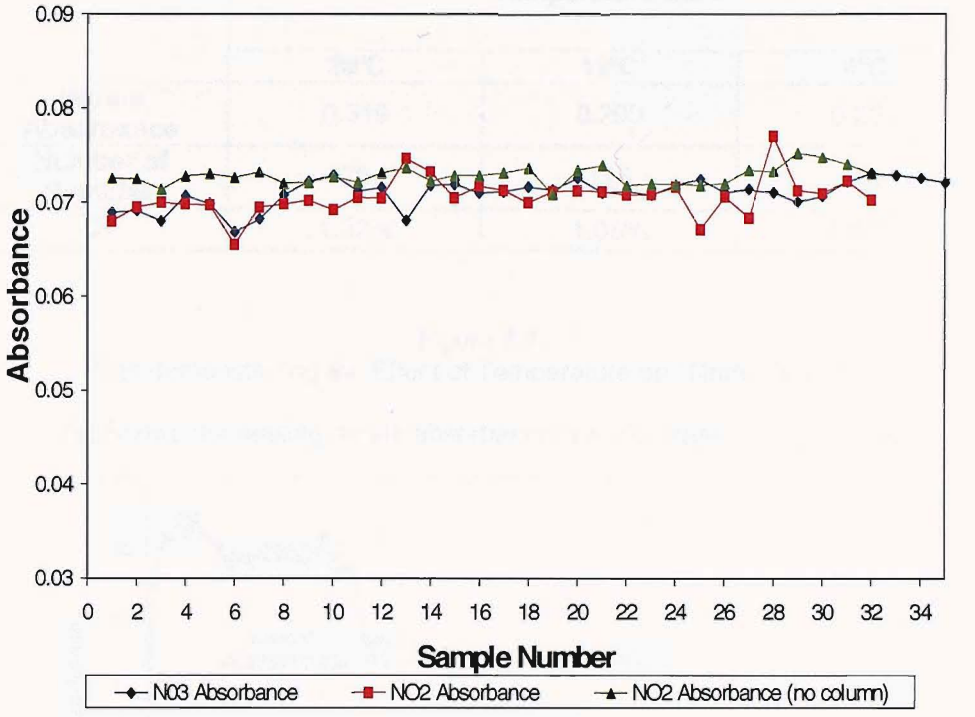
The standards were made up in the same way as described in Section 5.1.1. and the master nitrate macro described in Section 7.3.2 was used. The sampling rate was hourly.

7.3.4. Results of Granular Cadmium with New Chemicals

Table 7.4.
Summary of Cadmium Granular Reduction Column Results Obtained with the Master Nitrate Macro

	Granular Column		
	Average	**Standard Deviation**	**CV**
10 µM Nitrite Absorbance	0.0728	0.009	1.3
10µM Nitrate Absorbance	0.722	0.0018	2.44
Column Efficiency	99.17%		

Figure 7.3.
The Stability of the NO2 and NO3 Absorbance Results Obtained with the Final Cadmium Column Design and Master Nitrate Macro.



A reduction efficiency of almost 100% was achieved (Table 7.4, Figure 7.3) with no loss of nitrite. The column was also very stable with a CV of less than 2.5%. The next stage in this experiment was to test the effect of temperature on the column then field test the new cadmium reduction column and macro.

7.3.5. Temperature Effect on Granular Column

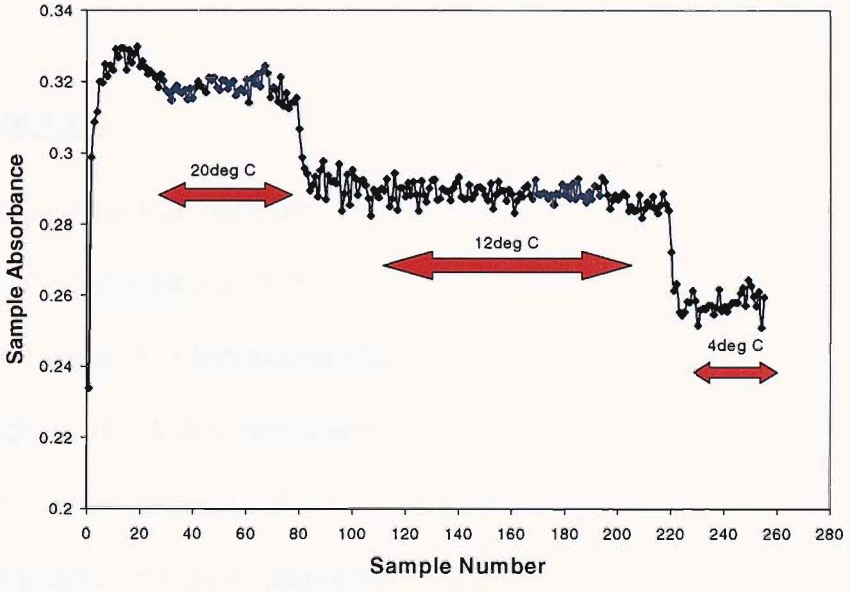
The instrument was set up to run in a temperature control room using the master nitrate macro and the granular column and was tested at three temperatures 20°C, 12°C and 4°C. These temperatures were limited by the performance of the temperature control room, which were found to operate within a range of 4°C to 20°C with a tolerance of ±3°C. The sample was a 50µM nitrate standard made up in 40‰ sodium chloride solution.

Table 7.5
Effect of Temperature on Nitrate Reduction

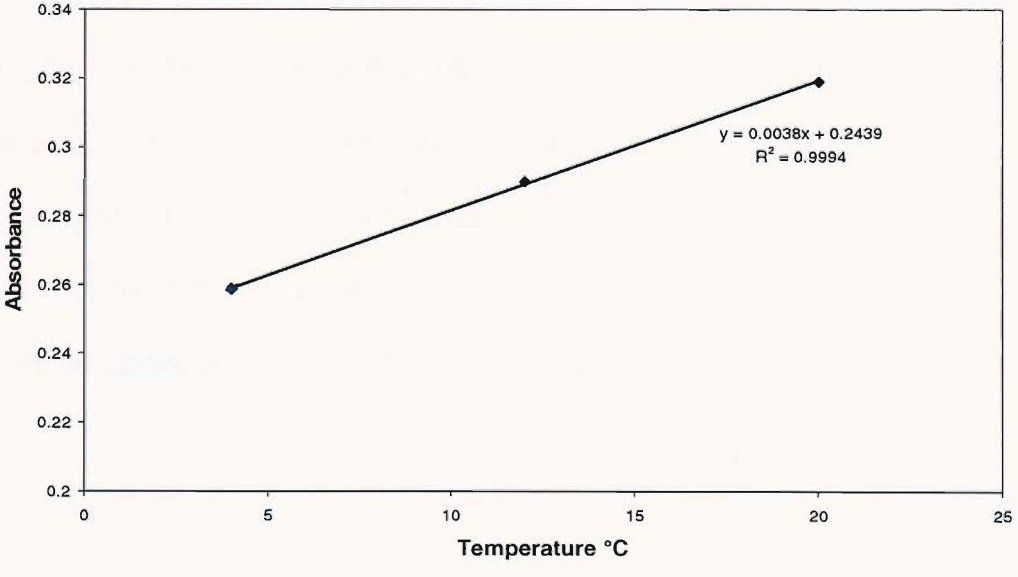
	Temperature ± 3°C		
	20°C	**12°C**	**4°C**
Nitrate Absorbance	0.319	0.290	0.259
Number of Samples	73	145	43
CV	1.32%	1.05%	1.47%

Figure 7.4.
Plots demonstrating the Effect of Temperature on Nitrate Reduction

(a) Shows decreasing nitrate absorbance with decreasing temperature



(b) Absorbance and Temperature



The results show that there is a temperature effect on nitrate reduction (Table 7.5), which is equivalent to a decrease in nitrate absorbance of 0.0037 per 1°C. This equates to a difference of 0.56µM for a 1°C change in temperature for a 50µM sample, which is a difference of 1.12%. As the standard is deployed with the instrument, the temperature will also affect the standard results in the same way, thus compensating for the temperature effect. However, this is only true if enough OBS are run more frequently than the changing temperature.

7.4. Summary

Initial results show that although the granular cadmium reduction column has a smaller reduction efficiency than the wire cadmium reduction column, it looses less nitrite, has increased stability and reaches its reduction potential more rapidly. Also, the construction of the granular column is simpler and consistently more reproducible than the wire column. The analysis macro for the granular column was optimised and resulted in an increase in reduction efficiency from 70% to 80%. Efficiency greater than 99% was achieved by changing the reagent concentrations for the NAS-2EN to those used in Auto-Analyser nitrate determination (Grasshoff *et al.*, 1999). It is possible that the efficiency of the wire column may also have been improved using these reagent concentrations, however the advantages of the granular column discussed above suggest that this robust and simple design is more desirable.

Finally, although there was a temperature effect on the reduction efficiency of the granular column (1.12% per 1°C) the OBS compensates for this effect (assuming enough OBS are run to follow the temperature effect).

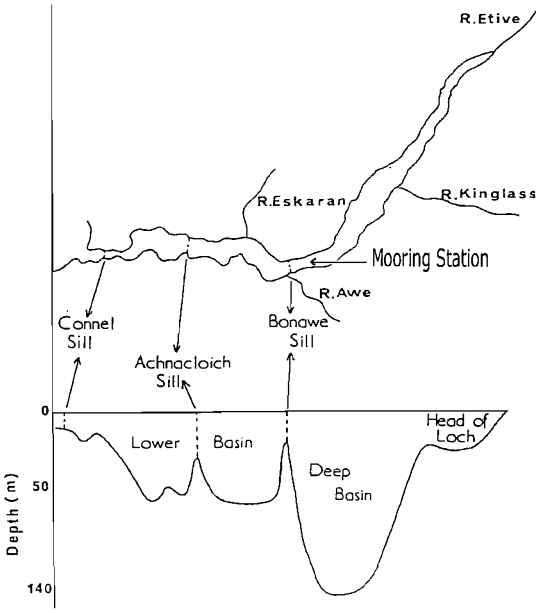
For the REES deployments in Loch Etive it was therefore decided to use the granular cadmium reduction column with the master nitrate macro and the chemicals described in Section 7.3.3.

8.0 Field Testing of Granular Column (REES Project)

8.1 Field Site

Field-testing of the WS Oceans NAS-2EN instrument using a method developed during bench testing was carried out in Loch Etive during the spring of 2000. This was carried out as part of a Scottish Association for Marine Science REES Project (Restricted Exchange Environment). Loch Etive is a fjord of glacial origin situated on the west coast of Scotland. It runs inland from the coast for approximately 24 km and is separated into three basins by the Connell Sill, Achnacloich Sill and Bonawe Sill (Figure 8.1).

Figure 8.1.
Map of Loch Etive
Adapted from (Edwards and Edelsten, 1977)



Loch Etive has an internal tidal range of 2m and a rainwater catchment of 1400km^2, which is larger than any other Scottish fjord and 7 times the mainland mean (Edwards and Edelsten, 1977). According to Solorzano and

Ehrlich, (1977), freshwater discharge into Loch Etive from the various rivers and streams has been calculated as 2.9km^3/y of which 50% is supplied by the River Awe. The flow of the Awe is altered and controlled by the hydroelectric scheme at Loch Awe. This fresh water flow is buffered by the 80Mm^3 storage in Loch Awe on a time scale of a few days during wet periods to a few weeks during dry spells (Edwards and Edelsten, 1977).

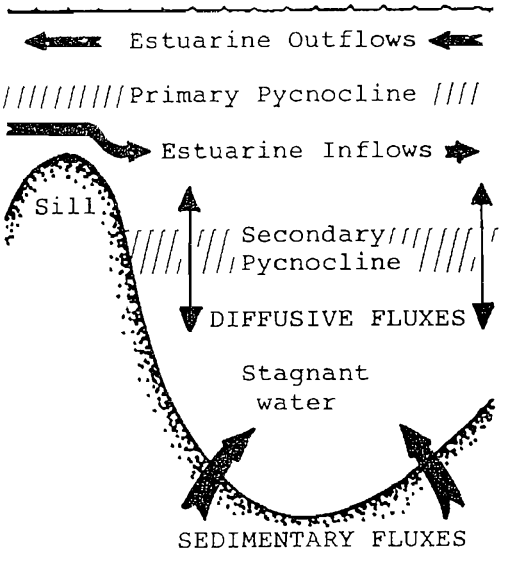
According to Solorzano and Ehrlich, (1977), in terms of nutrient behaviour, the Loch can be considered in three sections namely the lower basin, deep basin and head of the Loch. The three sections are inter-related but possess different chemical features.

The structure of the water column in the deep basin consists of three stratified layers (Edwards and Grantham, 1986). The upper layer, in which estuarine circulation develops, consists of out-flowing brackish water and a second layer of inflowing seawater. This is separated from the bottom water third layer by a secondary pycnocline at depth 30-100m (Figure 8.2). Due to its high density, the bottom water tends to be isolated and unaffected by the estuarine circulation above it. The bottom water is formed by occasional in-flows of higher salinity water from outside the Loch and is stagnant between renewal events.

During dry spells the density of the upper layers increases. If this event occurs during the flood of spring tides, the sill water flows down-slope as a turbulent plume, which destroys the secondary pycnocline (Edwards and Edelsten,

1977) and renews the bottom water. These renewal events may be separated by periods of months to years (Edwards and Grantham, 1986).

Figure 8.2.
Structure of Loch Etive Water Column.
(Adapted from Solorzano and Sharp, 1980)



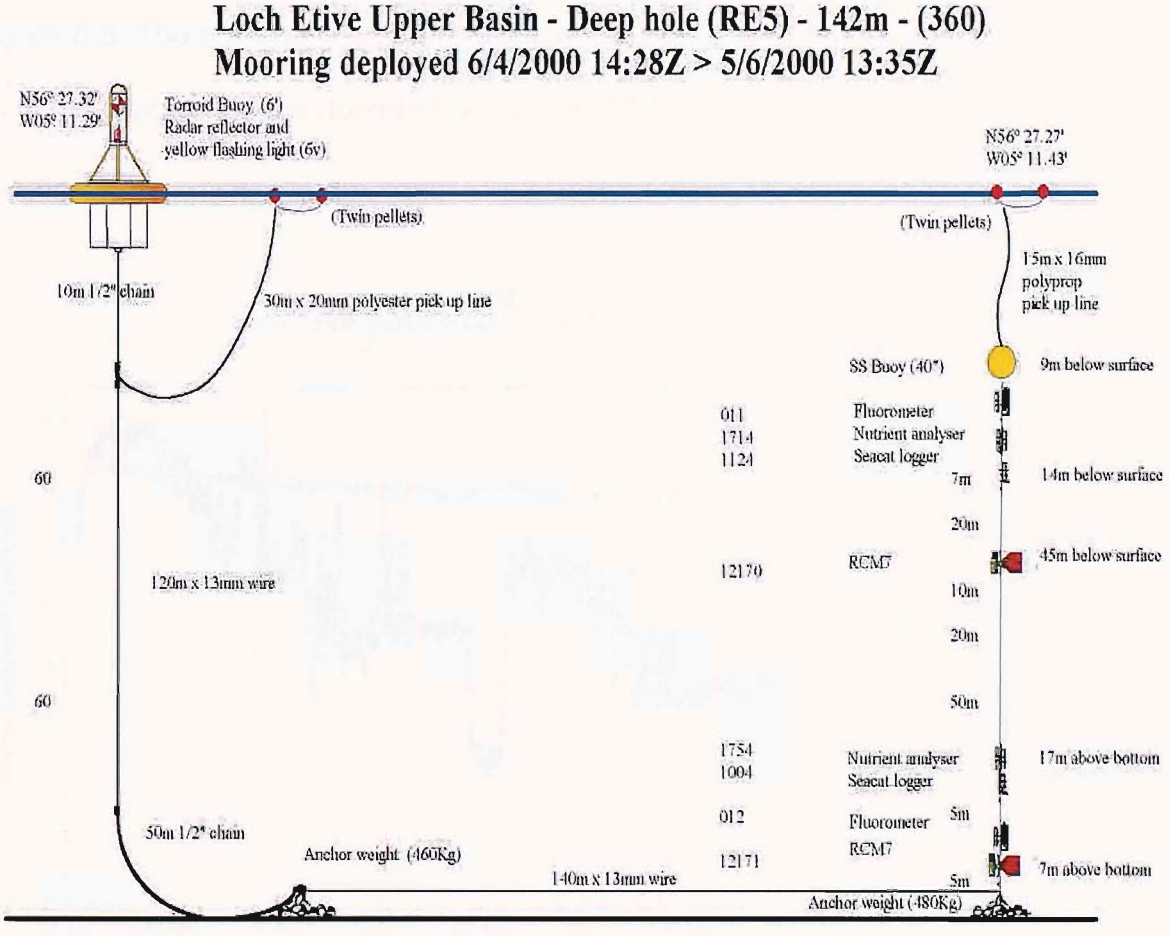
During periods of stagnation, bottom concentrations of oxygen decrease and the concentration of inorganic nutrients increase. The change in concentration of the oxygen and nutrients is due to both biogeochemical processes in the water column and diffusive exchange with the sediments.

Following a water renewal event, biogeochemical processes dominate as the vertical distribution of both nutrients and oxygen homogenises and therefore diffusion is negligible. As the bottom water stagnates, differences between the nutrient concentrations in the lower estuarine water and the bottom water increase, thus increasing diffusive fluxes. Over time the diffusive and biogeochemical fluxes become roughly equal and opposite and the stagnant water reaches a steady state (Edwards and Grantham, 1986).

8.2 Method

A mooring was deployed in the deep basin behind the Bonawe Sill. One NAS-2EN instrument was deployed approximately 12m from the surface and the second instrument deployed approximately 17m from the bottom (142m, Figure 8.3).

Figure 8.3.
Mooring Diagram (Supplied by Colin Griffith, DML).



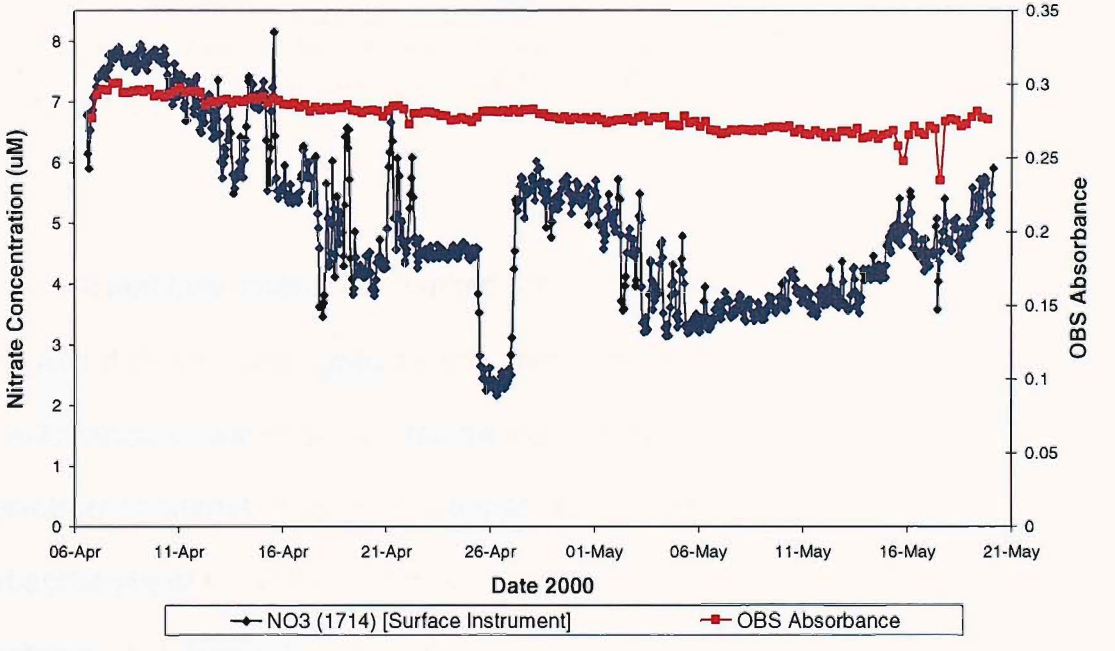
Both instruments were fitted with the cadmium granular column and set to run the "master nitrate" macro (see Appendix 1) as these were the "optimal"

parameters determined in Chapter 7. Samples were measured every hour with the 10µM nitrate on-board-standard (OBS) measured every six hours.

8.3 Loch Etive Results

Figure 8.4 shows the nitrate measurements from the NAS-2EN instrument deployed near the surface in Loch Etive. The temperature and salinity data from the surface Seacat logger from the same time period are also plotted in figure 8.5. The surface instrument worked successfully during the deployment until the reagents were depleted on the 21st May 2000 after 45 days (Figures 8.4 and 8.5).

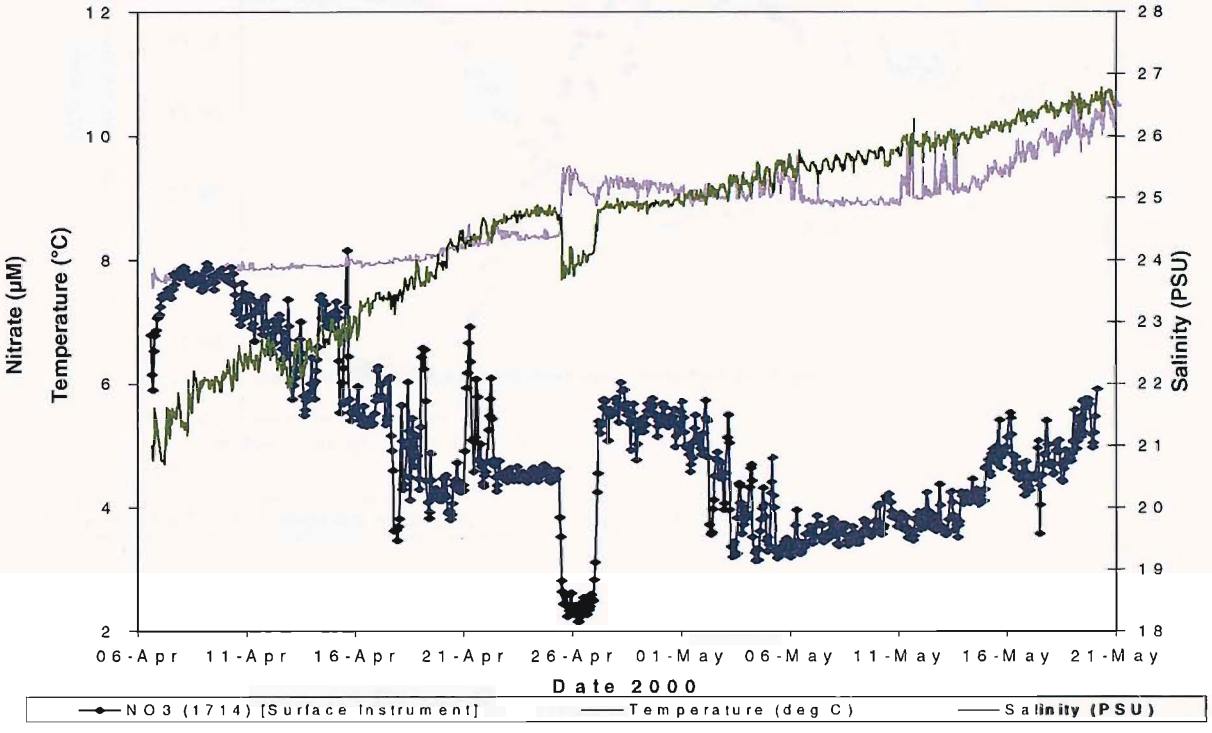
Figure 8.4.
Loch Etive Surface NAS-2EN Nitrate and OBS Data



The data collected indicate that the on-board standard data was significantly more stable for the REES deployment, which utilised the new column and

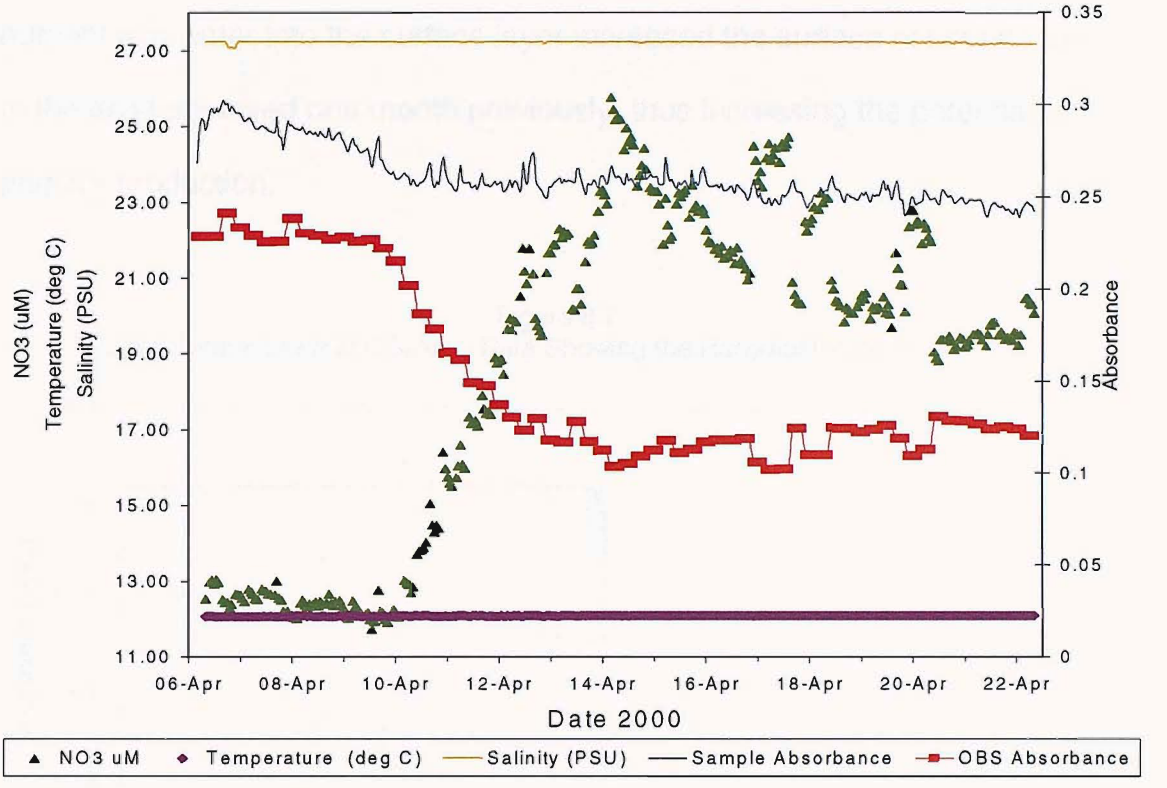
macro (coefficient of variation of 3.6% for OBS, over 900 seawater samples measured) than on the previous PROVESS deployments (See Section 6.2 and 6.3).

Figure 8.5
Loch Etive Surface Nitrate, Temperature and Salinity Data



The seabed instrument only worked for 16 days after which it switched off (Figure 8.6). On investigation it was found that the bottom instrument was faulty because water leaked into the compartment that contained the electronic controller boards. In contrast to the surface instrument, the absorbance of the OBS varied over time and fell from a value of 0.22 to 0.12 between 10th April 2000 and 14th April 2000.

Figure 8.6.
Temperature, Salinity, Nitrate, Sample and OBS Absorbance Data for the Seabed Instrument for the Loch Etive Deployment

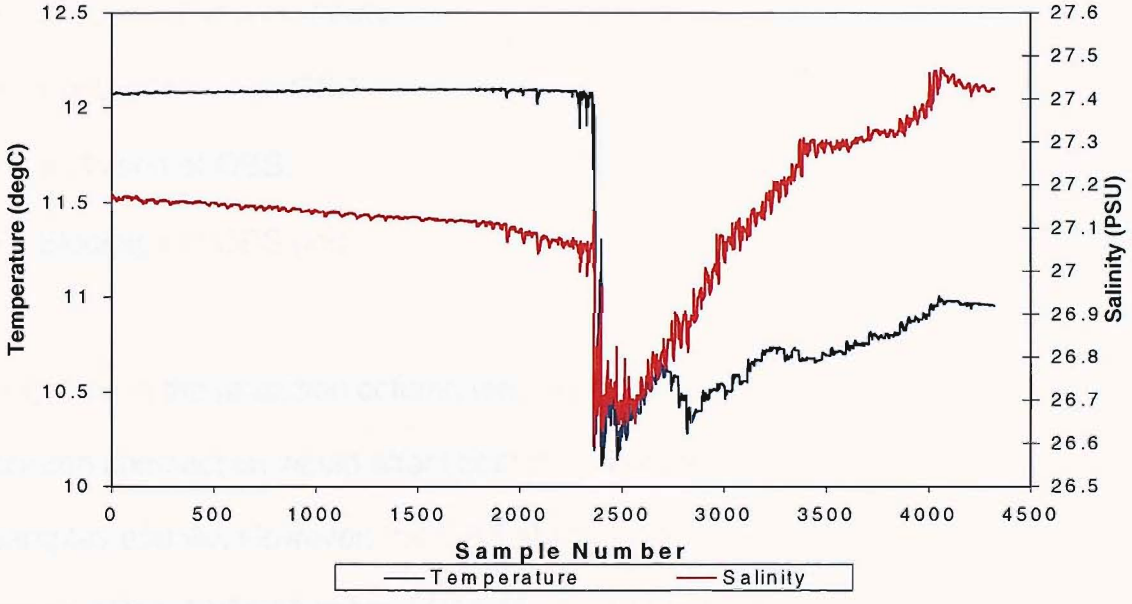


8.4 Discussion of Etive Surface Data.

The surface nitrate signal is complicated and displays many short period fluctuations (Figure 8.4). The fluctuations in the nitrate signal are not generated by the instability of the OBS signal as it has less than 4% variation (equivalent to 0.4µM). Fluctuations are also seen in the salinity and temperature data (Figure 8.5, data from DML) and is possibly attributed to surface mixing caused by wind and tides (the nitrate sensor was 14m below the surface). In general terms it can be said that the nitrate signal decreased in value (≈8µM to ≈4.5µm) from the start of the deployment until 25th April. During this period the water temperature and salinity increased. The decrease

concentrations from ≈3.5µM to ≈5.5µM after this event was probably due to the nitrate rich bottom water mixing up into the surface waters. This pulse of nutrient rich water into the surface layer increased the surface concentration to the level observed one month previously, thus increasing the potential for primary production.

Figure 8.7
Seabed Temperature and Salinity Data Showing the Renewal Event in Loch Etive



8.5 Discussion of Etive Seabed Data.

The results show (Figure 8.6) that there was an apparent loss in reactivity of the cadmium reduction column during the time period 9th April to the 14th April where the absorbance of the OBS fell from a value of 0.227 to 0.103. Also, during this time period there is an apparent increase in nitrate concentration from 2 µM to a maximum of ~26µM. As these two events happen simultaneously, it is probable that they are related to one another. Potentially

the rise in nitrate concentration of the seawater samples is solely attributed to the drop in the OBS absorption and is therefore not real. However it is possible that some event occurred that concurrently caused a loss in reactivity of the cadmium reduction column and an increase in nitrate concentrations.

The possible causes for the drop in OBS absorbance are: -

- Drop in column reactivity.
- Contamination of OBS
- A dilution of OBS.
- Blockage at OBS port.

Any drop in the reduction column reactivity caused by either contamination or column compaction would affect both the seawater samples and the OBS samples equally. However, the OBS absorbance fell by 50.1% during the time period of the 10/04/00 to the 14/04/00 whereas the seawater absorbance only fell by 1.8%. If the nitrate concentration of the seawater had remained roughly constant during this period then the seawater sample absorbance would also have fallen by approximately the same amount as the OBS absorbance. Therefore, the rise in nitrate concentration was not caused by a drop in column reactivity.

A dilution of the OBS would certainly cause a decrease in OBS absorbance. Ingress of the surrounding water into the standard bag or tubing is unlikely to cause a drop in OBS absorbance as independent sampling a week before the deployment indicated that the nitrate concentration in the surrounding water

was higher than the nitrate concentration in the OBS bag. This would have caused an increase in OBS absorbance.

Contamination of the OBS is unlikely as the OBS used in this instrument was made up at the same time as the OBS used by the surface instrument. Both bags of OBS were made up from the same artificial seawater and stock standard solution. The bag was new and sterile and the tubing clean. The bags were made up a least a week before deployment so if there had been any contamination inside the bag, the nitrate levels would most likely have fallen immediately rather than four days into the deployment.

Finally, it is possible that a partially blocked valve or tube connected to the OBS port could have caused the decrease in OBS absorbance. If the port or the tubing, which connects the bag containing the OBS to the OBS port, gets partially blocked or constricted then it is possible that a smaller volume of standard will be mixed with the ammonia chloride solution, reducing the OBS concentration and thus reducing the absorbance of the OBS samples. This is the most likely cause of the drop in OBS absorbance

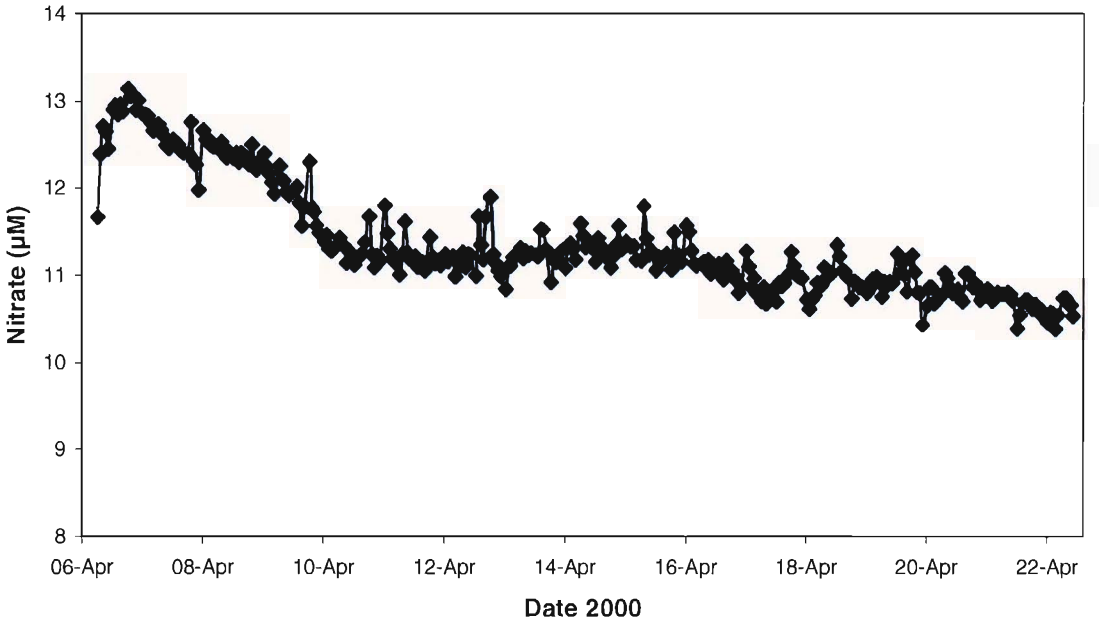
Nitrate concentrations during stagnation have been reported to be between 3.4µM and 8µM in the 1970,s (Solorzano and Sharp, 1980) and between 8µM and 12µM during 1980 and 1981 (Edwards and Grantham, 1986). Manual seawater samples collected from the bottom water on the 28th March 2000 had a nitrate concentration of 11.97µM, which is comparative to the levels of nitrate concentrations calculated by the NAS-2EN at the beginning of the deployment before the reduction in OBS absorbance.

It is unlikely that there was an increase of nitrate concentrations from ≈12µM to ≈25µM in the bottom waters during the deployment. As Edward and Grantham, (1987), stated nutrient concentrations reach a steady state during stagnation. In order for there to have been such a rise in nitrate concentration, the bottom waters would have to be mixed with another water mass rich in nitrate or there would have to have been a large increase of rapidly oxidising organic material reaching the bottom waters. The temperature and salinity data however show no change during this time indicating that the bottom waters were not mixed with another water mass. Also the current speed data show that during the time of the drop in OBS absorbance, current speeds were less than 45mm/s therefore, there were no fast turbulent currents turning over the sediments to cause a rise in nitrate levels. The surface nitrate levels were lower than the bottom water during this time period (they fell from ≈8µM to ≈5.5µM), therefore the large rise in nitrate concentration did not originate from the surface water.

It is therefore likely that the apparent increase in nitrate concentration of the bottom waters is solely attributable to the fall in the OBS absorbance and is therefore an artefact and was probably due to some type of blockage of the OBS bag or tubing. For future deployments it may better if a second OBS bag is connected to the instrument. This would be possible as the master nitrate macro used with the cadmium granular reduction column only uses seven ports thus allowing a free port to which an additional standard could be connected.

If the absorbance of the OBS is averaged from the start of the deployment (excluding first point) until 21:01GMT on the 9th April and this value is then used to calculate nitrate concentration, the results show a drop in nitrate concentration from 13.15µM to 10.39µM in a period of 369hours (Figure 8.8).

Figure 8.8.
Seabed Nitrate Sample Concentrations Re-Calculated Using an Averaged OBS Absorbance from the Start of the Deployment.



Although this crude technique does not take into account any natural drift in the OBS absorbance, the surface instrument showed minimal drift, so the introduced method is thought to be insignificant. This indicates that nitrogen is being lost from the bottom layer. Oxygen levels were low and fell from 40µM to 32µM during this period (data from DML) causing the oxygen to nitrogen ratio to increase from 3.07 to 3.2. This may indicate that denitrification was taking place. As the depth of the bottom layer was ≈70m and the apparent drop in nitrate occurred over 369 hours (≈ 12 days) then the denitrification rate was ≈13mM N m^{-2} d^{-1}. Although the denitrification rate is high compared with

the rates reported for coastal waters it is not unexpected for the anoxic bottom layer. Kristiansen and Schaanning, (2002), also reported a denitrification rate of 13mmol N $m^{-2}d^{1}$ in the water column of an intermittently anoxic fjord in southern Norway. It is expected that the denitrification rate would decrease after the renewal event as the oxygen levels of the deep layer is replenished.

9.0 Synthesis

Phytoplankton abundance is intimately linked to nutrients availability. When the light conditions are correct and nutrients are present, blooms occur. In temperate systems undisturbed by man, there is a natural periodicity to phytoplankton growth and decline in respect to light and available nutrients. Increased anthropogenic input of nutrients into the marine system can lead to eutrophication and can have a significant negative impact on some coastal marine ecosystems. The complicated biogeochemical and physical processes involved in the marine system make it difficult to monitor and predict future eutrophication events. Conceptual models, such as Cloern, (2002), (discussed in Chapter 3) need to be elaborated and turned into numerical models which will have the ability to predict how management steps can be undertaken efficiently to minimise the impact of the eutrophication. However, in order to understand the processes which cause these events, we need to be able to measure key *in-situ* biogeochemical parameters relevant to the phytoplankton such as nutrients, at a high temporal and spatial resolution.

Traditional techniques only allow limited monitoring of nutrients and are therefore likely to miss short-term transients (Section 3.4). There is therefore a clear requirement for *in-situ* analysers capable of measuring at a high temporal resolution. There are now a number of instruments available that claim to be able to reliably measure *in-situ.* nutrients. In Chapter 4 the traditional laboratory methods of nutrient analysis and a sample of the *in-situ*

instruments were examined. In order to be commercially viable and practical for marine field work the instrument requires the following qualities: -

- Stable, accurate and precise with a good response time (<30 minutes for coastal work).
- The ability to work autonomously for periods exceeding at least one month.
- Must be durable and robust.
- Must be mechanically simple.

The instruments investigated in Chapter 4 were *in-situ.* nutrient analysers but they either were not yet autonomous, had yet to be fully field-tested for long periods of time (at least a month) or had a very slow reaction time.
The NAS-2EN nitrate and NAS2-ES silicate analysers are autonomous instruments with low manual and chemical overheads. These instruments are relatively mechanically simple compared to the FIA and rFIA systems. They are self-calibrating using an on-board standard and can be deployed for up to two months. The instruments were field tested during the PROVESS experiment.

The PROVESS experiments showed that the performance of the cadmium reduction column in the nitrate instrument is erratic and full data sets were not collected. However, although there were stability problems with the cadmium reduction column, the nitrate results obtained when the column was stable was within the expected range of nitrate concentrations for the two sites

studied in the North Sea. Also, the data sets showed that the instruments were capable of recording short term transient events The silicate NAS-2EN experienced some technical problems but demonstrated that with further development work this instrument has the potential of being a valuable tool in helping to further the understanding of the role of dissolved silicon in the nutrient cycle.

Bench experiments investigating the reduction capability of the wire cadmium reduction column used by the nitrate NAS-2EN instrument, found that the expected reduction efficiencies of above 97% were not being achieved. The experiments showed that cadmium wire reduction columns only achieved reduction efficiencies of 75.6% for the standard columns and 83.43% of columns contained within fep lined tubing. Both wire cadmium columns also demonstrated that they required bedding-in time before becoming stable. The experiments demonstrated that the columns were increasingly not only reducing the nitrate to nitrite but were further reducing the nitrite. This may have been caused by the copper in the buffer solution adhering to the cadmium wire column and thus increasing the columns reactivity. By the end of the experiments the cadmium wire column had noticeably blackened in appearance which would also correspond with the excess copper adhering to the columns.

A cadmium reduction column was constructed using a glass rod containing Skalar copper coated cadmium graduals which were interspersed with copper flecks. Although this column had a lower reduction efficiency than the wire

columns, the experiments demonstrate that the nitrite was not being further reduced. A second column was constructed by replacing the glass rod with standard tubing to make the column more robust. Several macros were written to determine the optimal contact time for the sample and the reduction column. The optimal macro (called the master nitrate macro) was then tested on a new cadmium granular column with an increased bed volume. Also, this experiment used reagents made up to the concentration as described by Grasshoff *et al.*, (1999). A stable reduction efficiency of almost 100% was achieved which did not require the bedding in time that the cadmium wire column exhibited. Although the reduction efficiency of the column was affected by temperature, this was compensated for as the OBS was deployed with the instrument and would be exposed to the same temperature as the column. The granular cadmium column was far simpler to construct and easier to reproduce than the cadmium wire column which had to be wound by hand.

Therefore, the new cadmium granular reduction column, control macro (master nitrate macro) and chemicals were deployed as part of the REES project carried out by Dunstaffnage Marine Laboratory. The instrument deployed on the surface of Loch Etive worked successfully during the deployment until the reagents ran out 45 days. The on-board standard was stable with a coefficient of variation of 3.6% for over 900 seawater samples measured.

A second instrument, deployed on the seabed of Loch Etive, only worked for 16 days after which it switched off. On investigation it was found that the bottom instrument had leaked water into the compartment that contained the electronic controller boards. It was also found that from the 10 April 2000 to the 14th April 2000 the absorbance of the OBS fell from a value of 0.22 to 0.12. It is probable that either a blockage or some sort of constriction in the on-board standard tubing was the cause of this trend.

Although problems were encountered with one of the data sets retrieved from the REES program, this was probably due to a mechanical fault in the instrument. The surface data set and the bench tests show that the new cadmium granular column and control macro and chemicals represent a significant improvement of the instrument's performance. The stability of the reduction column and the amount of data collected during this deployment had not been previously achieved with this type of instrument. During the deployment a transient event, thought to be associated with the artificial control of the riverine input to the Loch, occurred and lasted for four days. This event was recorded by the surface instrument and had the nutrients been studied by traditional methods it is unlikely that the event would have been seen. This demonstrates the valuable contribution that such an instrument could make to a marine monitoring program.

For the future, further deployments using the improved method described in this thesis should be carried out in a range of environments to further ascertain the reliability on the method. However, it has been demonstrated

that the NAS-2EN used with this new method is capable of acquiring large, reliable data sets which was not been previously possible. The instrument is being further developed to allow it to be attached to a ships seawater flow system. This will not only allow the instrument to collect valuable data sets on scientific vessels but may mean it can be deployed on ships of opportunity, such as is case with the various Ferry Box programs which are currently being developed. A similar instrument is also being developed at the Southampton Oceanography Centre to measure ammonia using a fluorometric technique. This instrument is currently at the field testing stage and when fully developed will compliment the suite of *in situ* autonomous nutrient analysers.

Appendix 1. Master Nitrate Control Macro

NutrientDATA Macro – Description

Filename: Nitrate Master

This macro mixes the sample with ammonium chloride. Dilution 1 Ammonium Chloride: 1 sample.

Pushes 180 Steps (60 steps = 1ml) onto column pulls back 60 steps after 2 second delay then pushes 60 steps on and pauses for 2 seconds which is then repeated 9 times.

OBS on green port.

NutrientDATA Macro – Analysis Cycle

T - Seawater Analysis Routine
S - On Board Sample Analysis Routine

Filename: Nitrate Master

T Macro
S Macro
S Macro
S Macro
S Macro
S Macro

NutrientDATA Macro – S Macro (Seawater Analysis Routine)

Filename: Nitrate Master

Action	Argument	Port	Comment
Align rotary valve	NULL	Purple	Aligns valve
Move valve clockwise	1	Grey	Move to Seawater port
Retract plunger	60	Grey	Draw 1ml of seawater into syringe
Move valve clockwise	2	Red	Move to Ammonium Chloride port
Retract plunger	60	Red	Draw 1ml of Ammonium Chloride into syringe
Move valve anti-clockwise	4	Blue	Move to colorimeter port
Insert plunger	20	Blue	Mix
Retract plunger	20	Blue	Mix
Pause (seconds)	2	Blue	Mix
Insert plunger	20	Blue	Mix
Retract plunger	20	Blue	Mix
Pause (seconds)	2	Blue	Mix
Insert plunger	120	Blue	Push sample into colorimeter
Record data readings	1	Blue	Record reading (Blank Value)
Move valve clockwise	2	Grey	Move to seawater port
Retract plunger	30	Grey	Draw 0.5ml of seawater into syringe
Move valve clockwise	2	Red	Move to Ammonium Chloride port
Retract plunger	30	Red	Draw 0.5ml of Ammonium Chloride into syringe
Move valve anti-clockwise	2	Grey	Move to Seawater port
Retract plunger	30	Grey	Draw 0.5ml of seawater into syringe
Move valve clockwise	2	Red	Move to Ammonium Chloride port
Retract plunger	30	Red	Draw 0.5ml of Ammonium Chloride into syringe
Move valve anti-clockwise	1	Brown	Move to reduction column port
Insert plunger	120	Brown	Push 2ml sample onto column
Pause (seconds)	2	Brown	Pause
Retract plunger	60	Brown	Pull 1ml into syringe
Insert plunger	60	Brown	Push 1ml onto column
Pause (seconds)	2	Brown	Pause
Retract plunger	60	Brown	Repeat a further 9 times
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat

Action	Argument	Port	Comment
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Move valve clockwise	2	Orange	Move Valve to Sulphanilamide port
Retract plunger	4	Orange	Pull 0.06ml into syringe
Move valve clockwise	1	Yellow	Move to NED port
Retract plunger	4	Brown	Pull 0.06ml into syringe
Move valve clockwise	2	Blue	Move to colorimeter port
Insert plunger	20	Blue	Mix
Retract plunger	20	Blue	Mix
Pause (seconds)	2	Blue	Mix
Insert plunger	20	Blue	Mix
Retract plunger	20	Blue	Mix
Pause (seconds)	2	Blue	Mix
Insert plunger	20	Blue	Mix
Retract plunger	20	Blue	Mix
Pause (seconds)	5	Blue	Pause
Insert plunger	68	Blue	Push all of sample into colorimeter
Pause (seconds)	20	Blue	Pause
Move valve clockwise	4	Red	Move to Ammonium Chloride port
Retract plunger	60	Red	Pull 1ml into syringe

Action	Argument	Port	Comment
Move valve anti-clockwise	1	Brown	Move to reduction column port
Insert plunger	60	Brown	Push 1ml of Ammonium Chloride onto column
Move valve anti-clockwise	1	Grey	Move to seawater port
Retract plunger	100	Grey	Take in seawater to push sample out of colorimeter
Move valve anti-clockwise	2	Blue	Move to colorimeter port
Record data readings	1	Blue	Record Sample reading
Insert plunger	100	Blue	Push sample out of colorimeter
END ROUTINE			

NutrientDATA Macro – T Macro (On-Board Standard Routine)

Filename: Nitrate Master

Action	Argument	Port	Comment
Align rotary valve	NULL	Purple	Align valve
Move valve clockwise	1	Grey	Move to seawater port
Retract plunger	90	Grey	Draw 1.5ml of seawater into syringe
Move valve anti-clockwise	2	Blue	Move to colorimeter port
Insert plunger	90	Blue	Push 1.5ml seawater into colorimeter (rinse)
Move valve anti-clockwise	1	Green	Move to OBS port
Retract plunger	30	Green	Draw 0.5ml of standard into syringe
Move valve anti-clockwise	3	Red	Move to Ammonium Chloride port
Retract plunger	30	Red	Draw 0.5ml of Ammonium Chloride into syringe
Move valve anti-clockwise	4	Blue	Move to colorimeter
Insert plunger	20	Blue	Mix
Retract plunger	20	Blue	Mix
Pause (seconds)	2	Blue	Mix
Insert plunger	20	Blue	Mix
Retract plunger	20	Blue	Mix
Pause (seconds)	2	Blue	Mix
Insert plunger	60	Blue	Push sample into colorimeter
Record data readings	1	Blue	Record blank reading
Move valve anti-clockwise	1	Green	Move to OBS port
Retract plunger	30	Green	Draw 0.5ml of standard into syringe
Move valve anti-clockwise	3	Red	Move to Ammonium Chloride port
Retract plunger	30	Red	Draw 0.5ml of Ammonium Chloride into syringe
Move valve clockwise	3	Green	Move to OBS port
Retract plunger	30	Green	Draw 0.5ml of standard into syringe

Action	Argument	Port	Comment
Move valve anti-clockwise	3	Red	Move to Ammonium Chloride port
Retract plunger	30	Red	Draw 0.5ml of Ammonium Chloride into syringe
Move valve anti-clockwise	1	Brown	Move to reduction column port
Insert plunger	120	Brown	Push 2ml sample onto column
Pause (seconds)	2	Brown	Pause
Retract plunger	60	Brown	Pull 1ml sample off column
Insert plunger	60	Brown	Push 1ml sample onto column
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Insert plunger	60	Brown	Repeat
Pause (seconds)	2	Brown	Repeat
Retract plunger	60	Brown	Repeat
Move valve clockwise	2	Orange	Move Valve to Sulphanilamide port
Retract plunger	4	Orange	Pull 0.06ml into syringe
Move valve clockwise	1	Yellow	Move to NED port

Action	Argument	Port	Comment
Retract plunger	4	Yellow	Pull 0.06ml into syringe
Move valve clockwise	2	Blue	Move to colorimeter port
Insert plunger	20	Blue	Mix
Retract plunger	20	Blue	Mix
Pause (seconds)	2	Blue	Mix
Insert plunger	20	Blue	Mix
Retract plunger	20	Blue	Mix
Pause (seconds)	2	Brown	Mix
Insert plunger	20	Blue	Mix
Retract plunger	20	Blue	Mix
Pause (seconds)	5	Blue	Pause
Insert plunger	68	Blue	Push all of sample into colorimeter
Pause (seconds)	20	Brown	Pause
Move valve clockwise	4	Red	Move to Ammonium Chloride port
Retract plunger	60	Red	Pull 1ml into syringe
Move valve anti-clockwise	1	Brown	Move to reduction column port
Insert plunger	60	Brown	Push 1ml of Ammonium Chloride onto column
Move valve anti-clockwise	1	Grey	Move to seawater port
Retract plunger	100	Grey	Draw in seawater to push sample out of colorimeter
Move valve anti-clockwise	2	Blue	Move to colorimeter port
Record data readings	1	Blue	Record Sample reading
Insert plunger	100	Blue	Push sample out of colorimeter
Move valve anti-clockwise	2	Grey	Move to colorimeter port
Retract plunger	100	Grey	Take in seawater to push sample out of colorimeter
Move valve anti-clockwise	2	Blue	Move to colorimeter port
Record data readings	1	Blue	Record Sample reading
Insert plunger	100	Blue	Push sample out of colorimeter
END ROUTINE			

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