UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING, SCIENCE & MATHEMATICS

National Oceanography Centre, Southampton

School of Ocean & Earth Sciences

Studies of nitrous oxide and the nitrogen cycle in a temperate river-estuarine system*

by

Luciane Veeck

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ABSTRACT

FACULTY OF ENGINEERING, SCIENCE & MATHEMATICS

SCHOOL OF OCEAN AND EARTH SCIENCES

Master of Philosophy

STUDIES OF NITROUS OXIDE AND THE NITROGEN CYCLE IN A TEMPERATE RIVER-ESTUARINE SYSTEM

by Luciane Veeck

Nitrous oxide (N_2O), the third most important greenhouse gas in terms of anthropogenic climate forcing, is also one of the most important trace gases in driving atmospheric chemistry. It is a potent greenhouse gas, having a global warming potential per mole about 300 times that of carbon dioxide, and it is an intermediate in the destruction of stratospheric ozone. Unfortunately, the N_2O global budget, and in particular the cause of its steady rise over the past century, is not well defined. The natural and anthropogenic sources for its increase are probably not all identified and certainly not well quantified. Recent studies show that when estuarine and coastal regions are included in the global N_2O budget, a considerable portion of the global marine N_2O flux is from estuarine and coastal regions, mainly due to high emissions from estuaries. To examine the contribution of estuaries to N_2O emissions, nitrous oxide concentrations in the water were measured by gas chromatography on a monthly basis in the River Itchen and Itchen Estuary - UK, from November 2001 to December 2002. Water column concentrations of N_2O in both, river and estuary were supersaturated with respect to air (mean saturation 325% and 162%, respectively), indicating that they were sources of N_2O to the atmosphere. High N_2O concentrations in the river appear related to high concentrations in the groundwater. Highest N_2O concentrations in the estuary were generally observed at lower salinities (up to 79nM and saturation = 679%) when compared with concentrations at the high salinity (average saturation = 87%). Nitrite had the strongest correlation with N_2O for all surveys ($r=0.78$; $p<0.05$), suggesting that nitrite is linked to nitrous oxide production in estuaries. Fluxes from the River Itchen and Itchen Estuary extrapolated to the UK systems and compared with other anthropogenic sources of nitrous oxide to the atmosphere, showed that these systems are significant sources and should be included in the N_2O budget. Incubation experiments were done with sediment collected from the Itchen Estuary to investigate N_2O production. Initial experiments on a whole core showed the importance of temperature on N_2O production, and also the potentially complicating impact of biological activity. In subsequent experiments, homogenized and sieved sediment material were used, in which macro benthos were excluded. N_2O fluxes from the sediment were estimated and denitrification was suggested as the main process producing N_2O in the sediments of the incubated cores.

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DECLARATION OF AUTHORSHIP

I, Luciane Veeck, declare that the thesis entitled “Studies of nitrous oxide and the nitrogen cycle in a temperate river-estuarine system” 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.

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Chapter 1

INTRODUCTION

The nitrogen cycle is one of the more complex cycles of elements (e.g. relative to carbon, sulphur and oxygen). It includes a variety of important biological and abiotic processes that involve many compounds in the gaseous, liquid and solid phases (Schlesinger, 1997).

The atmosphere and hydrosphere are two major zones for nitrogen cycling on the surface of the Earth. In the atmosphere a minute fraction of nitrogen occurs in forms other than N_2. The quantitatively most important form of combined nitrogen in the atmosphere is nitrous oxide, which accounts for 99.5% of all combined nitrogen (Jaffe, 1992).

In recent years attention has been drawn to atmospheric nitrous oxide (N_2O) and the processes affecting its formation and destruction. Reasons for that lie on the global warming potential of nitrous oxide and its indirect involvement in the destruction of stratospheric ozone (O_3).

This chapter will present some background information about nitrous oxide, its significance and how it is formed through the biochemical pathways of the nitrogen cycle. Global sources and sinks will be discussed as well as the actual balance between them. Finally the current extent of knowledge on

aquatic sources will be explored and the objectives of this study will be presented.

1.1. Atmospheric significance of N_2O

The N_2O molecule is covalently bonded; it is a colourless gas with a boiling point of -90 °C. The gas is fairly soluble in water with a Henry's Law constant of 0.068 mol N_2O-N.l^{-1} atm^{-1} at 15 °C (Weiss and Price, 1980).

Because of its chemical inertness and the photochemical coupling of N_2O, NO_y* and O_3 in the stratosphere, N_2O has an atmospheric residence time of between 114-120 years (Prather, 1998). In the stratosphere it is the major source of nitric oxide radicals that play an important role in the depletion of stratospheric ozone (Crutzen and Schmailzl, 1983). The characteristic absorption of N_2O in the infrared range of the atmospheric window of the Earth makes it act as a greenhouse gas (Rodhe, 1990). Its contribution to the anthropogenic greenhouse effect was estimated to be 5-7% (Houghton et al., 1995).

Although a trace gas in the atmosphere, with concentrations around 314 ppb v/v (corresponding to a global burden† of 1510 TgN), N_2O has a global warming potential per mole some 296 times that of carbon dioxide (CO_2) over a 100 year period (Prather et al., 2001).

N_2O abundances are about 0.8 ppb greater in the Northern Hemisphere than in the Southern Hemisphere, consistent with about 60% of emissions occurring in the Northern Hemisphere. Almost no vertical gradient is observed in the troposphere, but N_2O abundances decrease in the stratosphere, for example, falling to about 120 ppb by 30 Km at mid-latitudes (Prather et al., 2001).

The present N_2O concentration has not been exceeded during at least the past thousand years. Concentrations in the atmosphere remained

* Thermodynamically unstable gases (NO, NO_2, NO_3, N_2O_5, HONO, HO_2NO_2, and HNO_3) in the stratosphere interchange with one another but have overall a relatively stable steady state concentration, and are designated NO_y.

† The burden is defined as the total mass of the gas integrated over the atmosphere and related reservoirs, which usually include just the troposphere and stratosphere.

constant for the centuries prior to the Industrial Revolution (pre-industrial levels were about 275 ppbv) and started increasing perhaps as recently as 50-80 years ago (Figure 1-1). The average rate of increase in the atmosphere was about 0.8 ppb v/v per year (0.25%/yr trend calculated for 1980 to 1998) (Zander et al., 1994).

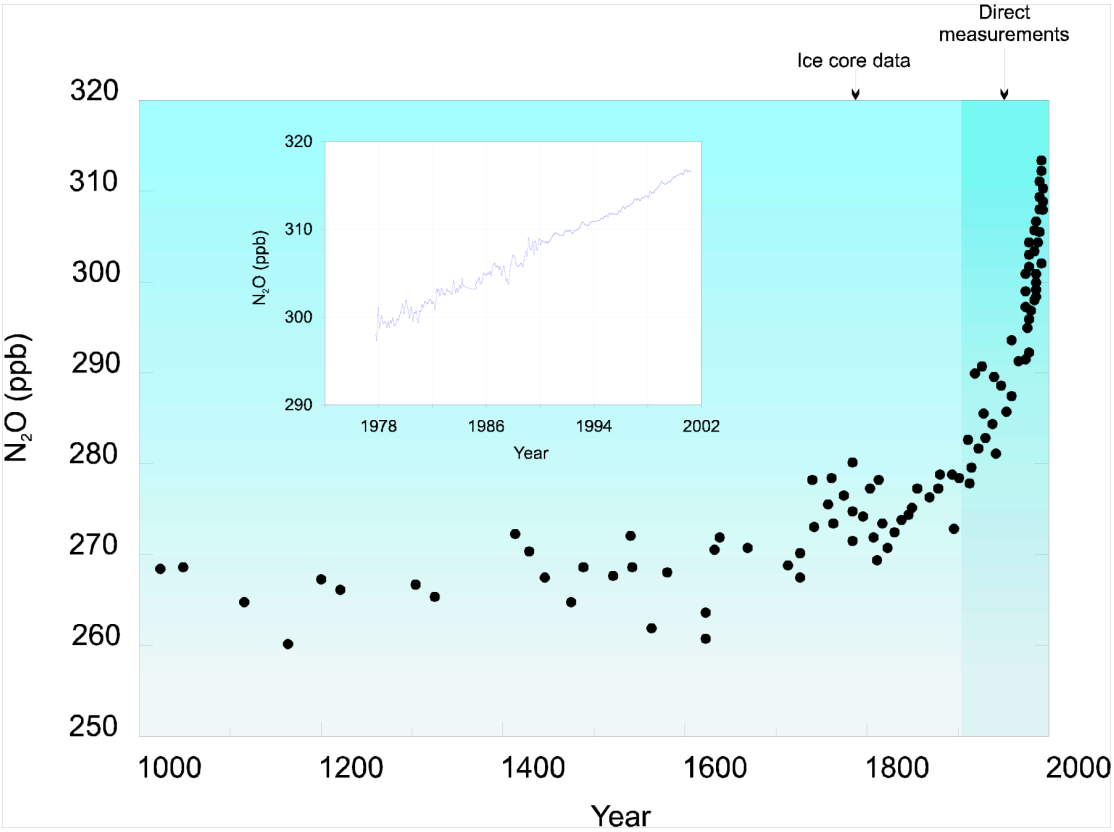

Figure 1-1. Change in N_2O concentrations for the last 1,000 years as determined from ice cores and air samples (adapted from Prather et al., 2001). Data sets are from: (Battle et al., 1996; Fluckiger et al., 1999; Langenfelds et al., 1996; Machida et al., 1995; Steele et al., 1996).

Significant interannual variations in the upward trend of N_2O concentrations are observed, e.g., a 50% reduction in annual growth rate from 1991 to 1993 (Thompson et al., 1994). Suggested causes are several-fold: a decrease in use of nitrogen-based fertiliser, lower biogenic emissions (Thompson et al., 1994), and larger stratospheric losses due to volcanic-induced circulation changes (Schauffler and Daniel, 1994). Since 1993, the growth of N_2O concentrations has returned to rates closer to those observed during the 1980s. While this observed multi-year variance has provided some

potential insight into what processes control the behaviour of atmospheric N_2O, the long term trends of this greenhouse gas remain largely unexplained.

1.2. N_2O and the nitrogen cycle

Atmospheric N_2 is the most abundant form of nitrogen at the surface of the Earth, and also the least reactive species of nitrogen. To be used by biota, N_2 must be first converted to one of the forms of fixed nitrogen by nitrogen fixing organisms. Once biologically available, nitrogen can be transformed by process like ammonia assimilation, nitrification, assimilatory nitrate reduction, mineralization, denitrification and dissimilatory nitrate reduction to ammonium (Figure 1-2).

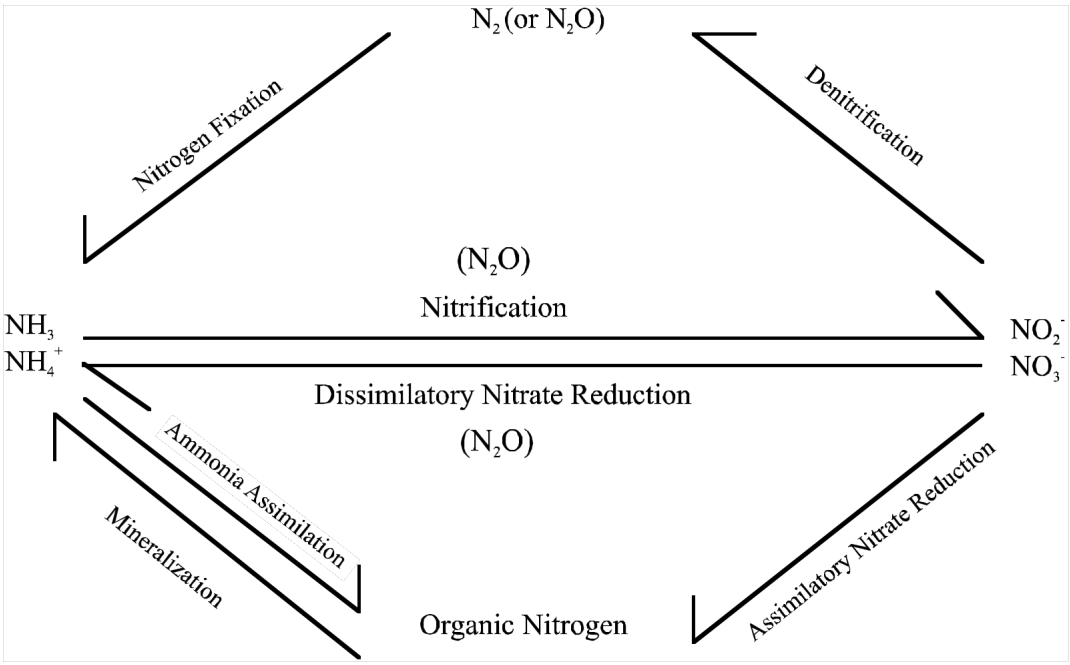

Figure 1-2. Biological transformations of nitrogen compounds (Adapted from Chameides and Perdue, 1997).

Denitrification is the major process which returns N_2 to the atmosphere. The balance between N-fixation and denitrification through geological time determines the nitrogen available to biota and the global nitrogen cycle (Schlesinger, 1997).

Human activities have had a dramatic impact on the global N cycle. N-fertiliser production and the fossil fuel combustion release about 60% of the total fixed N that is delivered from the atmosphere to the Earth's land surface every year (Smil, 1991). It is probable that denitrification has not kept pace with this new rate of fixation. Despite the fact that there is no concern over depletion of atmospheric nitrogen by human activity, other consequences of this increment of fixed nitrogen deserve scrutiny. These include problems of eutrophication, the concentration of nitrate ion in waters and food, acid precipitation, and, of particular interest here, the possibility of an increased atmospheric concentration of N_2O (Delwiche, 1981; Schlesinger, 1997; Vitousek, 1994).

Nitrous oxide is produced as a by-product of microbial oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) by nitrification, and an intermediate product of microbial reduction from NO_3^- to nitrogen gas (N_2) by denitrification. N_2O is also produced by other microbial processes such as dissimilatory nitrate reduction to ammonium (Goreau et al., 1980 ; Jorgensen et al., 1984; Knowles, 1982; Yoshinari, 1990). Nitrification and denitrification appear to be the dominant sources of N_2O in most natural systems (Firestone and Davidson, 1989).

Under aerobic conditions, the oxidation of NH_4^+ to NO_2^- is energy yielding. The further oxidation of NO_2^- to NO_3^- also yields energy and is also part of the nitrification process. In the oxidation of ammonium ion to nitrite there is undoubtedly an intermediate at the oxidation level of hyponitrous acid (HONNOH) or its anhydride N_2O, and stopping the reaction at this stage could be energetically advantageous, depending upon pH and other variables. Under alkaline conditions N_2O production actually gives a higher energy yield than does the production of nitrite. The difference is comparatively small, but in marine environments (a pH of 8.3 being typical) N_2O production would be slightly favoured in the first step of the nitrification reaction. There is also the added advantage that a nitrifying organism under conditions of low oxygen supply can, by liberating N_2O that is not readily available for further nitrification, exclude a competitive organism that would otherwise oxidise nitrite to nitrate, utilising some of the limited oxygen supply. A small difference

in energy yield is difficult to interpret, but the argument of competition, from an ecological point of view, is more convincing. Under circumstances of limited oxygen supply the evolution of a system favouring N_2O production in the nitrification process might be expected (Delwiche, 1981).

On the other hand, if the conditions become anaerobic, nitrate can serve as an electron acceptor for the oxidation of organic (and sometimes inorganic) compounds, with the yield of energy and the release of gaseous N_2 or N_2O.

Knowledge of the energy yielded in a particular reaction, although it is informative, does not necessarily assure an accurate prediction of what will happen. For example, denitrification with the production of N_2 yields more energy than does the production of N_2O. The difference in energy yield *per unit nitrate consumed* is appreciable, but, depending on the mechanism of the reaction, the cell may not be able to take advantage of the difference. Hyponitrous acid, one of the possible intermediates, is unstable, decomposing spontaneously to yield H_2O and N_2O. When nitrate ion is abundant and organic substrate limiting, the energy yield per unit carbohydrate would appear determinant. This difference is small but still favours the production of N_2.

Yet, N_2O is formed in the denitrification reaction. Under field conditions the yield of N_2O relative to N_2 ranges from negligible to 20% (Rolston et al., 1976; Stefanson, 1972; Stefanson, 1973). This suggests that other factors are involved. For example, when the concentration of nitrate is high compared with available organic substrate, N_2O is usually a larger fraction of the total denitrified gas. N_2O production by denitrification is also a function of pH. As pH is increased, the proportion of N_2O to N_2 appears to be favoured (Delwiche, 1981).

Despite the fact that N_2O is an intermediate product of denitrification, with possible further reduction to N_2, high N_2O fluxes are reported associated with incomplete denitrification. According to a recent study of N_2O emissions by forest soils (Vor et al., 2003), the final step of denitrification (the reduction of N_2O to N_2) will not take place as long as more efficient electron acceptors

(e.g. NO_3^-) are still available. Therefore large N_2O emissions occur mainly during intermediate aeration, which is probably the result of the coexistence of nitrification and denitrification. Hence soils influenced by alternating aeration should show higher N_2O emission rates than anaerobic or aerobic soils.

Formation of N_2O has also been observed in heterotrophic prokaryotes and fungi capable of nitrate reduction. These organisms are not classified as true denitrifiers as they are incapable of reducing nitrate completely to N_2 but may still produce N_2O and possibly NO. Smith and Zimmerman (1981) studied various dissimilatory nitrate reducers (e.g. *Citrobacter* and *Bacillus*) isolated from loam soils in a series of laboratory experiments. Ionic forms of nitrogen (either ammonium or nitrite) were the predominant products of nitrate reduction but significant quantities of N_2O were formed (up to 24%). Unlike true denitrifiers, further reduction of N_2O to N_2 was not observed and acetylene (used as a nitrification inhibitor) had no significant effect on the amount of N_2O recovered. Bleakley and Tiedje (1982) carried out laboratory studies to investigate production of N_2O by various nitrate-respiring bacteria, yeasts and fungi. N_2O production for nitrate-respirers only occurred during the stationary growth phase but up to 36% of the nitrate added was recovered as N_2O (*Escherichia coli*). Yeasts and fungi produced N_2O but in much lower quantities and maximum conversions of nitrate to N_2O were only up to 0.178% (*Hansenuela*).

Various green algae found in aquatic systems have also been found to produce N_2O. Weathers (1984) conducted a series of *in vitro* experiments on axenic cultures of *Chlorella, Scenedesmus, Coelastrum* and *Chlorococcum.* All species of algae studied produced N_2O (up to 122.6 nmol N_2O-N mg^{-1} cell dry weight) when grown on nitrite but not on nitrate. There was some evidence of oxygen influence on N_2O evolution but the exact role was unclear and the mechanism for N_2O production by green algae was not known.

1.3. Global sources and sinks of N_2O and the balance between them

The sources of atmospheric nitrous oxide are dominantly at the earth's surface and they are both natural and anthropogenic. Progress has been

made on quantification of N_2O sources, but as with other trace gases (e.g. methane), it remains difficult to assess global emission rates from individual sources that vary greatly over small spatial and temporal scales.

The total natural global emission (as considered by the Intergovernmental Panel on Climate Change) was estimated at 9.6 Tg N_2O-N.yr^{-1} (Kroeze et al., 1999). This value includes N_2O from soils under natural vegetation, oceans, aquatic systems, and formation in the atmosphere.

Biological processes in soils and oceans are the primary natural source of N_2O. From the total natural N_2O emission (Figure 1-3), 6.0 Tg N_2O-N.yr^{-1} are accredited to soils under natural vegetation, of which 4.0 Tg N_2O-N.yr^{-1} are from tropical soils (wet forest and dry savannas) and 2 Tg N_2O-N.yr^{-1} are from temperate soils (forests and grasslands) (Bouwman et al., 1993; Kroeze et al., 1999). Emissions from oceans were estimated at 3.0 Tg N_2O-N.yr^{-1}, of which 1.9 Tg N_2O-N.yr^{-1} are accredited to rivers, estuaries and continental shelves (Kroeze et al., 1999; Seitzinger and Kroeze, 1998). Finally, the global amount of N_2O that results from oxidation of atmospheric ammonia (NH_3) is currently estimated at 0.6 Tg N_2O-N.yr^{-1} (Dentener and Crutzen, 1994; Kroeze et al., 1999).

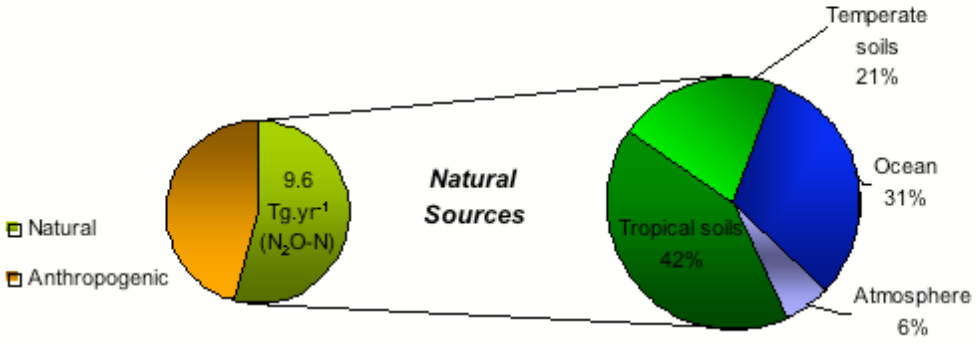

Figure 1-3. N_2O emissions from natural sources (data from Kroeze et al., 1999).

It is difficult to separate natural unperturbed biogenic emission of N_2O from additional biogenic emission resulting from fertiliser application and effluent inputs. The magnitude and global distribution of nitrous oxide emissions from natural soils and from agricultural soils have been investigated

(Bouwman et al., 2002; Bouwman et al., 1993; Bouwman et al., 1995; Matthews, 1994), showing that approximately 71% of total soil emissions is natural. According to Bouwman, 79% of the natural N_2O emission from soils comes from the tropical regions (equator ± 30°) and the remaining 21% from non-tropical regions pole ward of 30°. A comparable analysis of the magnitude and global distribution of nitrous oxide emissions in aquatic ecosystems due to natural and/or anthropogenic processes was made by Seitzinger and Kroeze (1998). According to their model, about 1% of the N input from fertilisers, atmospheric deposition, and sewage to watersheds is lost as N_2O in rivers and estuaries. Globally, rivers and estuaries could account for approximately 20% of the current global anthropogenic N_2O emissions. Approximately 90% of N_2O emissions from rivers and estuaries are in the northern hemisphere (in line with the regional distribution of dissolved inorganic nitrogen export by rivers), of which 50% are accounted to China and India.

The total anthropogenic global emission was estimated at 7Tg N_2O-$N.yr^{-1}$ (Perez-Ramirez et al., 2003). This value includes N_2O from agriculture, industry, transport, energy, waste and others (Figure 1-4).

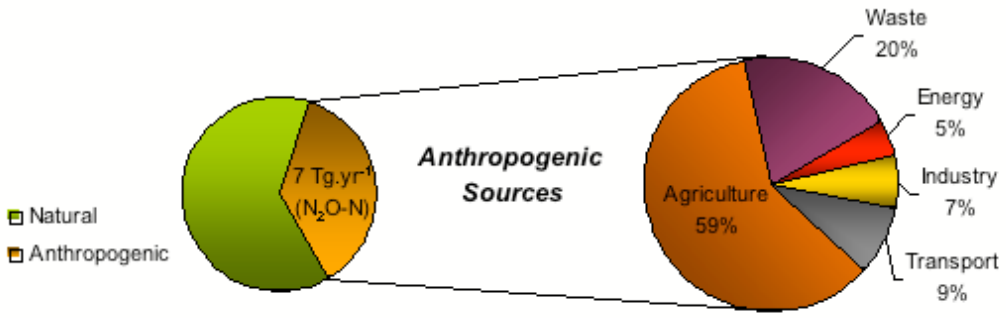

Figure 1-4. N_2O emissions from anthropogenic sources (data from Perez-Ramirez et al., 2003).

Agriculture, through soil cultivation, the use of nitrogen-fertilisers, and animal waste management systems, contributes to approximately 50% of the total anthropogenic emission (Kroeze et al., 1999; Perez-Ramirez et al., 2003). Recent discovery of a faster-than-linear feedback in the emission of

N_2O from soils in response to external N inputs is important, given the projected increases in N fertilisation and deposition increases in tropical countries (Matson et al., 1999). Tropical ecosystems, currently an important source of N_2O, are often phosphorus limited rather than being nitrogen limited like the Northern Hemispheric terrestrial ecosystems. Nitrogen fertiliser inputs into these phosphorus limited ecosystems generate N_2O emissions that are 10 to 100 times greater than the same fertiliser addition to nearby nitrogen limited ecosystems (Hall and Matson, 1999). In addition to N availability, soil N_2O emissions are regulated by temperature and soil moisture and so are likely to respond to climate changes (Frolking et al., 1998; Parton et al., 1998).

Emissions from chemical industry mainly apply to adipic acid and nitric acid production plants. Prior to legislation a number of industries have voluntarily initiated efforts to reduce N_2O emissions from adipic acid production, with a global reduction from 600Kt per year in 1994 to less than 100Kt per year currently. Other newly identified industrial sources are production plants of caprolactam, glyoxal, acrylonitrile, and in general, processes using nitric acid as oxidising agent or involving ammonia oxidation. Emissions from the latter N_2O sources are less significant and not quantified as yet (Perez-Ramirez et al., 2003).

The transport sector, a source that doubled between 1990 and 1998 (Perez-Ramirez et al., 2003), is a large uncertainty in emission inventories. The rapid increase seen in the nineties was thought to be a side effect of the introduction of the catalytic converters, but extrapolating measurements of N_2O emissions from automobiles in roadway tunnels in Stockholm and Hamburg during 1992 to the global fleet gives a source of only 0.24 ±0.14 Tg N_2O-N.yr^{-1} (Berges et al., 1993). More recent measurements suggest even smaller global emissions from automobiles, 0.11 ±0.04 Tg N_2O-N.yr^{-1} (Becker et al., 1999; Jimenez et al., 2000). However, emissions from road transport have increased in the United Kingdom (UK) and Europe by a factor of 3 and 2 respectively, between 1990 and 2001(Table 1-1).

Stationary combustion of fossil fuel is also a known source of N_2O. Combined N_2O emissions from static sources such as power stations and heating systems in 1990 was estimated at 0.5 Tg N_2O-N.yr^{-1} (Kroeze and

Bouwman, 1994). Unlike other pollutants, the nitrous oxide emission from public power in UK and Europe shows little variation over the period 1990 to 2001 in spite of the trend away from coal towards natural gas combustion (European Environment Agency, 2004). The emission factor for gas combustion is similar to that for coal combustion so no particular trend is apparent. However, these estimates are uncertain because there are very limited data on N_2O emissions from large turbines.

Sewage and waste disposal activities have been considered in the global N_2O budget since the late 1970s. (Kaplan et al., 1978) discovered that large N_2O supersaturations (up to 4000%) were associated with sewage discharges from urban areas in the lower parts of the Potomac and Merrimack rivers, USA. The global source strength of N_2O from waste-water plants was estimated at 0.2 to 1.6 Tg N_2O-N.yr^{-1}, assuming the Potomac to be globally representative.

Type of source	Global N_2O emissions[a] (Mt N_2O per year)	EU15 N_2O emissions[b] (Kt N_2O per year)		UK N_2O emissions[b] (Kt N_2O per year)	
		1990	2001	1990	2001
Natural	~ 13				
Soils	10				
Oceans	2.9				
Atmospheric chemistry	0.2				
Anthropogenic	~ 7	1319	1112	217	136
Energy	0.2-0.5	47	52	7	8
Industry	0.5	373	185	98	21
Transport	0.4-0.9	38	85	4	14
Agriculture	4.5	769	706	103	88
Waste	1.5	23	20	3	4
Other[c]		69	64	2	1
Total of all sources	~ 20				

[a] Global emissions from (Perez-Ramirez et al., 2003).
[b] EU15 and UK emissions/1990 and 2001 from http://dataservice.eea.eu.int/dataservice
[c] Fugitive emissions from fuel, solvent use, and land use change.

Table 1-1. Global, European and UK emissions of nitrous oxide.

Changes in the land use also can lead to increased N_2O emission. Forest clearance into pasture was estimated to increase the global tropical

forest N_2O strength from 2.4 Tg N_2O-N.yr^{-1} to 3.1 Tg N_2O-N.yr^{-1} (Matson and Vitousek, 1990), but the duration of these changes are still not well understood.

Regarding sinks of N_2O, there are no known important atmospheric reactions that lead to significant removal of this gas from the troposphere. The important atmospheric destruction is likely to take place by photochemical reactions in the stratosphere. Based on a combination of measurements and models, stratospheric loss of N_2O is reasonably well quantified in recent evaluations at about 13Tg N_2O-N.yr^{-1} to within ±20% (Prather et al., 2001). Ultraviolet photolysis comprises about 90% of the loss while photo-oxidation with an excited oxygen atom accounts for the rest (Toyoda et al., 2004).

The global N_2O budget has been the least well constrained of the global trace gas budgets. In both, 1990 and 1992 Intergovernmental Panel on Climate Change (IPCC) Scientific Assessments it was concluded that estimated ranges for known anthropogenic sources of N_2O could not explain the atmospheric increase. The most recent estimates of global N_2O emissions from Mosier *et al.* (1998) and Kroeze et al. (1999) provide a reasonable global loss rate, but uncertainties remain. The source strengths calculations are based on emissions inventories and different inventories vary widely. For example, the largest single anthropogenic source is agricultural soils, which is estimated to be 4.2 Tg N_2O-N.yr^{-1} but with a range of 0.6 to 14.8 Tg N_2O-N.yr^{-1}. Even the total source strength from emissions inventories has a range of at least ±50% (Kroeze et al., 1999; Mosier et al., 1998; Prather et al., 2001). The best constraint on the current source strength is based on the observed annual increase in surface abundance (Prinn et al., 2000; Weiss, 1981): i.e., the total source strength currently exceeds the sink by about 4Tg N_2O-N.yr^{-1} with an uncertainty of only ±10%.

In addition, there is not much known about nitrous oxide transport. By analogy to other trace gases, nitrous oxide is probably transported from the sediment to the overlying water or atmosphere by diffusion, gas bubble ebullition following stripping of nitrous oxide from sediment or plant supported transport (Martens and Chanton, 1989). Accordingly, any factor that affects

either directly or indirectly nitrous oxide production, consumption or transport may affect nitrous oxide emissions rates.

1.4. N_2O in aquatic systems

The contribution of the world's ocean to the global emissions of atmospheric N_2O was first estimated to be about 13% by Khalil and Rasmussen (1992), and then 20-30% by Nevison et al. (1995), and more recently 17% by Kroeze et al. (1999). As discussed previously, there is still significant uncertainty about the inventories and fluxes of nitrous oxide for marine systems, and especially in the coastal area, because estimates are based on few or no measurements.

In most oceanic water, N_2O is often found at levels in excess of atmospheric equilibrium leading to super-saturation, with "hot spots" of high concentration in coastal water. Coastal regions, although occupying only about 18% of the total ocean area, may contribute approximately 60% of the net marine N_2O flux, mainly due to high emissions from estuaries and upwelling areas (Bange et al., 1996).

Estuaries often receive high loading of nutrients and organic matter, while the tidal circulation generally causes a long residence time of the water. As a consequence, turnover of nitrogen and carbon usually is more intense in estuaries than either rivers or the open ocean. Since N_2O production is positively related to nitrogen and carbon turnover, estuaries potentially are strong sources of N_2O (de Wilde and de Bie, 2000; Firestone and Davidson, 1989; Law et al., 1992; Robinson et al., 1998).

A study by Law *et al.* (1992) investigated the N_2O emission from the water column along the Tamar estuary. The N_2O supersaturations measured in this study were attributed primarily to sediment release with water column production and freshwater input as secondary sources. The overall mean N_2O flux estimate of 820 nmol N_2O m^2 h^{-1} was multiplied by the total global area occupied by estuaries (1.4×10^{12} m^2) to give a global estuarine N_2O source of 0.44 Tg N_2O y^{-1}. This estimate did not account for sediment-air emission of N_2O from the intertidal zone

Nitrous oxide emissions from the intertidal estuarine zone were studied in the Scheldt Estuary (Middelburg et al., 1995), where annual N_2O emission rates were compared to annual nitrogen turnover rates based on mass-balance considerations. Results showed that the global riverine nitrogen input to estuaries (43×10^{12} g N y^{-1}) related to a global N_2O source of 2.5×10^9 g N y^{-1}, which was considered rather unimportant compared to other nitrous oxide sources which total 11.1×10^{12} g N y^{-1}.

Benthic denitrification is generally considered to be the primary estuarine source of N_2O (Bange et al., 1996; Butler et al., 1987; Delwiche, 1981; Law et al., 1991; Robinson et al., 1998), but the precise mechanisms of N_2O production are still unclear. Recently, progress has been made in determining the relative importance of nitrification and denitrification to estuarine N_2O production through the use of nitrification inhibitors such as acetylene (Bonin et al., 2002; de Bie et al., 2002). However, both nitrification and denitrification are to some extent sensitive to the same compounds (Bonin et al., 2002), therefore caution is required in the application and interpretation of inhibitor techniques.

Nitrification in the water column is also considered as an important source of nitrous oxide in estuaries (McElroy et al., 1978; Nixon and Pilson, 1983). de Wilde & de Bie (2000) showed that a major portion of N2O production in the Scheldt estuary results from nitrification in the water column, and that almost all of it is lost to the atmosphere within the estuary and is not transported out to sea.

Significant N_2O emissions have also been measured from N-enriched rivers (Seitzinger and Kroeze, 1998). Nitrogen leached from terrestrial ecosystems comes into contact with the riparian (streamside) ecosystems and then enters streams and rivers. Lowrance et al. (1997) when studying riparian forest buffers in Chesapeake Bay found that part of the N load which enters the ecosystem is processed to N_2O and released to the atmosphere before reaching the streams. A recent review by Groffman et al. (2000) showed that the riverine ecosystems are probably regional hot spots in N_2O production but their global N_2O release is unknown.

Inland freshwater lakes are another aquatic ecosystem potentially important as a regional source of N_2O. The pelagic regions of freshwater lakes and reservoirs are considered only to be minor sources, although their N_2O fluxes have shown extensive variability (Huttunen et al., 2001; Huttunen et al., 2003; Mengis et al., 1997). Instead, similar to the streamside ecosystems (Groffman et al., 2000) and wetlands receiving a high N load (Merbach et al., 2001; Silvan et al., 2002), the lake littoral zones with accelerated N cycling represent potential sites for substantial N_2O release.

The N-enriched rivers have been included in the recent global estimates of the aquatic N_2O emissions, whereas the N_2O emissions from inland freshwater lakes are still excluded (Seitzinger and Kroeze, 1998). The neglect of lakes and their littoral zones in the ecosystem N_2O exchange studies may raise serious uncertainties over estimates of the regional N_2O emissions, especially in northern, lake-rich landscapes.

Finally, nitrogen enriched groundwater has been proposed as an important anthropogenic source of atmospheric nitrous oxide. Dissolved N_2O concentrations in groundwater have been reported to be up to 3 orders of magnitude larger than the aqueous N_2O concentrations expected from equilibration with atmospheric N_2O (Muhlherr and Hiscock, 1998; Ronen et al., 1988; Smith et al., 1991; Ueda et al., 1993). Several relatively small areas have been studied (Ronen et al., 1988; Smith et al., 1991; Ueda et al., 1993), but the number of large-scale groundwater studies from which N_2O data are available is more limited (Muhlherr and Hiscock, 1998).

1.5. Aims of the project

To sum up, whilst some insights have been gained into the microbial processes responsible for nitrous oxide production, the details of mechanisms of N_2O production are still unclear. Additionally, it remains difficult to assess global emission rates of nitrous oxide from individual sources that vary greatly over small spatial and temporal scales. This lack of knowledge of sources leads to significant uncertainty in nitrous oxide inventories, especially in the coastal area. There are only very few or non existent data available for this

zone. Flux data from estuaries, of particular interest in this study, are also generally lacking.

Having considered the importance of nitrous oxide to the climate change process, and the lack of existing knowledge on nitrous oxide production and fluxes from coastal areas, the main aim of this project was to:

> Improve our knowledge of the relevance of estuaries as a source of N_2O to the atmosphere and in particular the contribution of the Itchen Estuary to UK emissions.

Specific objectives therefore are:

1. To calculate N_2O fluxes between water and air from the River Itchen and the Itchen Estuary;
2. To make preliminary estimate of N_2O fluxes between sediment and water from the Itchen Estuary using model systems.

Chapter 2

NITROUS OXIDE AND DISSOLVED NUTRIENTS IN THE ITCHEN RIVER AND ESTUARY

2.1. Introduction

As discussed in the previous chapter, estuaries may be significant sources of nitrous oxide to the atmosphere (de Wilde and de Bie, 2000; Delwiche, 1981; Firestone and Davidson, 1989; Law et al., 1991; Law et al., 1992; Robinson et al., 1998). Based on this argument an estuary (the Itchen Estuary) was chosen as a study site for this project.

The Itchen Estuary is a relatively small estuary if compared with other UK estuaries (e.g. Tamar and Great Ouse). In fact this characteristic is seen as a positive factor for this study, as it makes it possible to follow this system from its origin (at Cheriton Stream) to its end, when forming the Southampton Water. By studying the whole system, areas with nutrient inputs and high nitrous oxide concentration were identified and monitored during the thirteen months of sampling, providing a very good data set to discuss both, spatial and temporal variability.

The study sites, sampling, storage and analytical techniques used are described below. Data from the thirteen months sampling are presented and

discussed, and an overall summary is shown at the end of the chapter. The numerical data referent to this chapter is available in Appendices A (River Itchen) and B (Itchen Estuary).

2.2. The study site

The River Itchen rises on the Upper Chalk of the Hampshire Downs as three spring fed tributaries: the Candover Stream, the River Alre and the Cheriton Stream (Figures 2-1 and 2-2). The source of the Cheriton stream is considered by the Environment Agency to be the source of the River Itchen. The catchment area is 507 Km^2, with 44% of this land occupied by farms.

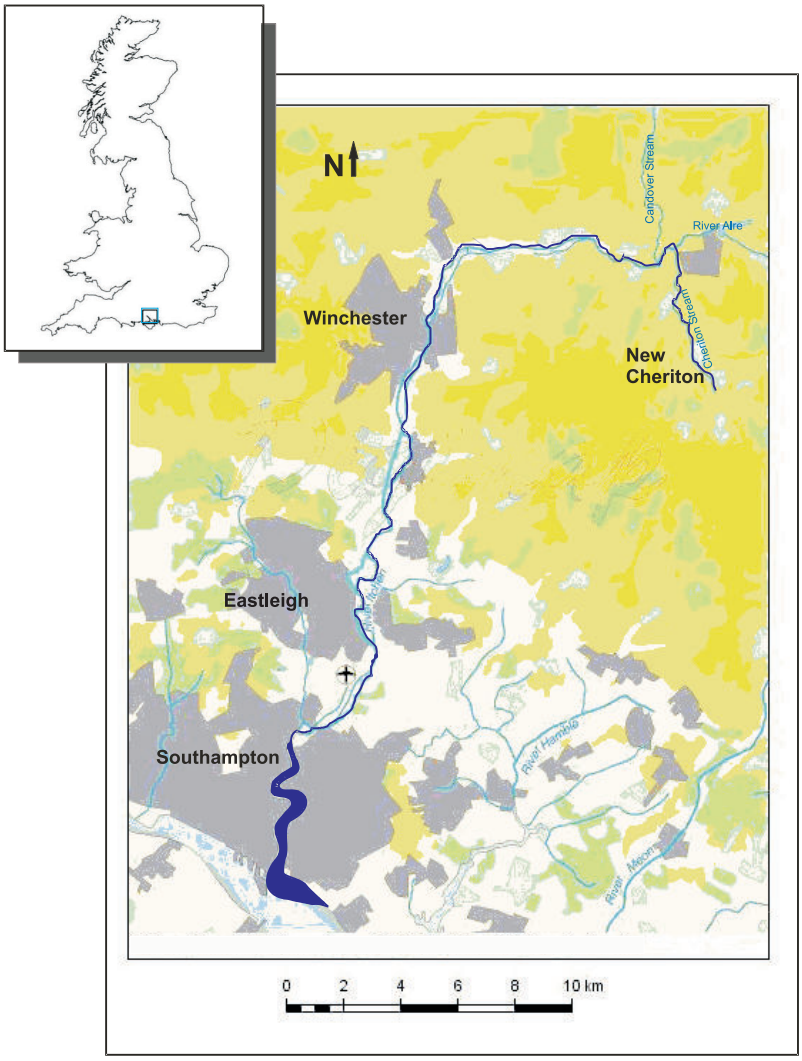

Figure 2-1. The River Itchen and Itchen Estuary.

There are a number of public and private sewage treatment works discharging into the river. The major discharges within the catchment are Eastleigh (30,000m^3/day), Winchester (7,600m^3/day, discharging directly to underground strata), and at Harestock (4,400m^3/day, discharging through a reed bed) (Whitehead and Mumford, 1996).

The River Itchen enters the Itchen Estuary at a tidal barrier at Woodmil, and at its southern extremity the Itchen Estuary mixes with water from the Test Estuary, forming Southampton Water (Figures 2-1 and 2-3). The maximum tidal range is 4.5m, and the surrounding area is highly urbanised. Two large sewage treatment works discharge directly into the estuary at Portswood (27,000m^3/day) and Woolston (15,000m^3/day) (Whitehead and Mumford, 1996).

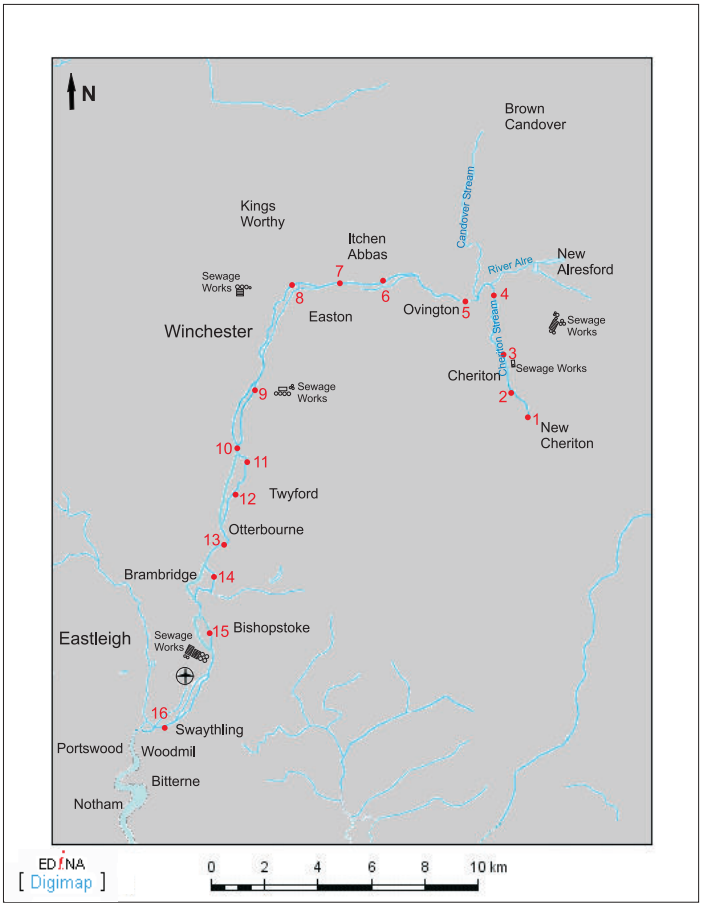

Figura 2-2. Sampling points in the River Itchen.

2.3. Sampling and storage

Water samples were collected from the River Itchen and Itchen Estuary on a monthly basis, from November 2001 to December 2002 (except March 2002). The study area was sampled at 16 sites along the River Itchen (Figure 2-2), and a maximum of 18 samples, were collected along the salinity gradient, from the Itchen Estuary (Figure 2-3). Surveys on the estuary were conducted over high tide, using an inflatable (RIB) boat (Ocean Adventure). The distances between the source of the River Itchen and each sample site are shown in Appendix C.

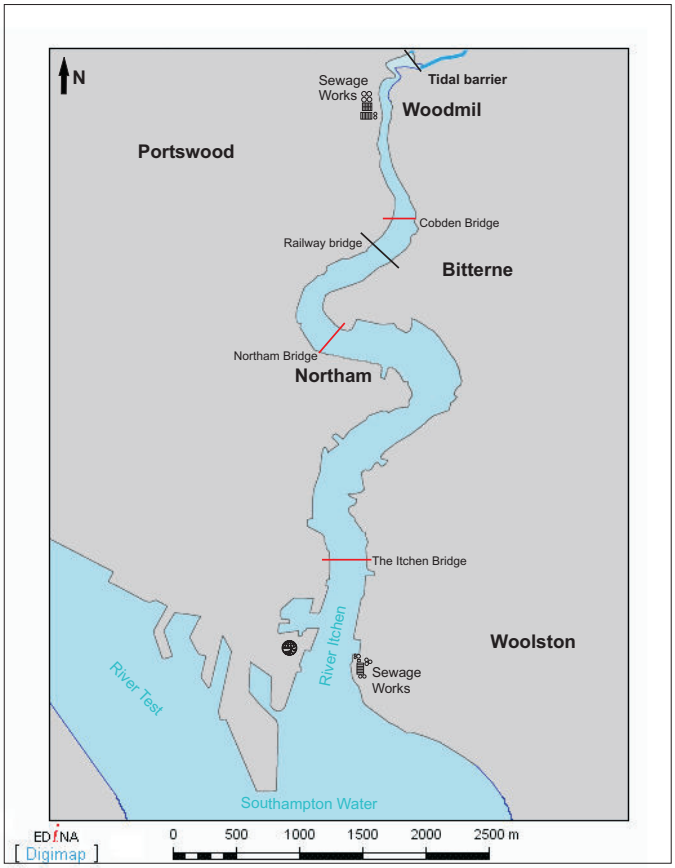

Figura 2-3. Itchen Estuary.

Surface water samples were collected using a plastic bucket. This water was then measured for salinity and temperature using a portable salinometer and samples filtered via syringe and GF/F filter (Whatman) into two 30ml plastic bottles for later analysis for dissolved nutrients. Separate

non-filtered samples (20 ml) were taken for N_2O analysis using a plastic hypodermic syringe, and dispensed into 30ml serum bottles, which were immediately closed with butyl rubber stoppers allowing 10ml of *in situ* air at the top. Air samples were also collected in each sample point for N_2O analysis, providing the initial concentration of N_2O for the headspace calculation. This was done by flushing in situ air into the serum bottles using a plastic syringe. Air temperature and pressure were also measured.

All samples were stored in a cool box. On return to the SOC, samples destined for nitrate, nitrite, phosphate and silicate analysis were all stored in the fridge until analysis (usually within 48 hours). Ammonium and nitrous oxide analyses were done on the same day.

2.4. Analytical methods

All chemicals used in the preparation of reagents and standards were analytical grade (Anala R), made up in high purity water (HPW) of 18MΩ cm^{-1}. Being aware that initially pure water which is in contact with the atmosphere can absorb relatively large quantities of ammonia, freshly deionised HPW was used where possible when preparing reagents and standards for ammonium analysis. When working with estuarine samples, saline solution (NaCl 40 gl^{-1}) was used as the wash, blank, matrix for the working standards and for diluting samples (when necessary). As estuarine measurements were carried out across a wide range of salinities, the use of saline solution minimises any salt effect that could occur (Stewart and Elliott, 1996). High purity water was used for the same purpose when analysing river samples.

2.4.1. Dissolved nutrients

Nitrate, nitrite, phosphate and silicate analyses were performed on an automated analytical system (Burkard Scientific SFA-2 Auto-analyser) linked to a Digital-Analysis Microstream data capture and reduction system. All these nutrients were analysed by colorimetric methods as described by Hydes (1984). The equipment was set up to measure nitrate concentrations up to 80

μM, nitrite concentrations up to 8 μM, silicate concentrations up to 40 μM, and phosphate concentrations up to 3 μM; and methods have a precision of about 1% at full scale. An overview of the methods is given below.

The analysis of nitrate requires the reduction of nitrate to nitrite, which is done using a cooper/cadmium column. Nitrite is determined by forming a diazo compound and then an azo dye, which is measured at 540nm. The linear portion of the calibration curve was extended by using a shorter path length of 15mm (Hydes and Wright, 1999).

Phosphate is reacted with acidified molybdate reagent to give a phosphomolybdate complex, which is then reduced to a highly coloured blue compound. Ascorbic acid is used as the reducing reagent with potassium antimonyl tartrate in a single reagent solution. The mixed reagent reacts rapidly with phosphate ions to give a blue-purple complex containing antimony and phosphorus in a 1:1 atomic ratio. Measurement is made at 880nm.

Dissolved silicate in water reacts rapidly in acidic molybdate solutions to form yellow silicomolybdic acid. This is reduced using ascorbic acid to give an intense blue coloured compound. Oxalic acid is added prior to the reduction step to prevent interference of the phosphate present in the sample and to stop the reduction of the excess molybdate. Measurement is made at 810nm.

Ammonium was analysed by a continuous-flow fluorometric technique adapted from Kerouel and Aminot (1997), by Breviere (2000). This method is based on the reaction of ammonia with orthophtaldialdehyde (OPA) in the presence of sulphite. The fluorescence produced by the reaction is measured by a fluorometer equipped with a detector with a 370nm UV LED (light emitted diode) and a 430 nm emission filter. Additional modifications to this technique were made in order to obtain better results when measuring the different concentrations expected in river samples (e.g. < 10μM) and estuarine samples (e.g. 20 - 80μM). Experiments were made to find the best temperature for an additional heating bath placed on the last mixing coil, just before the fluorimeter. The use of different temperatures for this water bath (52°C and 37°C) permits the use of different range of standards (5-20μM and

20-80 µM, respectively) with a better resolution of the resulting peaks. Experiments were also made to improve the quality of the blanks, and these showed that the use of water produced by the Milli-Q deioniser on the same day of analysis is necessary to obtain good blanks.

All analytical runs were calibrated upon the basis of four mixed secondary standards run in duplicate at the start of each run (see Appendix D for the range of standards used for each nutrient analysed). The secondary standards were prepared fresh for every analytical run. Drift standards and blanks were also measured at regular intervals during and at the end of each run. The calibration coefficients for each run were generally higher than 0.999 for nitrate, nitrite and silicate; and 0.998 for phosphate and ammonium. Examples of standard curves can be seen in Appendix E. All samples were analysed in duplicate. Whenever needed, samples were diluted using saline solution (estuary samples) or high purity water (river samples).

2.4.2. Nitrous oxide

A Shimadzu GC-14A gas chromatograph, equipped with a ^{63}Ni electron capture detector (ECD) was used for N_2O analysis. The GC-ECD was fitted with a pre-column (two meters long) and a main separation column (four meters long), both packed with 60/80 mesh Porapak Q. The carrier gas used was argon/methane (95%/5%). Optimal temperature settings were 60°C for the column and 340°C for the detector (as suggested in Butler and Elkins, 1991), and the detector standing current was set to 2nA. The retention time for N_2O is just over 3 min and the detection limit of the ECD under these conditions was 3 pmol N_2O (calibration curve in Appendix F).

Calibration curves were constructed using a range of different volumes of standard air (0.25, 1, 2, 3, 4 and 5 ml of NOAA-67707). To minimise the effect of the tailing oxygen peak that appears on chromatograms when injecting large volumes of samples (particularly with sample sizes in excess of 0.5 ml), a cold trap was used. The trap was a stainless steel loop (10cm x 1/8 in od tube) packing material (Unibeads 2S; 80-100 mesh). Temperature control was achieved using a liquid nitrogen bath for trapping and a heated metal block for

the desorption temperature. The placing of the hot block and cold bath was done manually, with appropriate computer prompts being given to the operator. The standards were automatically injected and carried onto the trap where they were frozen. After completion of trapping, the standard was re-vaporised by using the hot block at the temperature of 200°C. Once re-vaporised the standard was carried trough the pre-column and finally the main column. The pre-column was used as a filter for unwanted high boiling point compounds. The trap was left on column flow for just sufficient time for the compounds of interest to pass through the pre-column onto the main column. Once the streams were switched, higher boiling point components were left on the pre-column and were then flushed to waste by the trap flow (Boswell and SmytheWright, 1996).

Cold trapping with liquid nitrogen had the advantage that sample sizes of 1ml or larger could be analysed without loss of resolution from oxygen overloading the ECD. Small sample sizes of 250 μl (volume of gas used to analyse samples from the river and estuary), are not affected by tailing oxygen peak. The gas sub-samples (250 μl) were taken from the headspace of bottles containing water from the river and estuary using a gas tight syringe. These sub-samples were injected into the sample port of the GC-ECD, going directly to the main column.

Peak areas were integrated using the software Borwin (Version 1.21.60) and quantified by comparison with known standard (NOAA-67707) and laboratory air. N_2O concentration in water samples were back-calculated from the measured headspace concentration according to Weiss and Price (1980), adjusted for salinity, air pressure and temperature.

An experiment was undertaken to investigate the possible loss of N_2O within sample bottles due to adsorption or diffusion through the stopper. Results showed that N_2O concentrations in the equilibrium bottles had less than 1% variation for up to 12 hours.

Another important concern regards the use of mercuric chloride ($HgCl_2$) or formaldehyde as inhibitors of microbial activity that might change the concentration of nitrous oxide. Studies made by Kieskamp et al. (1988) and

Garrido et al. (1998) showed that the use of these compounds when preserving samples could increase the production of N_2O. Based on these studies (Garrido et al., 1998; Kieskamp et al., 1988) and the restriction of time to analyse samples (within 12 hours from sampling), a decision was made to not use $HgCl_2$ or formaldehyde to preserve samples. To check on stability of nitrous oxide in samples under these conditions, a 12 hour experiment was made using estuarine water samples that were analysed every hour to detect changes in N_2O concentration. No changes were observed over this time scale.

In addition to the measured concentrations, N_2O was also expressed as a percentage of the air-equilibration concentration (percentage of saturation). The concentration at which the sample would be considered saturated with N_2O was calculated from solubility coefficients corrected for temperature and salinity.

2.5. Results and discussion

2.5.1. River Itchen

2.5.1.1. Dissolved nutrients

Nitrate concentrations in the River Itchen were very similar from sample sites 5 to 16, and higher from site 1 to 4, indicating a strong spatial variation. Maximum values at Site 1(source of the river) were observed from November/01 to May/02 and also November/02 and December/02. Figure 2-4 shows nitrate concentrations for each sample site relative to their distance from the source of the river. Maximum value at Site 1 was 574µM, in February/01.

The high values of nitrate concentration observed at the source of the river could be caused by agricultural pollution of the aquifer, as 44% of the catchment area is farmland. High nitrate concentrations like these have been associated with intensively farmed areas in UK (Gooddy et al., 2001; Hiscock et al., 2003; Knapp, 2005). The geology of the Itchen catchment is mainly Chalk, a porous, fine-grained limestone which outcrops over the whole of the

valley to the north of Eastleigh. Rain soaks into the Chalk rock rather than running off and then gradually percolates through the pores and small fissures in the Chalk until it runs out from springs of nitrate rich groundwater (Halcrow, 2004).

Lower nitrate concentrations at Site 1 were generally observed from July/02 to October/02. This period represents the driest time of the fourteen months of survey and at the source of the river the water was coming from the ground at an extremely small flow when compared with the other months. This is typical of chalk streams, as the springs tend to seasonally migrate up and down their valleys with fluctuating water-table level (Berrie, 1992). A marked increase in the nitrate concentration from Site 1 to Site 2 was observed during this period, showing that groundwater is not the only source of nitrate to this part of river, and input still occurs as the river streams through this intensively farmed area. The importance of non-point source runoff to the nitrate concentration in rivers in rural areas is highlighted in many studies (Arheimer and Liden, 2000; Howarth et al., 2002; Mayer et al., 2002; Wernick et al., 1998).

The downstream decrease in nitrate concentration generally observed from Site 1 to Site 4 is likely to be in part due to dilution, as many small tributaries join the river in that area. In addition, the biological consumption of nitrate also plays an important role. There is a thriving cress industry within the Itchen catchment, with ten large and a number of smaller watercress farms, most of them situated within the area between sites 1 to 4. The water quality implications of modern watercress growing have been investigated (Casey and Smith, 1994) and show that nitrate concentrations in the outflow water from watercress farms are lower than the stream values, because nitrate is removed by the growth of watercress. Nitrate concentrations from sites 5 to 16 were relatively uniform and in accordance with previous study (Whitehead and Mumford, 1996).

Nitrite concentrations showed a different distribution when compared with nitrate. Nitrite was depleted at the first four sites; increasing from site 5 to 9 and then dropping again until Site 16, where values were generally high (Figure 2-5). A maximum concentration was observed in September, at Site

16 (7.4μM). The low nitrite concentrations measured within the first four sites reflect the low concentration in the groundwater. Studies undergone in a chalk aquifer in Cambridgeshire, in an area dominated by arable farming where the application of nitrogen based fertilizers is widespread, also reported low levels of nitrite (Hiscock et al., 2003).

The increased concentrations found from site 5 to 8 may be explained by the presence of fish farms. Agriculture and fish farms are the main activities in the area comprising sampling sites 5 to 8. This may explain the high concentration of nitrite (and also ammonium concentrations at Site 6), as those activities are reported to significantly affect the water quality of this area (River Itchen Steering Group, 2004). High concentrations of nitrite and ammonium have been reported in waters discharged from fish farms and were mainly attributed to the food supplementation regime, which is partly transformed into fish biomass and partly released into the water as suspended organic solids or dissolved matter such as carbon, nitrogen and phosphorus (Gutierrez-Wing and Malone, 2006; Karousos et al., 2005; Lyssenko and Wheaton, 2006)

The high concentrations generally found at sites 9 and 16 may reflect the influence of the sewage treatment works located close to these sampling sites (Figure 2-2). It has been reported that streams receiving the outflow from sewage plants can be subject to continual nitrite and ammonium pollution (Berenzen et al., 2001).

Ammonium concentrations at sites 1 to 4 were generally low (< 2μM) except in February and October, when peaks of 8 and 9μM (respectively) were observed at Site 1 (Figure 2-6). The generally low concentration is in agreement with low ammonium concentration in groundwater reported in other studies (Hiscock et al., 2003)

High peaks were observed at Site 6, in December (16μM), April (10μM), and May (16μM) (as discussed above). In order to obtain a better visualisation of the spatial variability of the data set, Figure 2-6 only shows ammonium concentrations up to 10μM. The full data set including ammonium concentrations up to 16μM can be seen in (Appendix G). In addition, with

exception of December/01 and January/02, Site 16 shows an increase in ammonium if compared with the previous site. As in the nitrite data, this feature relates to the influence of the sewage treatment works discharged in the area.

Phosphate was also generally low at the first four sites (average for all surveys is 0.5μM). An increase in concentration was observed at Site 5, about 8 km from the source of the river. This increase is possibly related to the watercress farms upstream this site. Addition of fertilisers to the watercress beds are generally reflected in increasing concentrations of phosphorus downstream of the beds (Casey and Smith, 1994). In addition, the release of solid wastes, phosphorus and nitrogen from fish farms in this area is significant (River Itchen Steering Group, 2004). Phosphate waste outputs are a great concern in freshwater since phosphorus is generally the most limiting factor for plant (algae) in that environment (Cho and Bureau, 2001).

Higher concentrations of phosphate were observed from Site 9 (about 19 km away from the source) downstream. Site 16 showed higher concentrations than the other sites in all surveys, reaching the maximum value in September (12μM) (Figure 2-7). This was expected as the area comprising sites 9 to 16 includes two of the main towns of the catchment (Winchester and Eastleigh) and also a number of large sewage treatment plants. The sources of phosphate entering surface waters in the UK have been estimated to be approximately 45% domestic, mainly reaching rivers through sewage treatment works (Morse et al., 1993).

The high concentrations at Site 16 suggest phosphate input from the largest sewage treatment works (Eastleigh) located about 500m upstream from the sample site. The difference between the ranges of concentrations found around Site 5 and the concentrations downstream of Site 9 may be explained by the different amounts of phosphate typically contained in sewage effluents and agricultural drainage waters. In general, phosphate concentrations in sewage effluent are higher by at least one order of magnitude than concentrations in agricultural drainage waters (M. Vighi and Chiaudani 1987). This leads to a situation in which rivers at low flow have the highest concentrations of phosphate, contributed mainly by sewage treatment

works; while the instantaneous load of phosphate is highest at high flows, and contributed by diffuse sources like agriculture.

Silicate concentrations were generally in a range of 150μM to 200μM for all sites on all surveys, with the exception of April, when samples from Site 4 to 16 were lower than 150μM (Figure 2-8). Concentrations higher than 200μM were observed at Site 1 in September and October (253 and 245μM, respectively). The fact that no significant changes were observed in the concentration of silicate throughout the river was expected as silicate loads tend to reflect the catchment mineralogy, and are relatively independent of anthropogenic influences (Hessen, 1999).

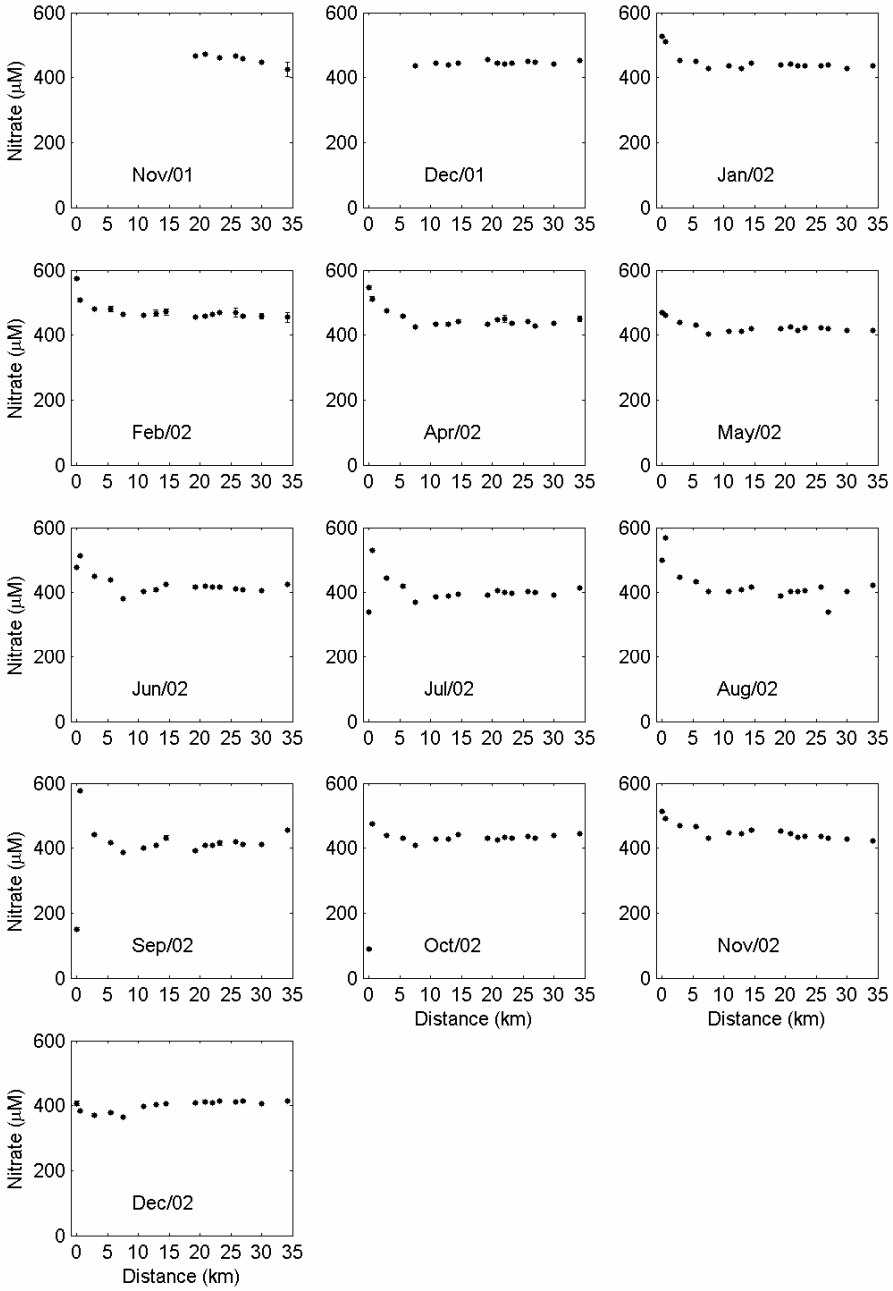

Figure 2-4. Nitrate concentration in the River Itchen. Horizontal axis represents the distance (km) from the source of the river. Error bars show ± 1 standard deviation (n=3).

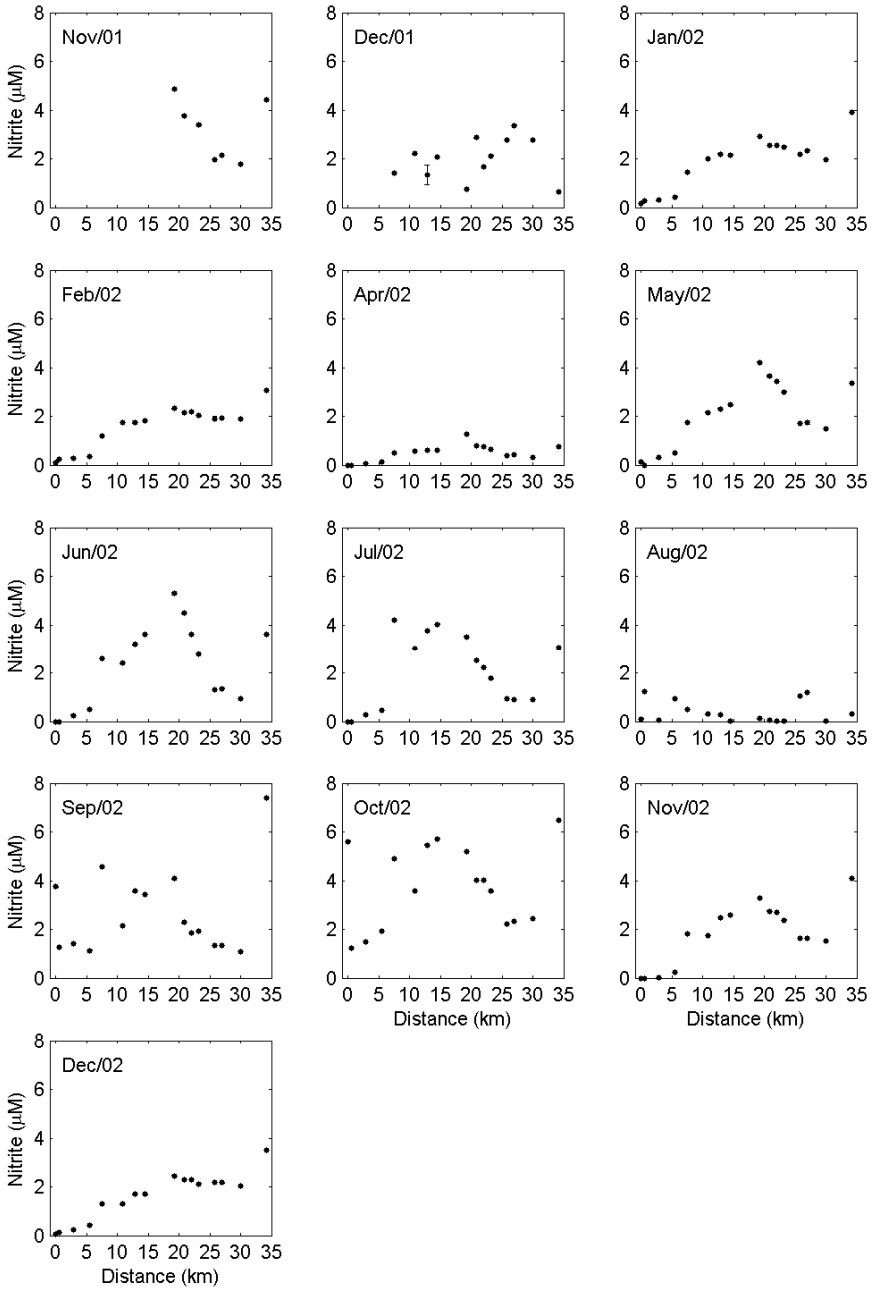

Figure 2-5. Nitrite concentration in the River Itchen. Horizontal axis represents the distance (km) from the source of the river. Error bars show ± 1 standard deviation (n=3).

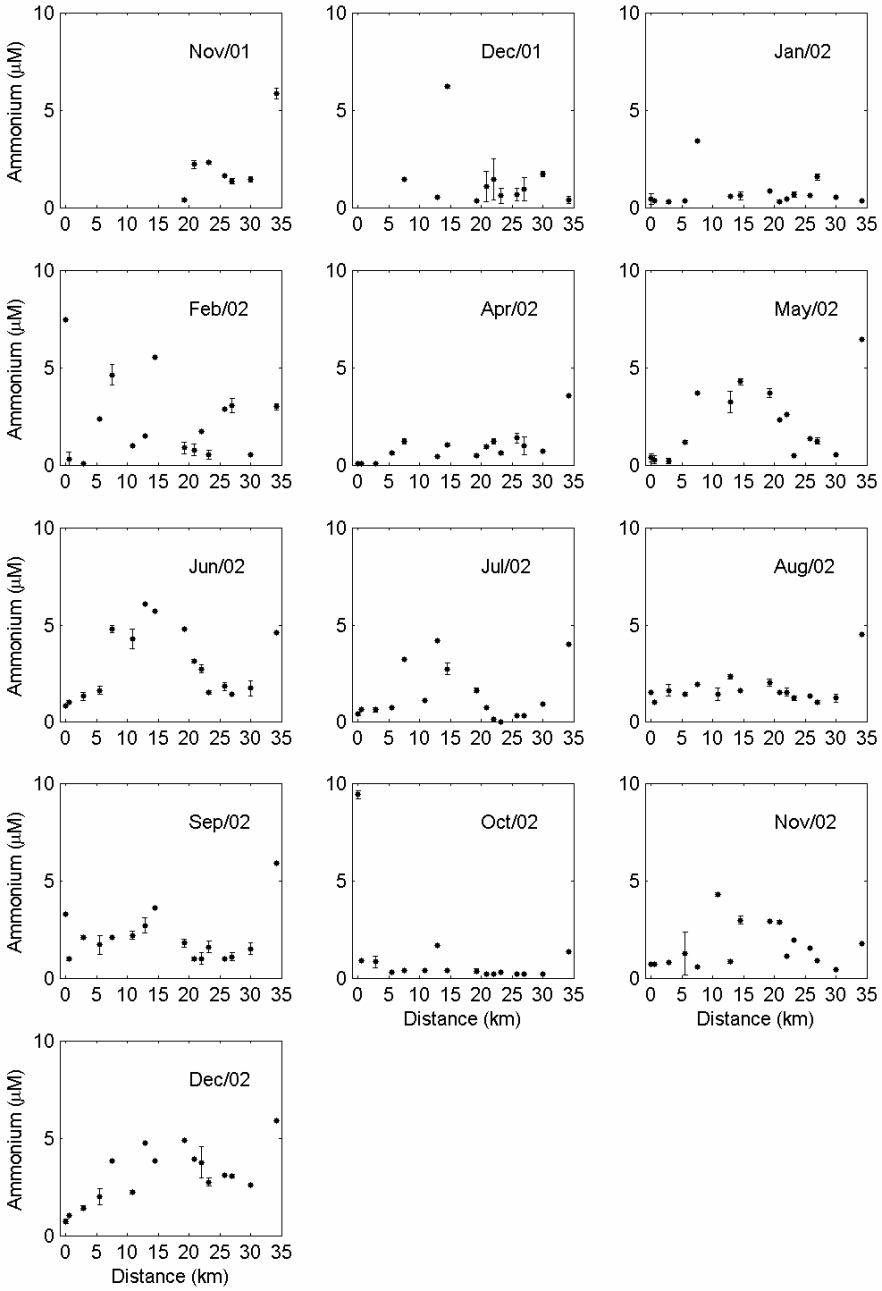

Figure 2-6. Ammonium concentration in the River Itchen. Horizontal axis represents the distance (km) from the source of the river. Error bars show ± 1 standard deviation (n=3).

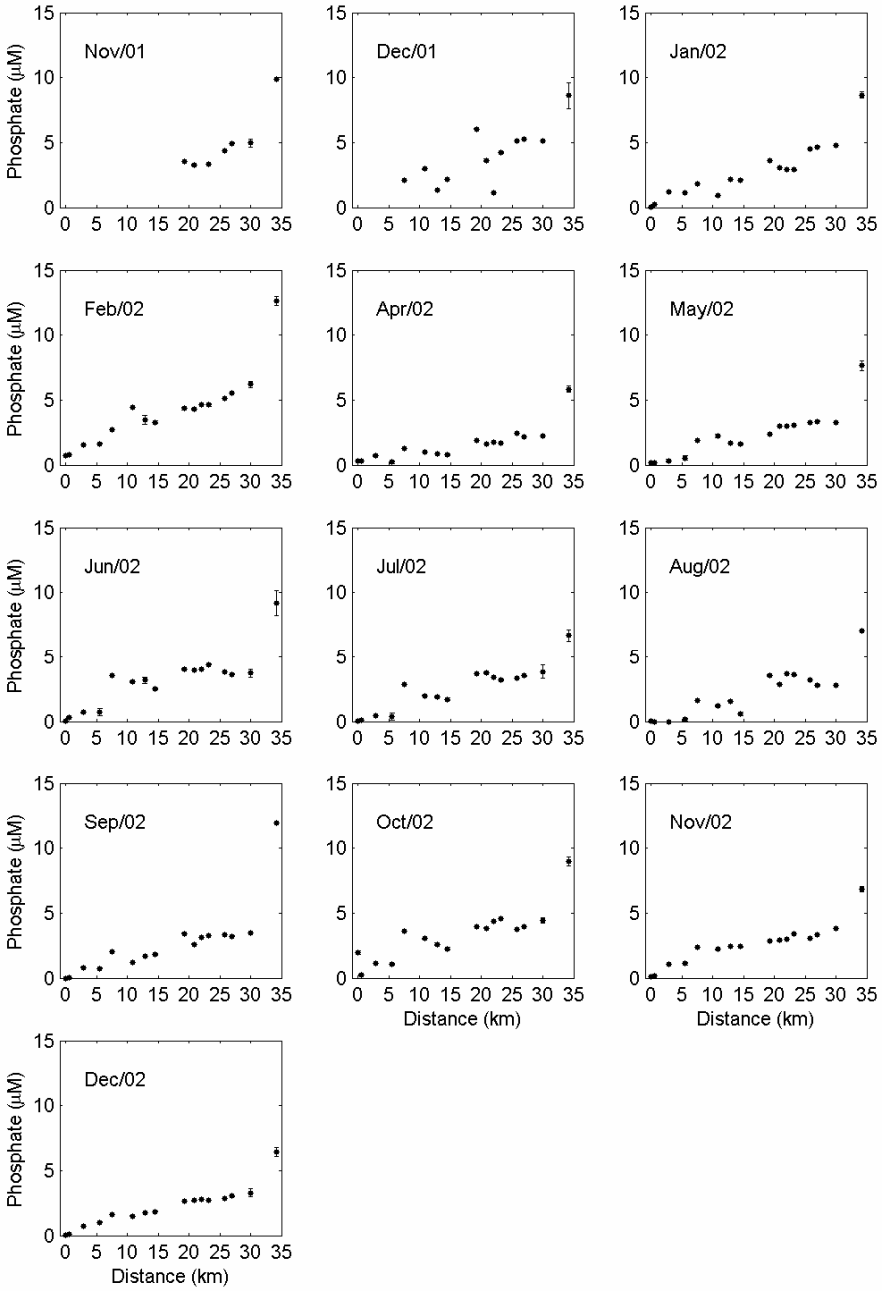

Figure 2-7. Phosphate concentration in the River Itchen. Horizontal axis represents the distance (km) from the source of the river. Error bars show ± 1 standard deviation (n=3).

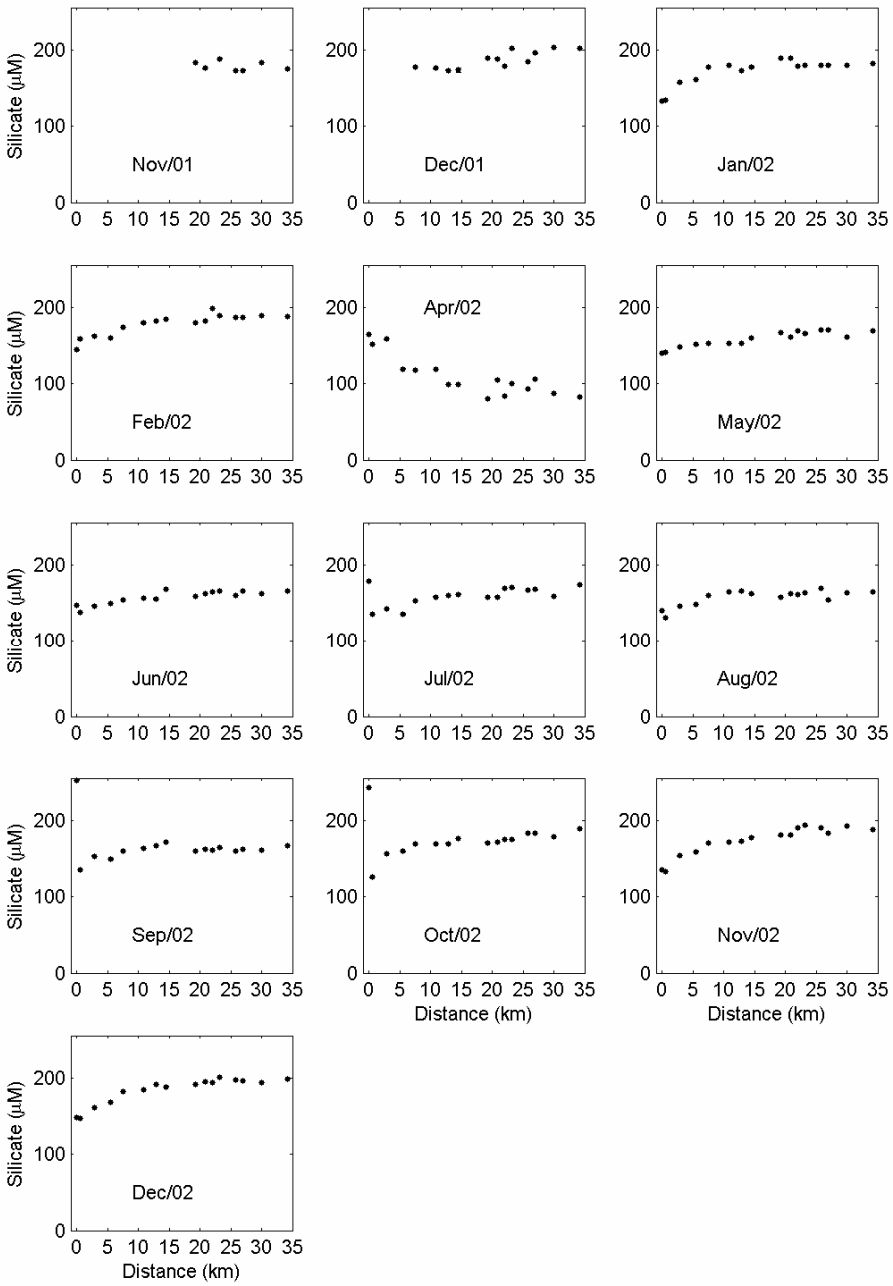

Figure 2-8. Silicate concentration in the River Itchen. Horizontal axis represents the distance (km) from the source of the river. Error bars show ± 1 standard deviation (n=3).

2.5.1.2. Nitrous oxide

Nitrous oxide concentrations had a distribution pattern along the river which was generally maintained for all surveys (Figure 2-9). Concentrations were higher at Site 1 (source of the river), with values decreasing to Site 4 and then increasing again to Site 5.

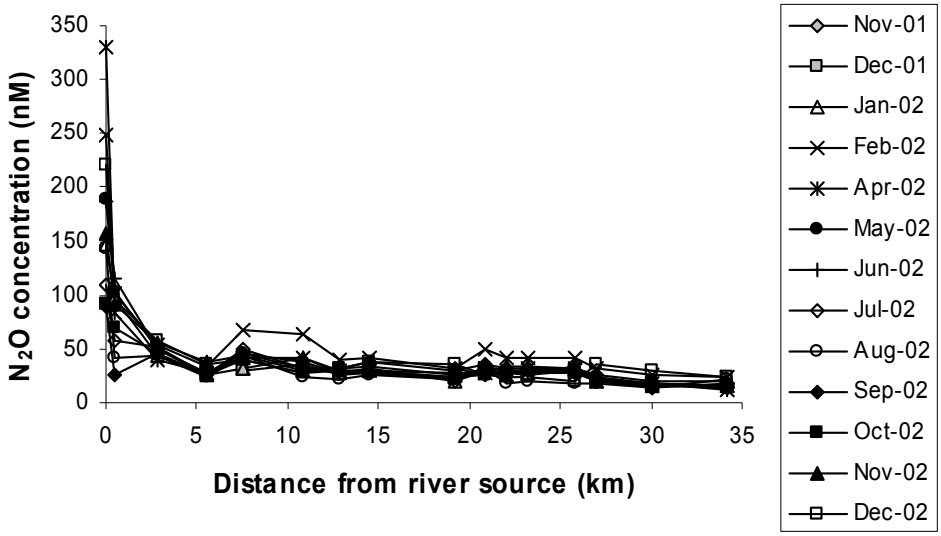

Figure 2-9. Nitrous oxide concentrations in River Itchen from November 2001 to December 2002.

The high concentrations observed at the source of the river (from 90 to 331 nM) are most probably directly related to the N_2O concentration in the groundwater. A preliminary assessment of nitrous oxide in groundwater in Cambridgeshire, UK (Muhlherr and Hiscock, 1997) reported strongly oversaturated samples, with concentrations ranging from 172 to 3856nM.

The lower N_2O concentrations at the source of the river correspond to the lower concentrations of nitrate measured in the dry season (July to October). High nitrate and nitrous oxide concentrations at the source correlated well ($r=0.69$, $P<0.05$) but varied greatly seasonally. Since agriculture is likely responsible for the elevated groundwater nitrate concentrations, N_2O in groundwater appears linked to the high use of nitrogen fertilizers. The hypothesis that land use affects N_2O concentrations in the groundwater was confirmed by Mcmahon et all*.,* (2000), when studying large aquifers in the

Central High Plains in the United States. Nitrification was suggested as the process producing nitrous oxide in the aquifer. In addition, concentrations of N_2O in chalk groundwaters in the Cambridgeshire area, were reported to be at least 1 order of magnitude greater than the atmosphere-water equilibrium value (Hiscock et al., 2003). The authors also suggested that the high concentrations of nitrate and nitrous oxide found in the groundwater were produced by nitrification of ammonium in the soil zone.

The fact that both, nitrate and nitrous oxide concentrations are higher during the months in which the soil is soaked around the spring, points out the possibility of dissolution of nitrate and production of nitrous oxide within the wet soil. N_2O production in nitrate rich soils was also reported by Davidson and Swank (1990), but this pathway appears important only in recently disturbed soils.

The dramatic decrease in nitrous oxide concentrations generally seen from Site 1 to Site 4 indicates that rapid degassing occurs once the water is released from the ground. The rapid degassing of nitrous oxide to the atmosphere has been reported by other authors (Bowden and Bormann, 1986; Clough et al., 2006; Reay et al., 2003) and highlights the need for caution when basing N_2O fluxes estimates on measurements made at widely spaced sampling points.

The overall increase in N_2O concentrations measured at Sites 5 and 6 suggests some input or situ production (Figures 2-10). The magnitude of the differences between the concentrations from Site 5 to 16 was not as great as it was from Site 1 to Site 5. The increased nitrous oxide concentrations measured at Sites 5 and 6 may be related with the fish farming in that area. The high load of suspended matter, characteristic of fish farm discharges allows the presence of suboxic microzones in the oxygenated water column which, together with the high nitrogen concentrations would favor the production of N_2O by both, denitrification and nitrification processes. Nitrous oxide production was reported to occur in biofilters used in aquaculture (Haug and McCarty, 1972; Lee et al., 2000). The uncertainty regarding the potential nitrous oxide emission by aquaculture was investigated by Aubin, *et al.* (2006).

Emissions of N_2O from water to atmosphere can be inferred for the whole river area, provided saturations were in excess of 100% on all surveys, at all sites (Figure 2-10). The maximum N_2O concentration was observed in April at Site 1 (331nM), which is approximately 28 times the air-equilibrated saturation concentration. The observed drop in N_2O concentration generally observed between sites 1 and 4, was not maintained between sites 4 and 16, despite the water still being over-saturated with N_2O. This suggests that the N_2O flux from the river surface was matched by N_2O inputs over this reach of the river, but further work is required to identify the source(s) and scale of these N_2O inputs.

Nitrous oxide emissions have been measured from only a few rivers (most of them tidal) and, among these rivers, emissions were highly variable (0.2–8.0 μmol N m^2 h^{-1}) (Cole and Caraco, 2001 and references therein). A broad correlation between annual mean nitrate concentration and annual mean N_2O emissions was found among these rivers over a range of 4–400 μM nitrate (Cole and Caraco, 2001). Significant correlation between concentrations of nitrate and nitrous oxide in the surface waters of River Itchen were found in ten out of the thirteen months sampled during this study (see Appendix H). Ammonium and nitrate did not correlate to nitrous oxide at the same extent (Appendices I and J). This suggests nitrification as the main process producing nitrous oxide in the river. Although nitrification appears to be the dominant process, denitrification at the anoxic sediment-water interface may be the major source of the N_2O concentrations in the river immediately downstream of sewage treatment works and fish farms.

There is a lack of data for sample sites 1 to 8 in November/01, and sites 1 to 4 in December/01. The reason is that sampling from the source of the river was not planned from the beginning of this project, and started only in January, when it became apparent the river might be an important source of nitrous oxide.

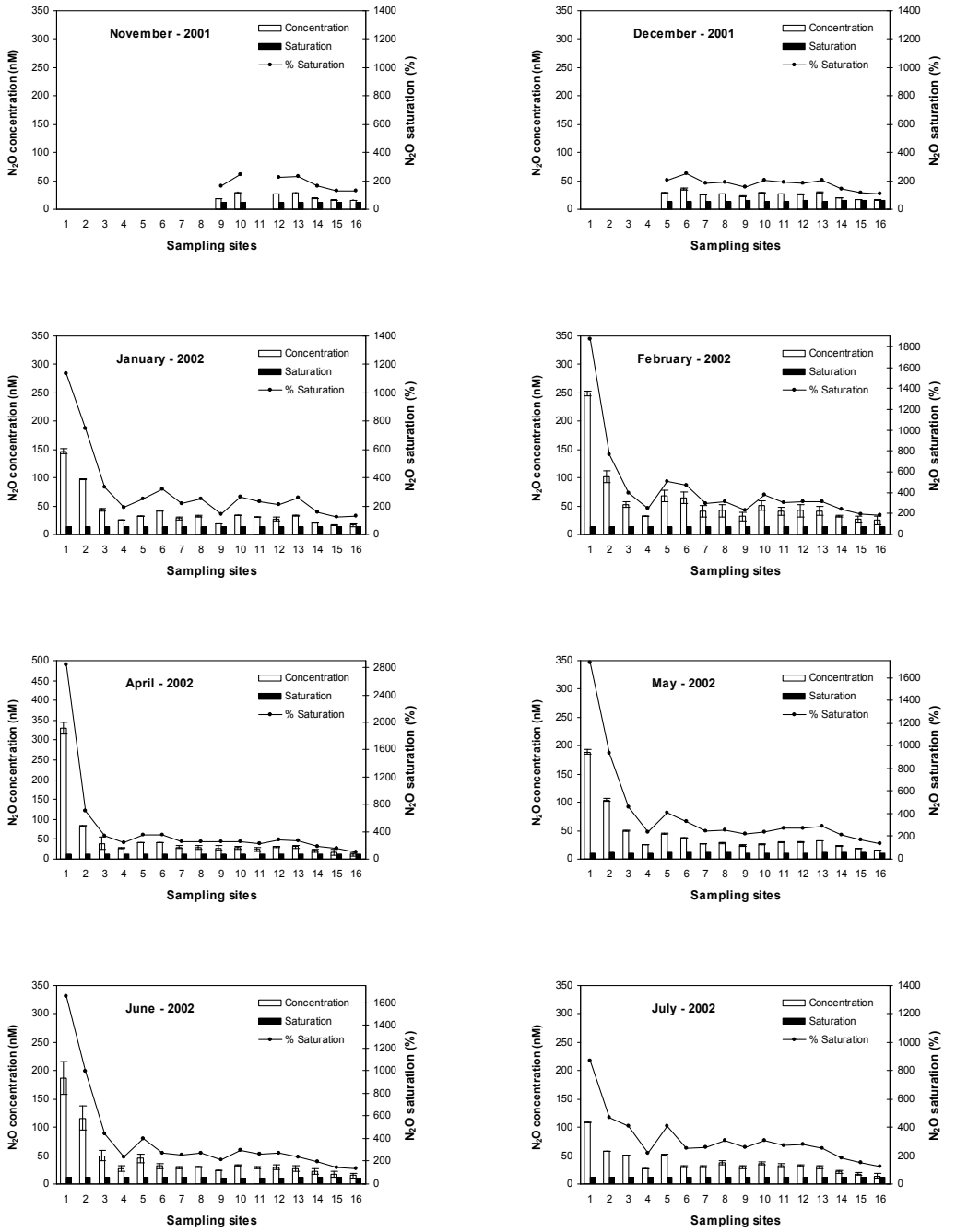

Figure 2-10. Nitrous oxide concentrations in the River Itchen. White columns represent measured concentrations (error bars show ± 1 standard deviation, n=3), black columns represent calculated saturation value (at in situ temperature and salinity), and percentage saturation is shown by black line (right hand side y axis).

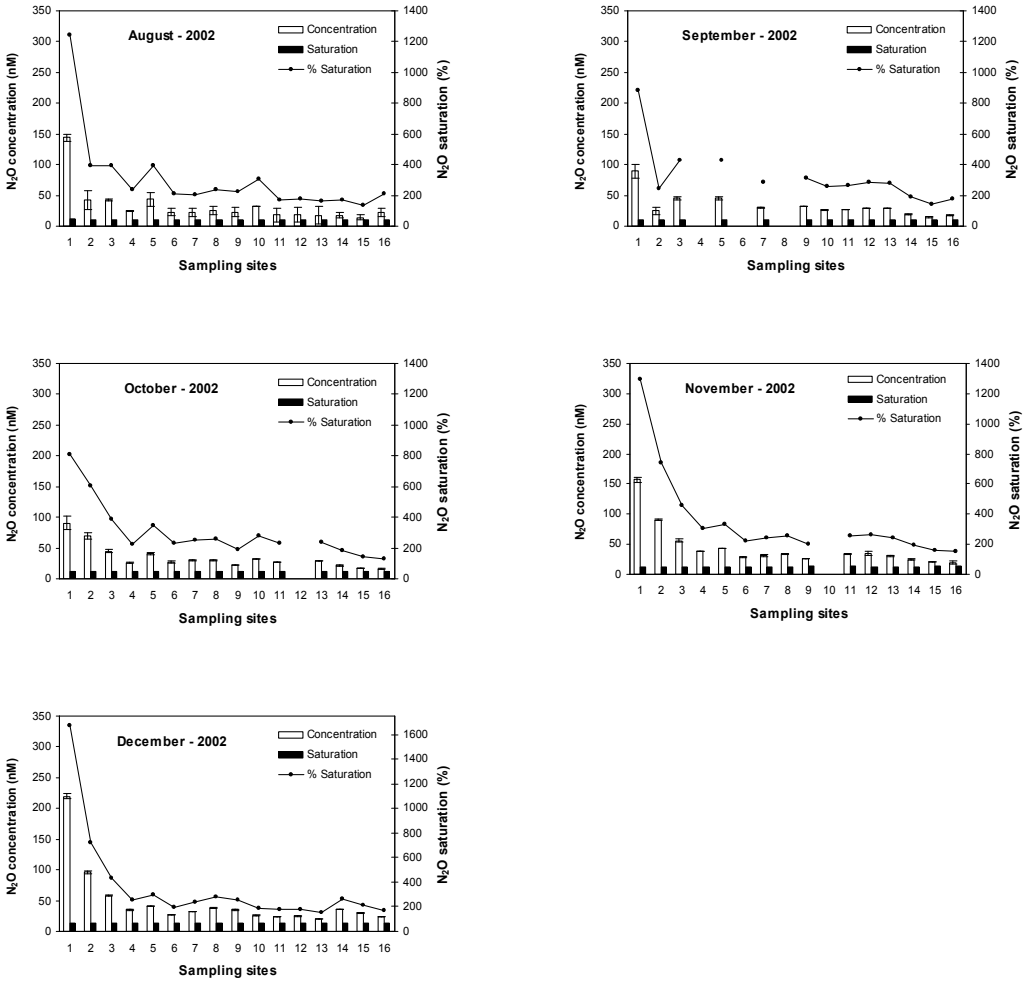

Figure 2-10. (continued) Nitrous oxide concentrations in the River Itchen. White columns represent measured concentrations (error bars show ± 1 standard deviation, n=3), black columns represent calculated saturation value (at in situ temperature and salinity), and percentage saturation is shown by black line (right hand side y axis).

2.5.2. Itchen Estuary

2.5.2.1. Dissolved nutrients

In the estuary, there was an inverse relationship between nitrate and salinity on all surveys and this was essentially linear at all times (Figure 2-11). Theoretical dilution lines (TDL) were estimated using the highest and the lowest salinity samples in each month. These lines can be seen in Figures 2-11 to 2-15, and show the expected change in nutrient concentration assuming linear mixing. The highest concentration of nitrate (677μM) was found at low salinity, in August. Points of low nitrate concentration at low salinity (relative to usual high concentration at low salinity) were measured during the April, May, June, July, September, November/02 and December/02 surveys. These are probably related to the discharge from the sewage treatment works (which is low in nitrate), as the sampling points in question were always close to the effluent outfall and a discharge flux was clearly noticed during these months. Similar observations of overall conservative behaviour of nitrate in the Itchen Estuary (Wright and Hydes, 1997), suggested that there were no significant point sources releasing nitrate into the estuary. The conservative behaviour of nitrate, as observed in this study, is commonly seen in other estuaries (Balls, 1994; Uncles et al., 2003). No significant variation was found in the range of nitrate concentrations from November/01 to December/02 except from the August survey, when the highest concentration (677μM) was observed close to the effluent outfall.

Nitrite concentrations were generally about 2 orders of magnitude lower than nitrate (with the exception of high nitrite concentration peaks of 24μM in May, 30μM in June, 44μM in August and 26μM in November/02; Figure 2-12). Plots of nitrite against salinity indicate that nitrite has a less conservative behaviour than nitrate. The high concentrations mentioned above were measured at the upper end of the estuary, close to the sewage treatment works outfall. Distributions downstream of this point source appear to be conservative.

Ammonium concentrations were much higher than nitrite (Figure 2-13). High concentrations of ammonium were measured in April (983μM), May

(449μM), June (314μM), July (300μM), August (440μM), September (461μM) and November/02 (270μM) in the upper estuary. In order to obtain a better visualisation of the spatial variability of the data set, Figure 2-13 only shows ammonium concentrations up to 300μM. The full data set including ammonium concentrations up to 1000μM can be seen in (Appendix K). Similarly to nitrite, high concentrations of ammonium were measured at the low salinity end of the estuary, suggesting the same point source. Distributions downstream of the sewage treatment outfall are generally conservative.

Phosphate also showed very high peaks in April (77μM), May (41μM), June (134μM), July (109μM), August (27μM), September (79μM) and November/02 (43μM) in the upper estuary. A similar feature has been described by Ormaza-Gonzalez (1990) when studying the Itchen Estuary. This author observed high phosphate concentrations in the upper estuary and reported a dramatic decrease (25-50%) in concentration within the salinity range of 7 to 10; suggesting removal of dissolved phosphates, especially in the maximum turbidity zone. The high phosphate concentrations measured in the upper estuary suggest the same source as the high ammonium and nitrite concentrations (presumably the Portswood sewage treatment works). Correlations between phosphate and ammonium, and phosphate and nitrite in the upper estuary can be seen in Figures 2-16 and 2-17. Accordingly, the present data showed an inverse relationship between phosphate concentration and salinity (Figure 2-14). Comparison with the TDL indicates that once the input of phosphate from the sewage treatment plant gets well mixed with the water from the upper estuary, the dilution of this nutrient could be explained by simple mixing.

Silicate concentrations were higher (maximum value of 199μM, in November/02) at low salinity. An inverse relationship between silicate and salinity was observed on all surveys and this was essentially linear at all times (Figure 2-15). No significant variation was found in the range of silicate concentrations from November/01 to December/02, with the exception of April, when concentrations were lower in the upper estuary (maximum 83μM) with a high peak (141μM) at the sewage treatment works. This overall

conservative behaviour has been reported by Burton et al (1970) for the Test and Hamble estuaries and by Hydes and Wright (1999) for the Itchen estuary.

Nitrite, ammonium and phosphate distributions clearly show the influence of the sewage treatment works, indicating inputs of these nutrients at Portswood. A consented discharge of 27000 m^3 is received by the Itchen estuary on a daily basis (Whitehead and Mumford, 1996). The influence of this discharge is better observed during the summer months, when the river flow is generally lower, further reducing the capacity for dilution of the sewage effluents, resulting in elevated nutrient concentrations. The impact of sewage effluents discharged in UK estuaries was also discussed by other authors (House and Denison, 1997; Mainstone and Parr, 2002; Uncles et al., 2003).

Plots of nitrate against salinity (Figure 2-11) suggest production of nitrate by nitrification in November/01 and August/02, as measured values were higher than the estimated TDL. In agreement with that, ammonium removal was also evident from the mixing plot in November/01, despite the high ammonium concentration measured close to the sewage treatment works outfall. In the same way, possible removal of nitrate can be inferred for the months of June/02, September/02 and December/02. This may be removal by denitrification or just the mixing with the low nitrate sewage at low salinity.

It should also be appreciated that the impact of a nutrient load on an estuary will also be a function of its residence time within the estuary. This is indicated by the fresh-water flushing time (Alber and Sheldon, 1999; Balls, 1994; Nedwell et al., 1999). In the Itchen estuary, flushing times of about 26 hours for spring tides and 76 hours for neap tides were estimated (Wright and Hydes, 1997). During this study, sampling was done mostly during spring tides, with only five trips during neap (Dec/01, Jan/02, Feb/02, May/02 and Sep/02). No significant differences were observed as a result of different flushing times.

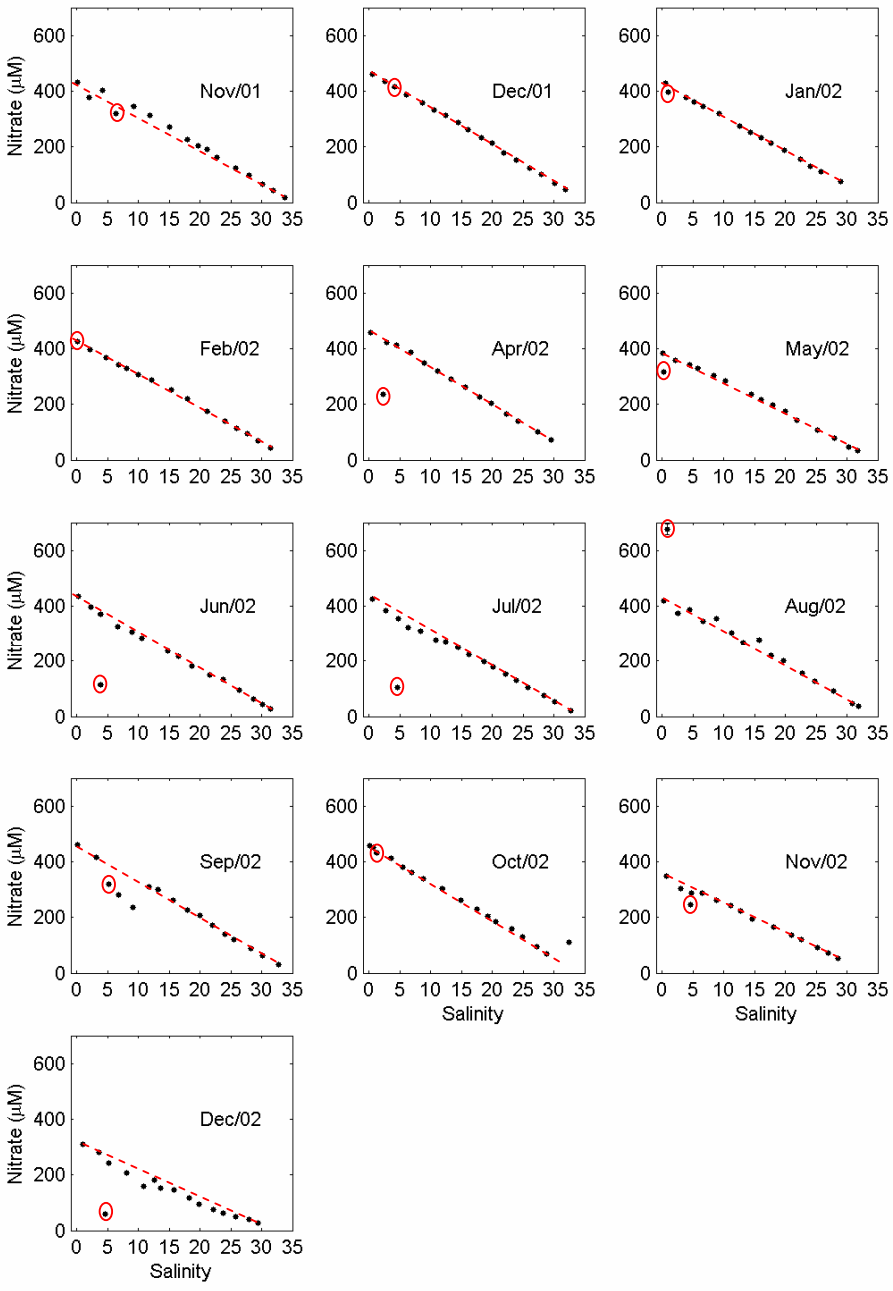

Figure 2-11. Nitrate concentration in the Itchen Estuary. Error bars show ± 1 standard deviation (n=3). Theoretical dilution line is represented by the red dotted line. Sample collected in front of the STW effluent outlet is circled in red.

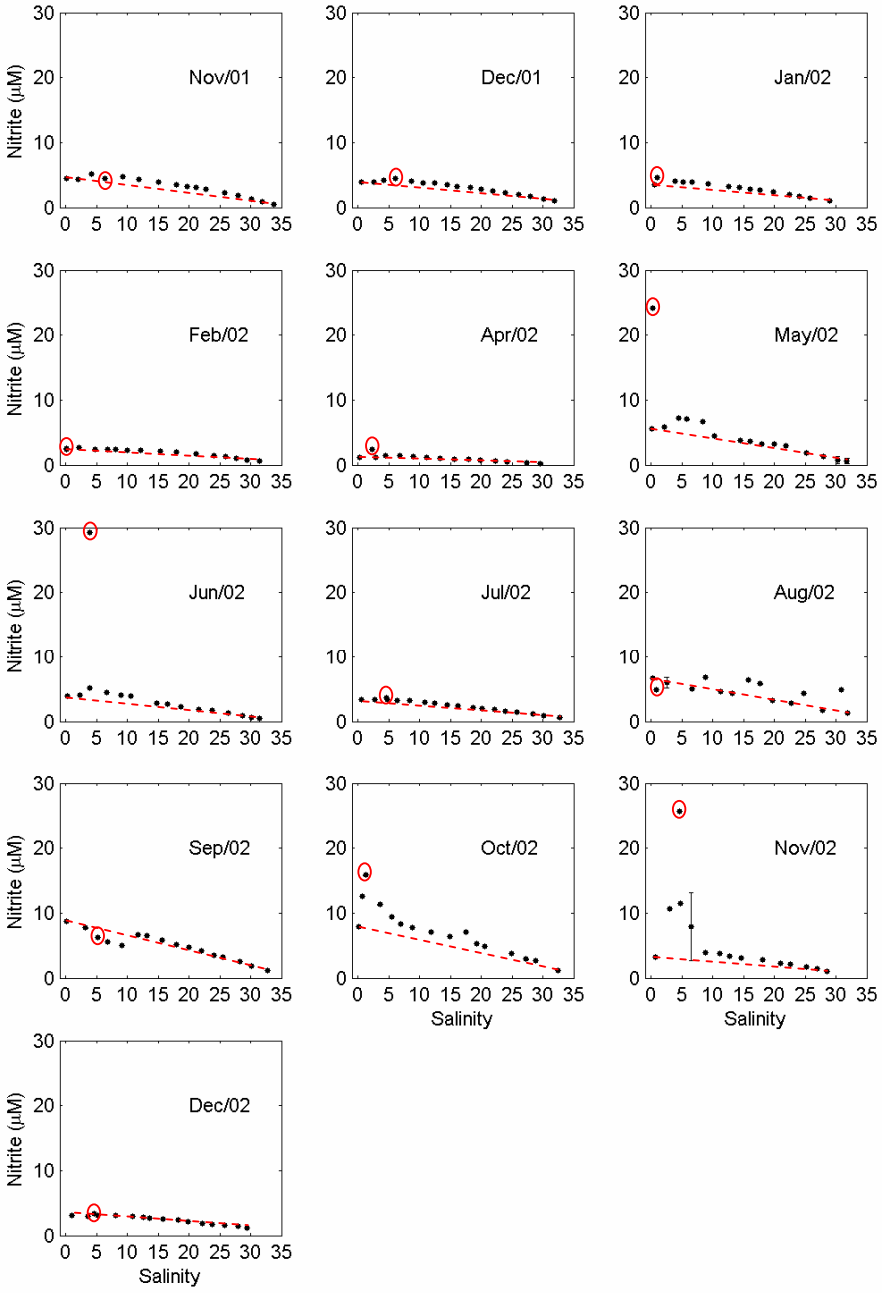

Figure 2-12. Nitrite concentration in the Itchen Estuary. Error bars show ± 1 standard deviation (n=3). Theoretical dilution line is represented by the red dotted line. Sample collected in front of the STW effluent outlet is circled in red.

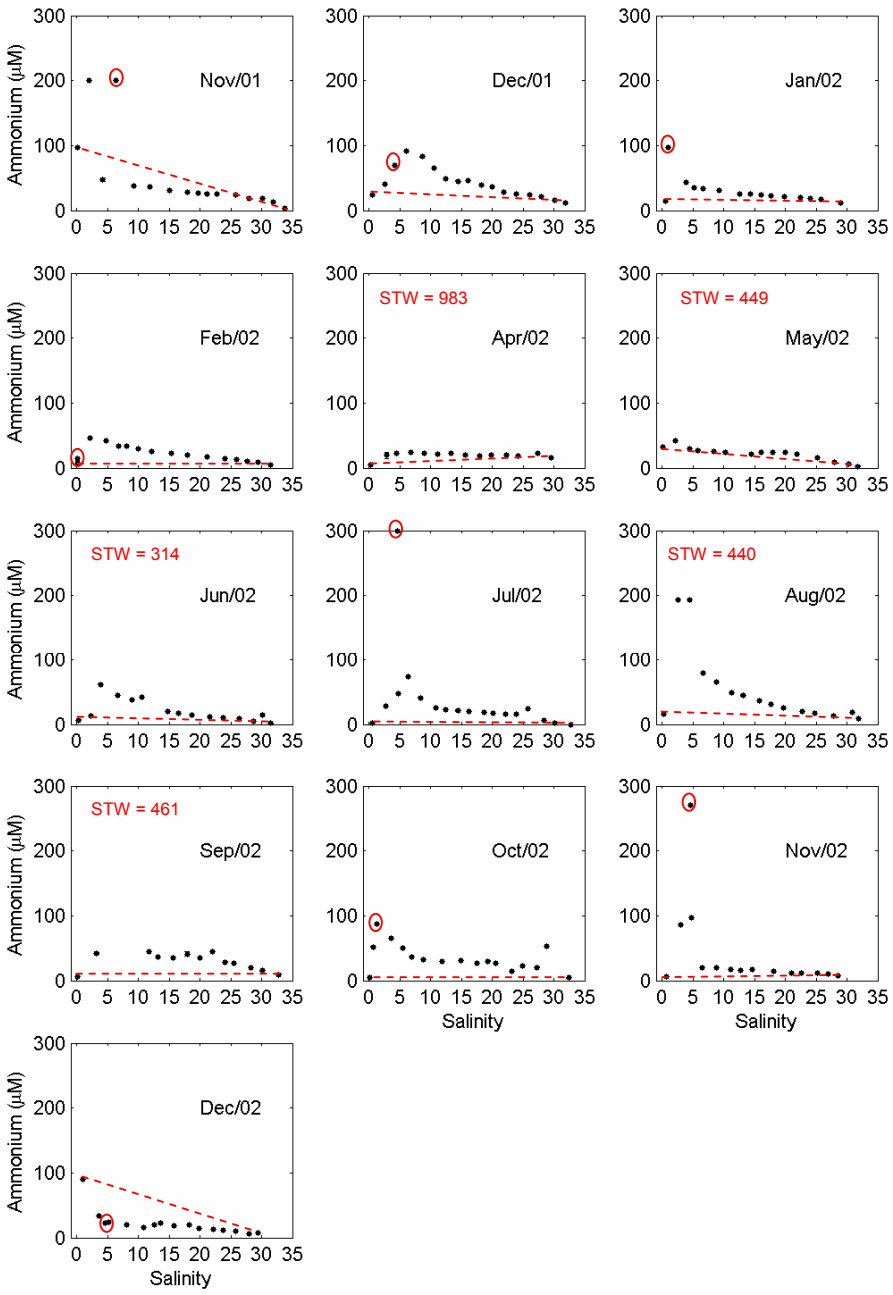

Figure 2-13. Ammonium concentration in the Itchen Estuary. Error bars show ± 1 standard deviation (n=3). Theoretical dilution line is represented by the red dotted line. Sample collected in front of the STW effluent outlet is circled in red or indicated at the top left (if out of scale).

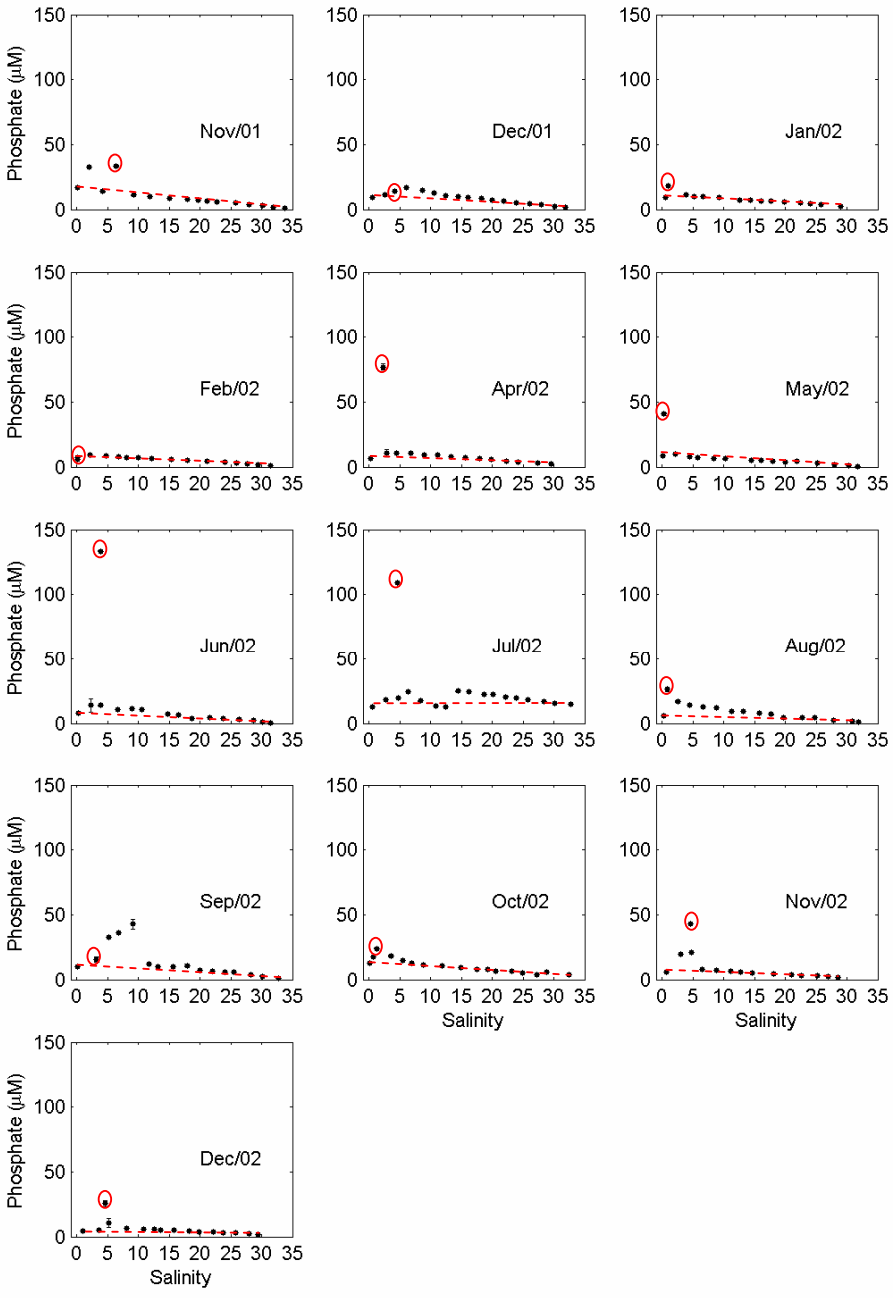

Figure 2-14. Phosphate concentration in the Itchen Estuary. Error bars show ± 1 standard deviation (n=3). Theoretical dilution line is represented by the red dotted line. Sample collected in front of the STW effluent outlet is circled in red.

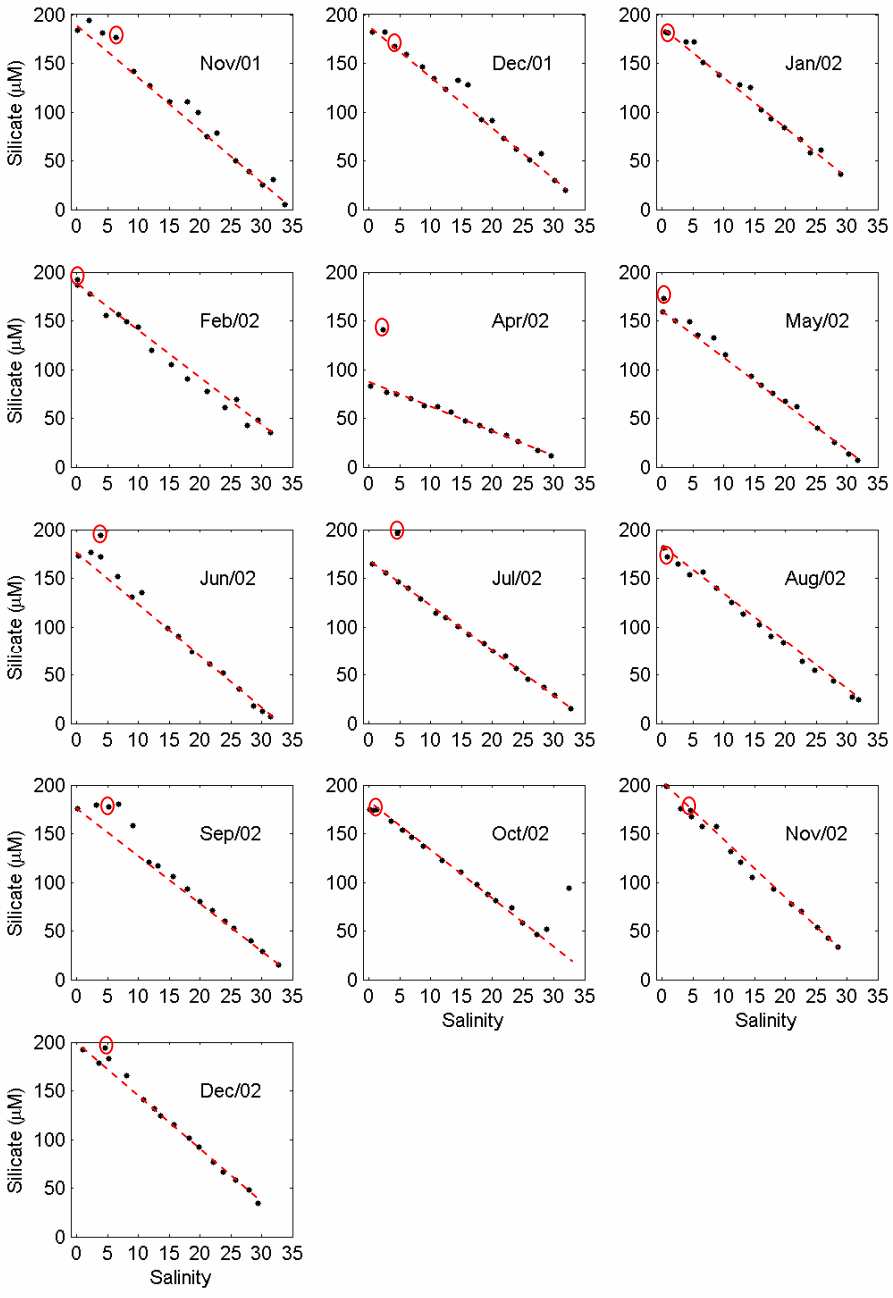

Figure 2-15. Silicate concentration in the Itchen Estuary. Error bars show ± 1 standard deviation (n=3). Theoretical dilution line is represented by the red dotted line. Sample collected in front of the STW effluent outlet is circled in red.

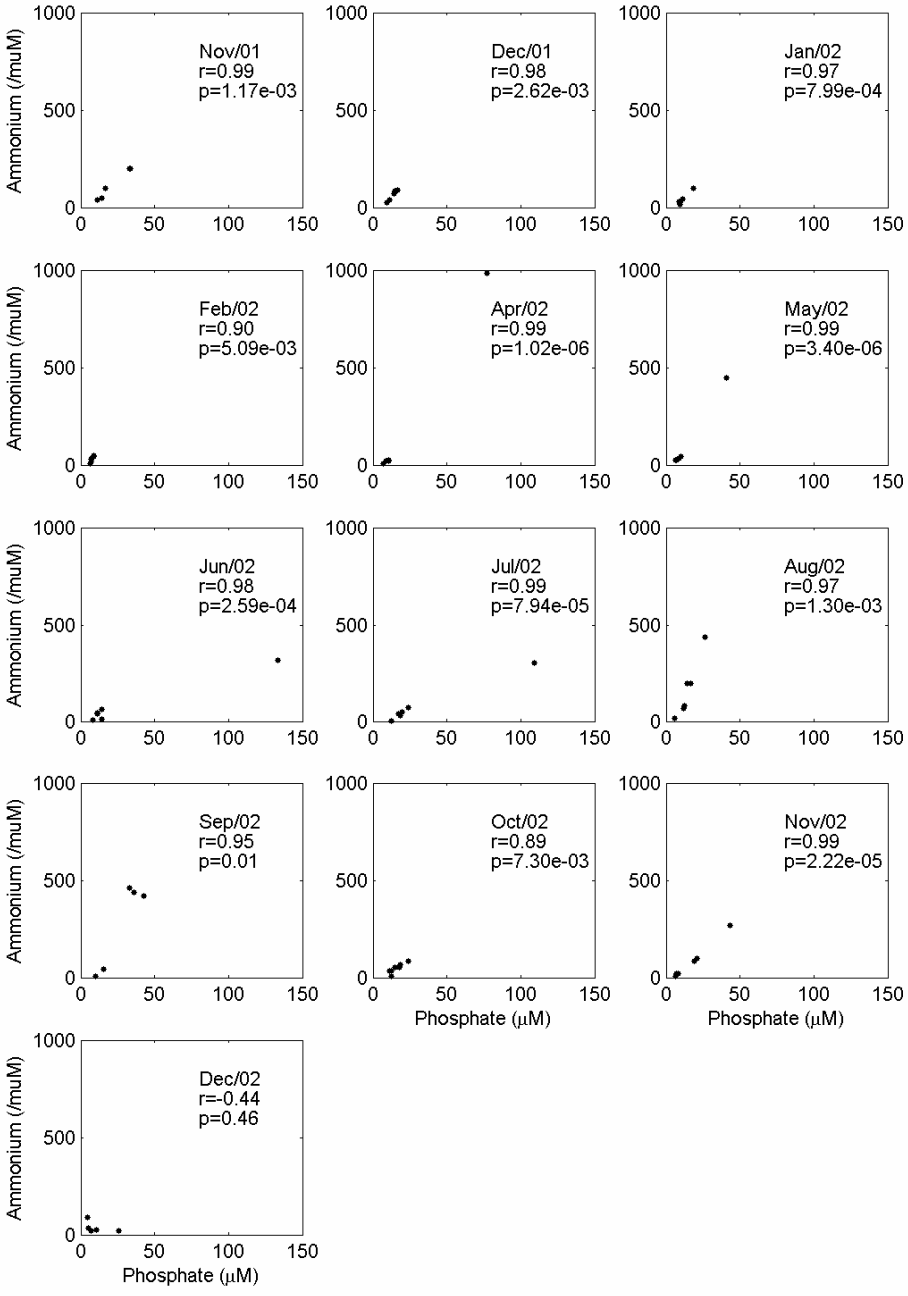

Figure 2-16. Correlations between ammonium and phosphate concentrations in the surface water from the upper estuary (salinity range 0 to 10).

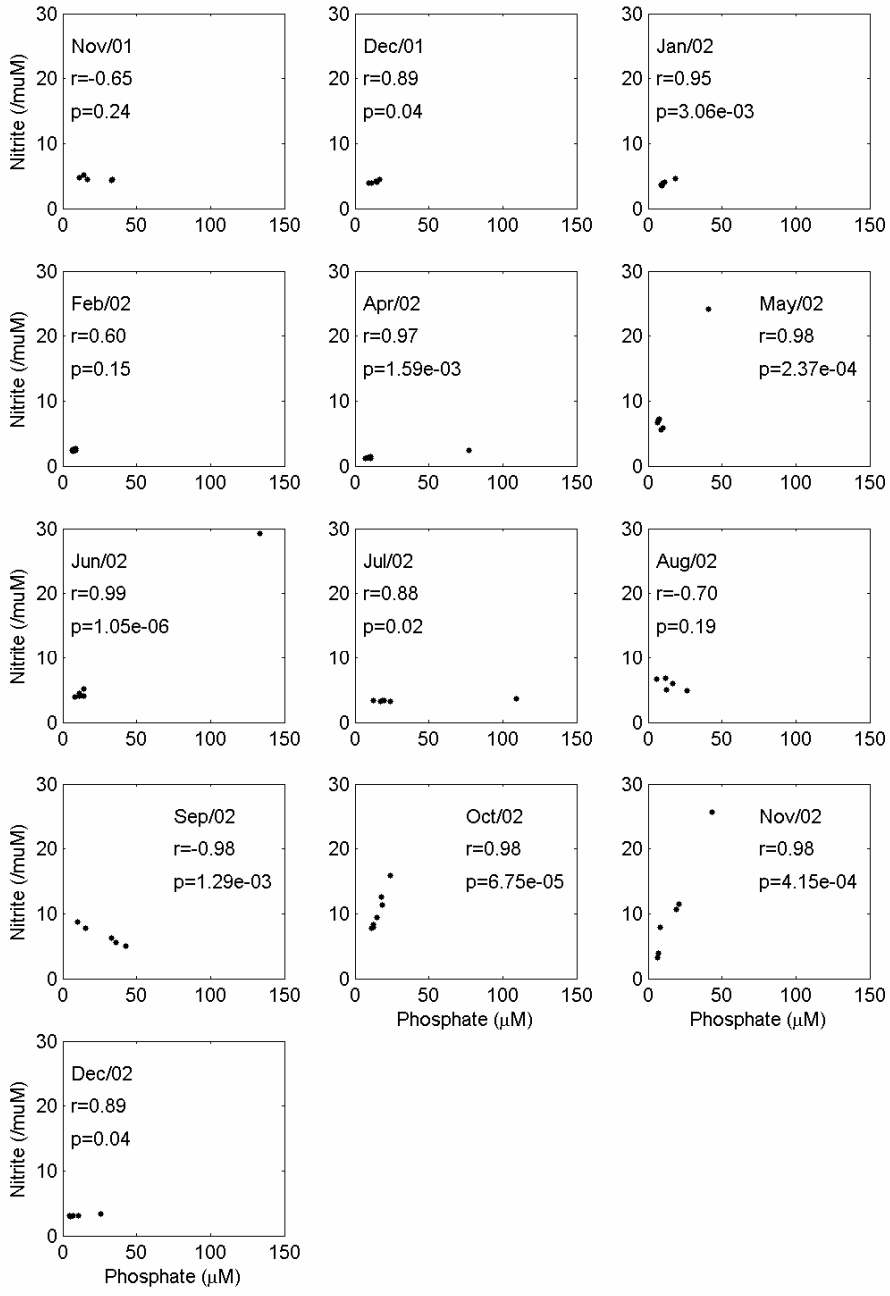

Figure 2-17. Correlations between nitrite and phosphate concentrations in the surface water from the upper estuary (salinity range 0 to 10).

2.5.2.2. Nitrous oxide

Higher N_2O concentrations were generally observed in the upper estuary when compared with concentrations in the lower estuary (Figure 2-18). This general distribution was expected since it has been observed in many estuaries (Barnes and Owens, 1998; Nedwell and Trimmer, 1996; Robinson et al., 1998). The highest N_2O concentration in each survey was found at the sampling site closest (just after) the outlet of the sewage treatment plant (Figure 2-19), suggesting input or production of nitrous oxide in that area of the estuary. It is well known that N_2O is emitted by the wastewater treatment processes, especially biological nitrogen removal (Inamori et al., 2003; Kaplan et al., 1978). Further diffuse inputs in this estuary are suggested by the relatively uniform values observed until high salinities are reached. This behaviour is clearly observed when comparing N_2O concentrations measured in the estuary with the theoretical dilution line (TDL) (Figure 2-18 and Appendix L). The TDL indicates the expected change in nitrous oxide concentration assuming linear mixing and indicates whether N_2O

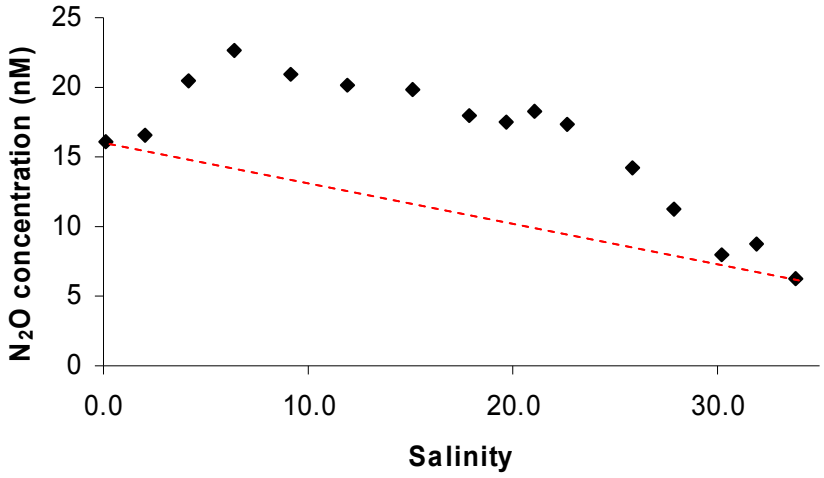

Figure 2-18. Nitrous oxide concentrations in the Itchen Estuary in November 2001. The red dotted line represents the theoretical dilution line.

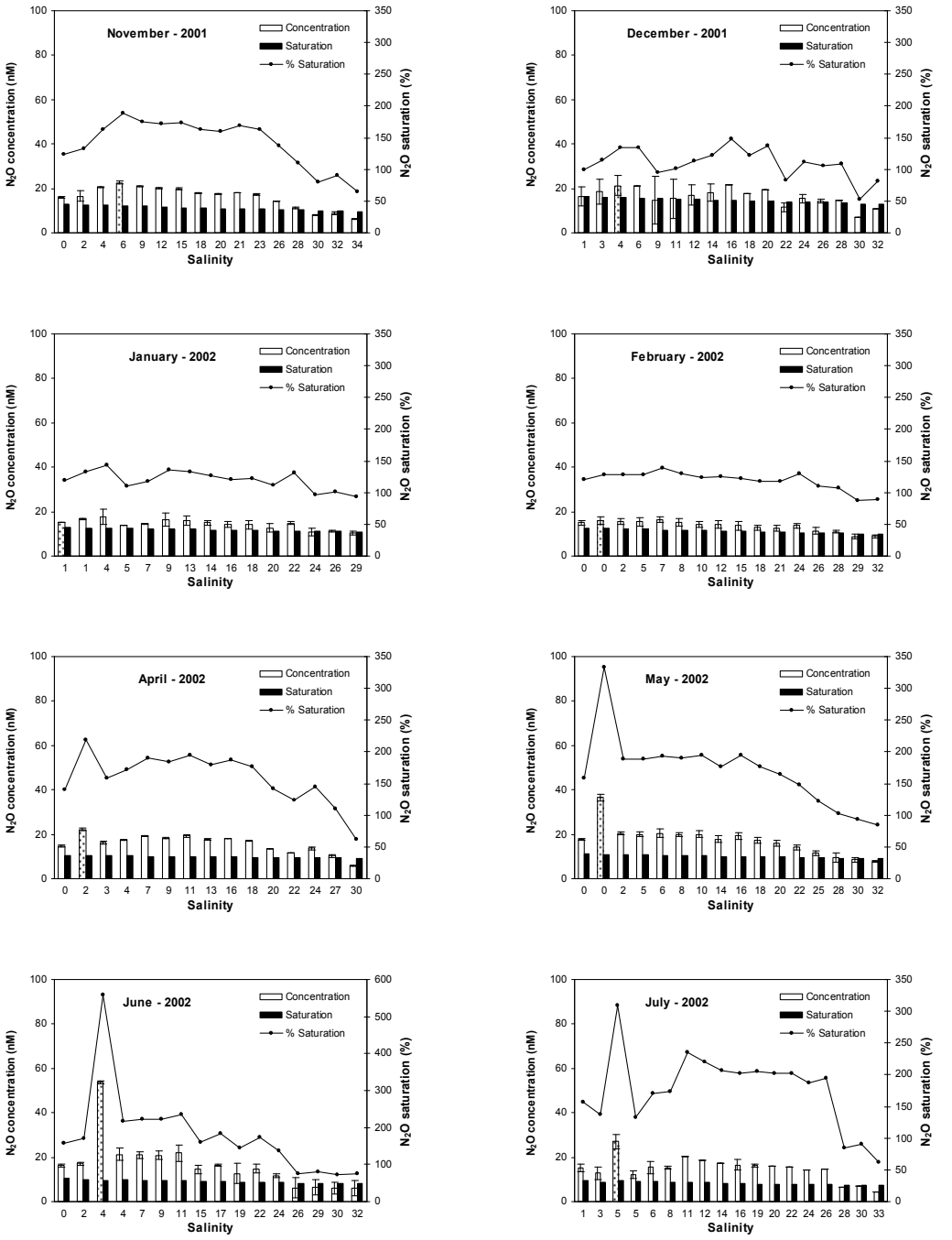

Figure 2-19. Nitrous oxide concentrations in the Itchen Estuary. White columns represent measured concentrations (error bars show ± 1 standard deviation, n=3), black columns represent calculated saturation value (at in situ temperature and salinity), and percentage saturation is shown by black line (right hand side y axis). Dotted columns represent the sampling site closest to the outlet of the sewage treatment plant.

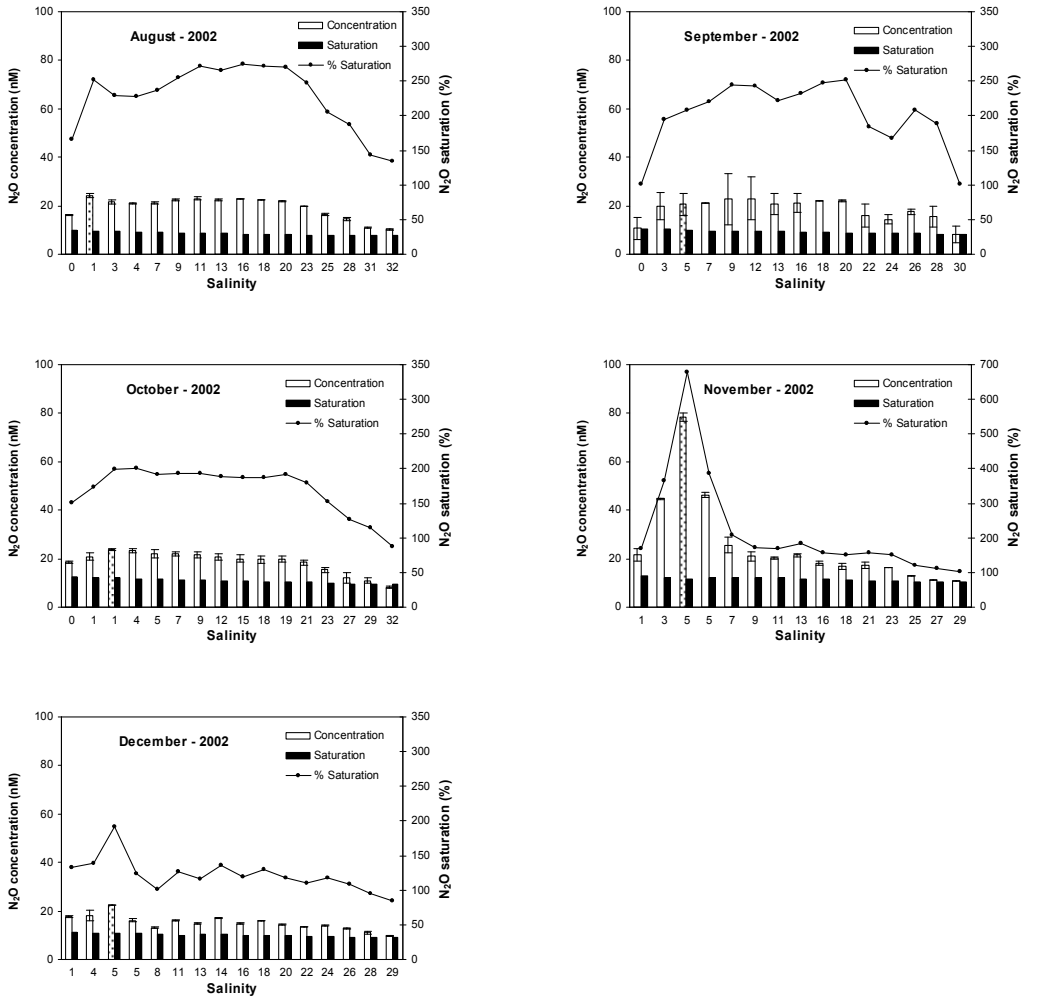

Figure 2-19. (continued) Nitrous oxide concentrations in the Itchen Estuary. White columns represent measured concentrations (error bars show ± 1 standard deviation, n=3), black columns represent calculated saturation value (at in situ temperature and salinity), and percentage saturation is shown by black line (right hand side y axis). Dotted columns represent the sampling site closest to the outlet of the sewage treatment plant.

mixes with salinity conservatively. Theoretical dilution lines were estimated using the highest and the lowest salinity samples in each month. The full set of plots for the thirteen months sampling can be seen in Appendix L.

Maximum N_2O concentration and saturation were observed in November/02 (79nM and 679%, respectively) (Figure 2-19). Mean saturation

for all surveys was 162% which can be compared with a mean saturation of 200%, generally observed in other UK estuaries by Nedwell (personal communication). A comparison with 11 other estuaries located in Europe and North America (Bange et al., 1996), confirms that N_2O saturation in the Itchen is relatively low (Table 2-1). However, N_2O emission from water to atmosphere was expected on all surveys, from salinity zero to salinity around 28, where saturations were in excess of 100% (average = 171%). Samples with salinity higher than 28 presented N_2O concentrations lower than the air equilibrium saturation concentration (average saturation = 87%).

Estuaries	N_2O Saturation (%)	Mean N_2O Saturation (%)
Itchen	61 - 679	162
Gironde	106 – 165	132
Amvrakikos Golf	94 – 107	101
Tamar	100 – 330	215
Elbe	199 – 1600	900
Schelde	120 – 3000	1560
Yaquina Bay	100 – 400	250
Alsea Bay	90 – 239	165
Hudson	117 – 700	409
Chesapeake Bay	95 – 130	113
Merrimack	117 – 455	286
Potomac Estuary	100 - 5000	2550

Table 2-1. Compilation of N_2O saturations in estuaries (Adapted from Bange et al., 1996).

It is also important to note that the nitrous oxide concentration measured in the water is the result of the balance between input and or production of N_2O in the estuarine system and the loss of N_2O from water to air caused by the air-equilibration. Based on the data acquired and the calculated percentage saturation it is possible to establish when this balance causes the emission from water to air; but it is not possible to establish the boundaries of nitrous oxide production or input within the estuarine system.

Nitrite had the strongest correlation with N_2O on all surveys (average r = 0.88; $p<0.05$), with exception of September/02 and December/02, when no significant correlations were found between N_2O and all measured nutrients; and August, when an unusual high concentration of nitrate was measured in the upper estuary (Figure 2-20). A similar correlation was reported in a study on the Colne Estuary (Dong et al., 2002), where strong correlations of nitrous oxide effluxes from the sediment were observed with nitrite concentrations in the overlying water and with nitrite influx into the sediment. In addition, the same study found increases in N_2O production from sediments about 10 times greater with the addition of nitrite to the overlying water than with the addition of nitrate during an incubation experiment.

The correlations between N_2O and nitrate concentrations were higher than for N_2O and ammonium concentrations, with exception for May, June, July and November/02 surveys (Figures 2-21 and 2-22). On these surveys a low nitrate and high ammonium point is noticed close to the sewage treatment plant outlet (very characteristic of the effluent released – see Figures 2-11 and 2-13). If these measurements are considered outliers (which is justified by the fact that it only appears when the effluent is being released), the correlation between N_2O and nitrate concentrations will be always higher than for N_2O and ammonium concentrations. The fact that the correlations between N_2O and nitrite and nitrate concentrations in the water were generally higher than that for N_2O and ammonium suggested that direct denitrification was more important than coupled nitrification-denitrification in the production of nitrous oxide. It also suggested that on the occasions of sewage release, coupled nitrification-denitrification may happen and the intensity of both processes should vary along the estuary. An estudy in the Colne Estuary showed that the rate of denitrification and rate of N2O production decreased down the estuary to the mouth, following the decreasing gradients of nitrate and nitrite concentrations (Dong et al., 2006).

Both processes of denitrification (as well as, to a lesser extent, dissimilatory nitrate reduction (Conrad, 1996; Kelso et al., 1997; Smith and Zimmerman, 1981)) and nitrification, at low ambient oxygen tension (de Wilde and de Bie, 2000; Dong et al., 2002; Jorgensen et al., 1984; Poth and Focht,

1985; Punshon and Moore, 2004; Wrage et al., 2001), are known to cause emission of nitrous oxide as an intermediate product (Miller et al., 1993).

An important point to notice is that the correlations between N_2O and all the nutrients analysed are stronger if working only with data from the salinity range 10 to 35 (Table 2-2). This shows clearly how the sewage input influences the relationship indicated by the correlation analysis discussed above.

The plots of nitrous oxide against salinity (Appendix L) clearly show the production of N_2O in every month sampled. Those plots also show that production was relatively lower from January/02 to July/02 and again in November/02 and December/02. The N_2O production observed up to salinity 10 (especially in Dec/01, Jun/02, July/02, Oct/02 and Nov/02) could be related not only to the sewage effluent as previously discussed, but also to the maximum turbidity zone. In the Itchen Estuary, the maximum turbidity zone is situated within the salinity range of 7 to 10 (Ormaza-Gonzalez, 1990). In this zone, the high load of suspended matter would allow the presence of suboxic microzones in the oxygenated water column which, together with the high nitrogen concentrations would favor the production of N_2O by both, denitrification and nitrification processes.

In addition, the correlations calculated for surface water concentrations may not reflect the process producing nitrous oxide as, in estuaries, the main production of nitrous oxide is believed to take place within the bottom sediments. Simultaneous studies of concentration and production of N_2O in the coastal waters of the Mediterranean Sea did not show a direct relationship between the gas concentration and production in the water (Marty et al., 2001). The authors suggested that the dissolved nitrous oxide was not necessarily produced in situ, in surface waters, but could be either produced by bacteria in deep layers, and transported to the surface waters, or originated from anthropogenic activity.

The proportioning of the nitrous oxide produced by nitrification or denitrification in the Itchen Estuary is a question that requires further investigation.

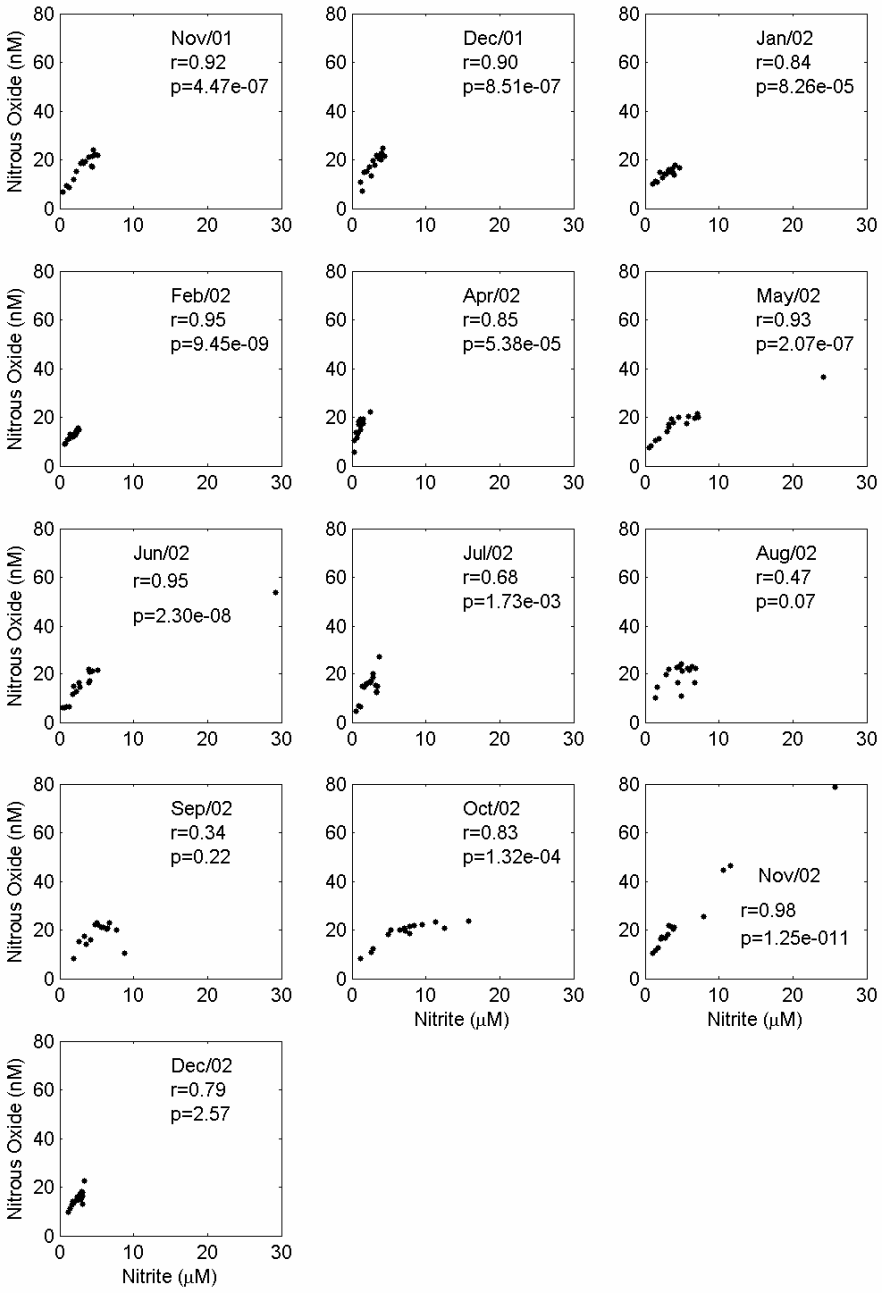

Figure 2-20. Correlation between nitrous oxide and nitrite concentrations in the surface waters shown for each of the thirteen sampling months.

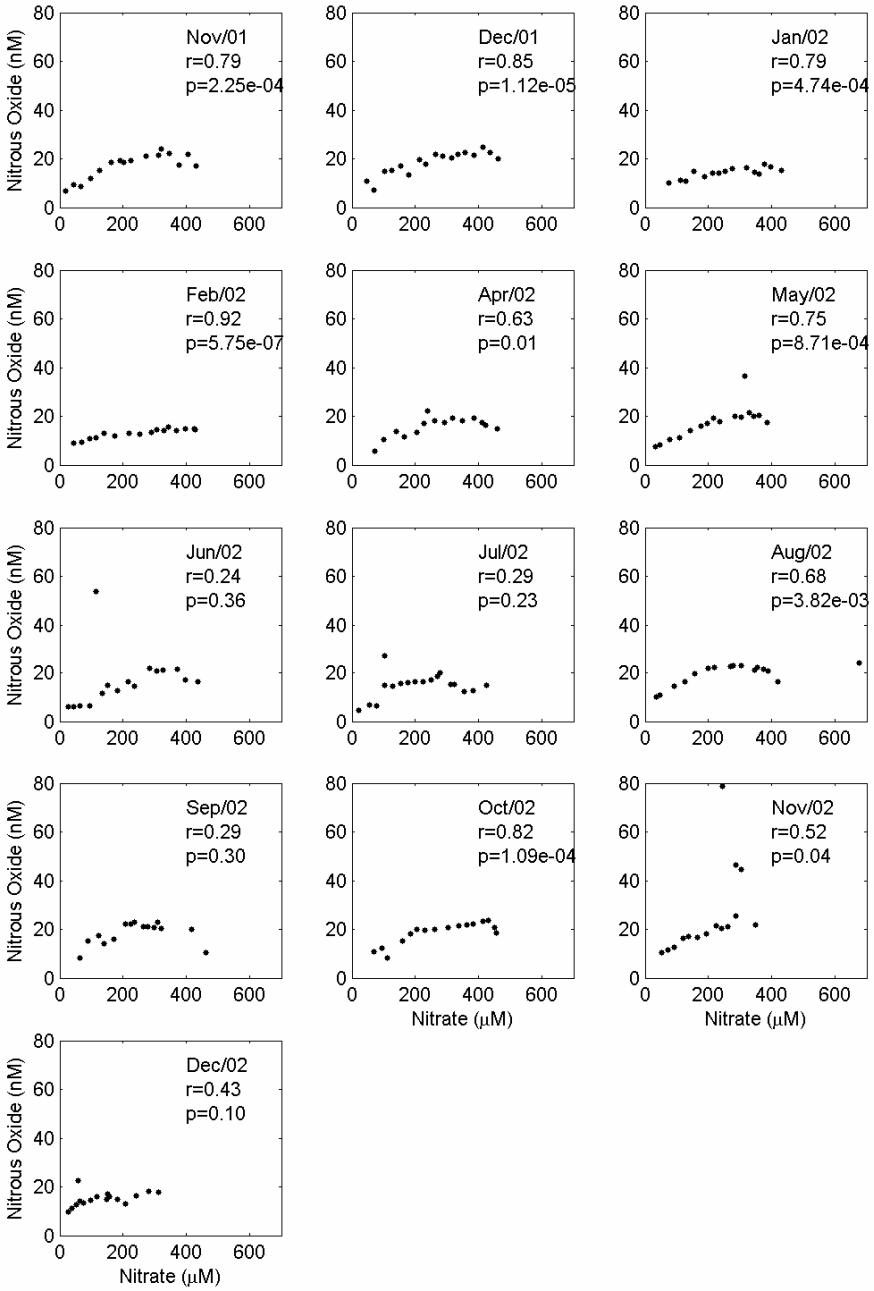

Figure 2-21. . Correlation between nitrous oxide and nitrate concentrations in the surface waters shown for each of the thirteen sampling months.

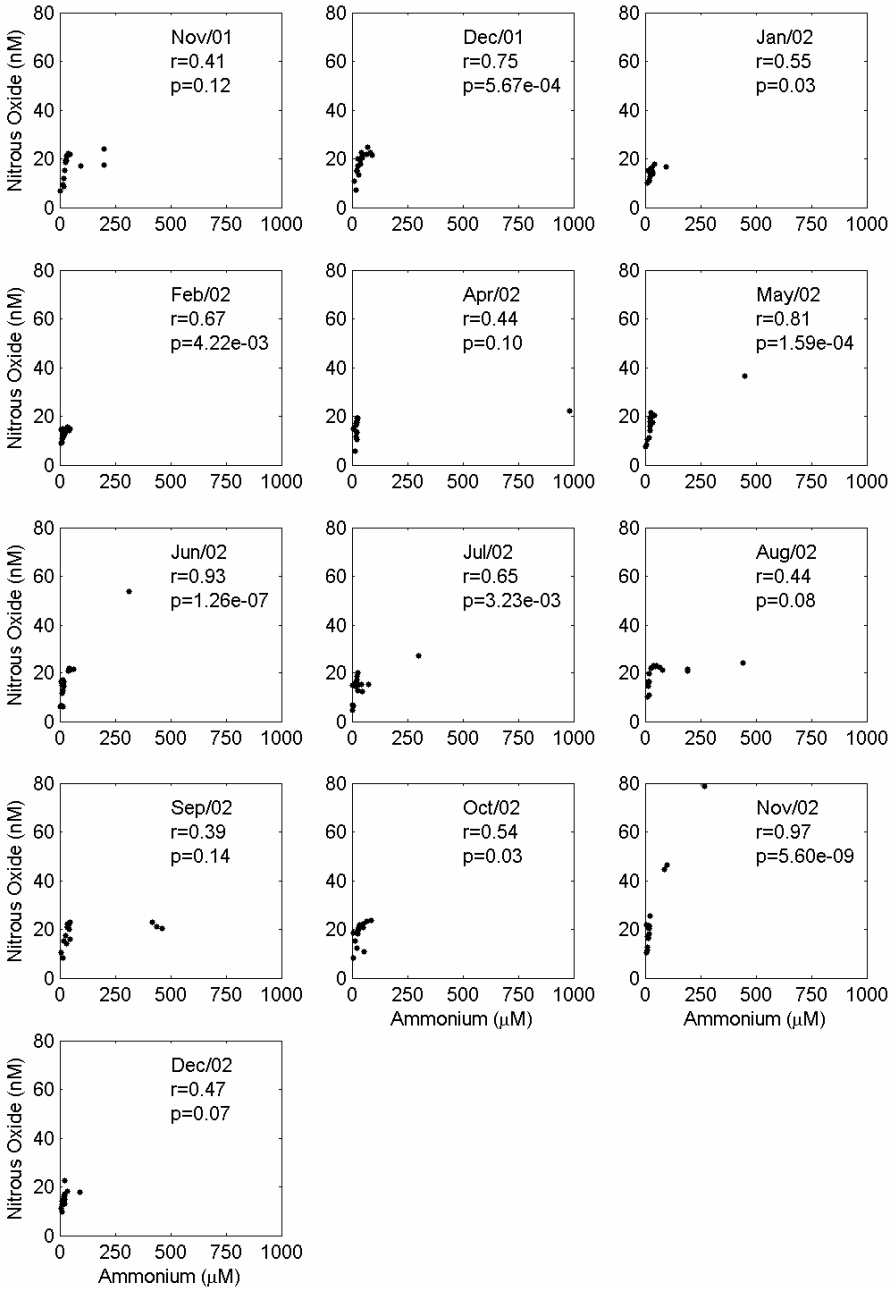

Figure 2-22. . Correlation between nitrous oxide and ammonium concentrations in the surface waters shown for each of the thirteen sampling months.

	Average r Salinity range 0-35	Average r Salinity range 10-35
Nitrate	0.62	0.91
Nitrite	0.78	0.90
Ammonium	0.62	0.79
Phosphate	0.75	0.85
Silicate	0.74	0.89

Table 2-2. Comparison of average correlations between Nitrous oxide, nitrate, nitrite, ammonium, phosphate and silicate, using data from salinity ranges 0 to 35 and 10 to 35.

2.6. Summary

1. Dissolved nutrients in the water column of River Itchen showed the influence of land activities, such as agriculture, fish farms and watercress farming, within the first 15 km (from the source of the river to sampling Site 8).

2. From Site 8 to the tidal barrier at Woodmill, nutrient concentrations in the water reflect the input of treated sewage to the river water, especially downstream of Eastleigh and Winchester STW.

3. Nitrous oxide concentrations in the river water were higher at the source, followed by a dramatic decrease in concentration within the next 7 km from the source. This indicates a rapid degassing and suggests the importance of groundwater as a source of nitrous oxide to the atmosphere.

4. River water was consistently supersaturated with N_2O, ranging from 104 to 2800% saturated. The high concentration of N_2O in the River Itchen strengthens the argument that rivers are an important contributor to emissions of this greenhouse gas.

5. Overall, dissolved nutrients in the water column of the Itchen Estuary showed a typical estuarine behaviour, with the higher concentrations in the upper estuary decreasing towards the sea. Significantly high concentrations of phosphate, ammonium and nitrite at the upper estuary show the influence of the sewage discharge by the Portswood STW.

6. Nitrous oxide concentrations in the estuarine water were higher in samples collected close to the sewage outlet, suggesting input or production in this area. Production of nitrous oxide within the estuary was also suggested as concentrations in the water were higher than the predicted by the traditional dilution line.

7. Water within salinity range 0 to 28, from the Itchen Estuary, was consistently supersaturated with nitrous oxide (average saturation = 171%), indicating emission of nitrous oxide from water to atmosphere. The supersaturation measured in this study further contributes to the argument that estuaries are sources of nitrous oxide to the atmosphere.

Chapter 3

NITROUS OXIDE FLUX ESTIMATES TO THE ATMOSPHERE

3.1. Introduction

Nitrous oxide emissions from oceans were estimated at 3 Tg N_2O-N.yr^{-1}, of which 1.9 Tg N_2O-N.yr^{-1} are accredited to rivers, estuaries and coastal waters (Kroeze et al., 1999; Kroeze and Seitzinger, 1998). Despite the existence of estimations, assessing global emission rates from individual sources that vary greatly over small spatial and temporal scales remain difficult, and additional data for comparisons across a range of rivers and estuaries are warranted.

In order to have an estimation of what the nitrous oxide fluxes from River Itchen and Itchen Estuary are, average values of N_2O excess in water were applied to a flux model for each survey. The nitrous oxide data used for this estimation was presented and discussed in chapter 2.

Based on the estimated fluxes, and knowledge of the surface area of these waters, emissions were calculated and compared with other systems. An extrapolation of the Itchen estuary's emission to the global estuarine area allows the comparison of data acquired for this study with other global estimations, giving an idea of how representative this system is in a global picture.

3.2. The thin film flux model

A thin film flux model (Scranton, 1983) was used to estimate N_2O fluxes from water to air. The model is based on the assumption that a dissolved chemical has a uniform concentration throughout a surface water body, due to turbulent diffusion, except in a very thin layer at the water's surface. A similar assumption is made concerning the chemical concentration in overlying air. Within a few micrometers or millimeters of the water-air interface, it is assumed that the eddies responsible for turbulent diffusion are suppressed; therefore, chemical transport in this thin layer (or film) can only occur by molecular diffusion, which is considered to be the rate-limiting step of air-water exchange (Figure 3-1) (Liss and Slater, 1974).

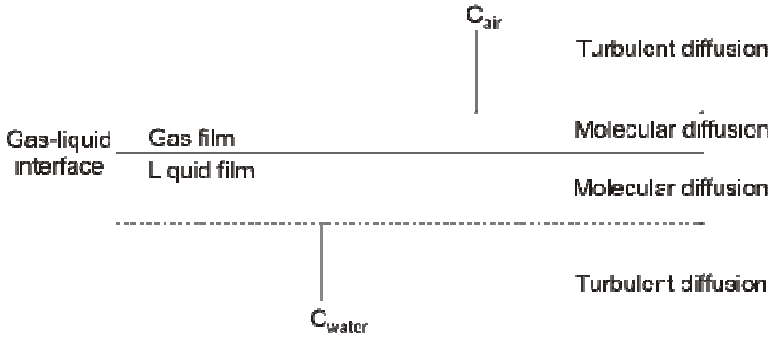

Figure 3-1. Schematic of the thin film model (adapted from (Hemond and Fechner-Levy, 2000).

The following equation is used to calculate the water-air flux:

$$F = D \cdot \frac{(Cw - Ca)}{(Zw - Za)}$$

where:

F = water-air flux (nmol N_2O m^{-2} h^{-1})

D = temperature-corrected N_2O diffusivity in water (m^2 h^{-1})

Cw = N_2O concentration in water (nmol N_2O m^{-3})

Ca = N_2O concentration in water from air equilibration (nmol N_2O m^{-3})

Zw = lower limit of thin film (m)

Za = upper limit of thin film (m)

N_2O diffusion coefficients, directly proportional to water temperature, were estimated using the following approximation based on measured diffusion constants (Broecker and Peng, 1974):

$$D = 10^{((-1010/T)+B)}$$

Where: *D* = N_2O diffusion coefficient in water ($cm^2\ s^{-1}$); *T* = water temperature (K); *B* = constant (-1.24).

Thin film thickness (Zw – Za) was estimated from a wind speed dependent relationship (Upstill-Goddard et al., 1990) using wind speed data from the SOC/MetOffice. The relationship was based on two wind regimes, one for a rough surface at low wind speeds and one for breaking waves at high wind speeds:

$K = 1.11U + 0.35$ for $U < 9.5$ m s^{-1}

$K = 2.53\ U - 13.09$ for $U > 9.5$ m s^{-1}

Where: *K* = gas transfer velocity for CO_2 at 20°C (cm h^{-1}); *U* = wind speed (m s^{-1}).

The thin film thickness was then calculated using *K* by:

$$(Z_w - Z_a) = \frac{D}{K}$$

Where: $Z_w - Z_a$ = thin film thickness; *D* = diffusion coefficient for CO_2 at 20 °C (5.904×10^{-2} $cm^2\ h^{-1}$) and *K* = gas transfer velocity.

As can be seen from the above equation, thin film thickness is highly dependant on gas transfer velocity (*K*). While measurement options and models exist for choosing a value for the gas transfer velocity, the determination of *K* is by far the most problematic term in the flux equation, resulting in a large uncertainty when estimating the exchange of any gas in

aquatic systems, particularly river and estuary systems (Raymond and Cole, 2001; Zappa et al., 2003).

Rivers and estuaries represent a case in which both wind forcing and boundary friction can generate turbulent energy, and therefore such turbulence depends on the depth, mean tidal velocity, and wind regime of a given system (Cerco, 1989; Mcintyre et al., 1995). The situation is further complicated by the time dependence of the tidally-driven currents and changes in fetch, water depth, or stratification that have spatial heterogeneity in most river-estuary systems.

Given the complex interplay of wind speed and hydraulic conditions, and also the lack of direct *K* measurements for the River Itchen and Itchen Estuary; for the purpose of this study, *K* has been estimated from a wind speed relationship only. Therefore, it is important to keep in mind that the estimated value carries uncertainties that may be better constrained by future studies.

3.3. Applying the model to the River Itchen

Nitrous oxide concentrations in the river water were much higher near the source, where the springs are located (sampling sites 1 and 2 - average concentration = 127 nM N_2O), than in the remaining river (sampling sites 3 to 16 – average concentration = 30 nM N_2O).

As the average N_2O concentration in the water is used to calculate the fluxes from water to air, using the average concentration for the whole river can generate a significant error. This can be seen in Appendix M, where fluxes calculated separately for the two segments of the river (sampling sites 1 and 2, and sites 3 to 16) can be compared to the fluxes calculated for the river as a whole (using average N_2O concentration from sites 1-16).

The flux calculated with the average N_2O concentration for the whole river would greatly influence the final emission result, for which the area of each segment of the river must be applied to the respective flux. The first

segment of the river has a very small area (0.01 km^2) when compared with the rest of the river (0.6 km^2), resulting in very low emissions, even with the high fluxes. Therefore, the annual emission for the total area of the river is found to be about 46% greater if the fluxes are calculated using the average N_2O concentration for the whole river (Appendix N). This reinforces the importance of spatial variability in nitrous oxide studies and indicates that caution is needed when interpreting results from spatially variable data.

With the intent to present reliable estimates, fluxes and emissions were calculated using the river segmented into two blocks; from Site 1 to Site 3, and from Site 3 to Site 16. Emissions from these two segments were then added to express the total emission from River Itchen.

3.3.1. N_2O fluxes from surface river water

As expected, N_2O fluxes from water to air were much higher at sites 1 and 2 than sites 3 to 16 (average flux of 6600 and 960 nmol N_2O m^{-2} h^{-1}, respectively). Table 3-1 shows that the flux from the river source area was lower in September (1980 nmol N_2O m^{-2} h^{-1}) and at its maximum in February (12900 nmol N_2O m^{-2} h^{-1}). Maximum flux from sites 3 to 16 was also observed in February (2300 nmol N_2O m^{-2} h^{-1}), but the lowest flux was observed in November/01 (335 nmol N_2O m^{-2} h^{-1}).

The maximum fluxes estimated in February resulted from the high average N_2O excess and thin interface films that were observed in both sections of the river (Table 3-1). The flux from water to air, as calculated in this study, depends on the combination of these variables.

N_2O excess is also highly dependent on water temperature. The N_2O excess is calculated by the difference between the N_2O concentration measured in the water sample and the theoretical N_2O concentration in water that is 100% saturated with N_2O. Like other gases, N_2O saturation in water will vary according to the water temperature.

Survey	N_2O excess in water		Average wind speed	Thickness of the film	N_2O water-air flux	
	Sites 1 to 2	Sites 3 to 16	Sites 1 to 16	Sites 1 to 16	Sites 1 to 2	Sites 3 to 16
Nov/01	*	10	2.9	165	*	335
Dec/01	*	11	3.0	160	*	342
Jan/02	109	16	4.9	102	5989	857
Feb/02	162	29	7.3	70	12938	2318
Apr/02	195	19	4.3	115	10349	994
May/02	135	19	5.5	91	8947	1258
June/02	140	18	4.5	110	8009	1032
July/02	71	20	5.0	100	4546	1268
Aug/02	82	17	3.2	151	3490	714
Sept/02	47	14	3.2	151	1978	605
Oct/02	69	16	4.3	115	3545	832
Nov/02	112	20	4.8	104	5856	1029
Dec/02	145	18	4.4	113	6905	885

Table 3-1. N_2O excess in water (nmol N_2O l^{-1});Average wind speed (m s^{-1}); Thickness of the film (μm); N_2O flux (nmol N_2O m^{-2} h^{-1}). * No data available as sites 1 and 2 were included in the sampling scheme only from January onwards.

Wind speed, as discussed in Section 3.2, is the variable that controls the gas transfer velocity, which is used to calculate the thickness of the interface film. Therefore, the higher the wind speed the higher the gas transfer velocity and thinner the interface film. As the interface film acts as a rate-limiting step for water-air exchange, it is expected that higher fluxes will be calculated when thinner films are present.

In addition, it is to be realised that wind speed and N_2O concentration in the water are strongly coupled. High wind speeds will result in rapid exchange of the N_2O from water to the atmosphere and consequently decreasing N_2O concentrations in the river. In contrast, low wind speeds will result in a low exchange rate and accumulation of the produced N_2O.

Fluxes presented in this document were estimated using the average wind speed for each sampling month. As the N_2O concentration measured in the water is supposed to be strongly coupled with the wind speed, calculating the flux using the average wind for the month and the instantaneous N_2O concentration may not be ideal; but will present a better monthly figure than just assuming that the instantaneous picture is representative of the whole month. This may be clearer if looking at the wind speed data for each month

(Appendix O). Taking Nov/2001 as an example, the wind speed at the sampling time was one of the strongest for that month, and does not represent the most common situation for that particular month.

Nitrous oxide fluxes for the River Itchen are of the same order of magnitude as those estimated for the South Platte River (Mcmahon and Dennehy, 1999). Median fluxes from the nine channel cross sections at the South Platte River ranged from 3180 to 47300 nmol N_2O m^{-2} h^{-1}. Denitrification and nitrification within the stream/aquifer system were considered to be the main sources of N_2O in this nitrogen enriched river.

3.3.2. N_2O emission from the river

Total N_2O emissions from the River Itchen were calculated by applying the previously calculated fluxes to the estimated area of each section of the river (i.e. sites 1 to 3, and sites 3 to 16). The surface area of the River Itchen was estimated using the database of the Geodata Institute – Southampton University.

Survey	N_2O Flux		N_2O Emission		
	Sites 1 and 2	Sites 3 to 16	Sites 1 to 3 Area=0.01 km^2	Sites 3 to 16 Area=0.6km^2	Total emission for the river
Nov/01	*	335	*	6	6
Dec/01	*	342	*	6	6
Jan/02	5989	857	3	16	19
Feb/02	12938	2318	6	44	50
Apr/02	10349	994	5	19	23
May/02	8947	1258	4	24	28
Jun/02	8009	1032	4	20	23
July/02	4546	1268	2	24	26
Aug/02	3490	714	2	14	15
Sep/02	1978	605	1	11	12
Oct/02	3545	832	2	16	17
Nov/02	5856	1029	3	19	22
Dec/02	6905	885	3	17	20

Table 3-2. Nitrous oxide fluxes (nmol N_2O m^{-2} h^{-1}) and emissions (kg N_2O) from the River Itchen for each month.

Despite the very high N_2O fluxes estimated for sites 1 and 2, emissions from this area of the river were much lower than for the remaining parts of the river (average = 3 and 18 kg N_2O $month^{-1}$, respectively; Table 3-2). That is

only because the surface area from Site 1 to Site 3 is much smaller than the area from Site 3 to Site 16 (0.01 and 0.6 km^2, respectively).

The N_2O emission estimated for the total length of the river, from the source to the tidal barrier, was 262 kg N_2O per year. This estimation was made using the twelve months survey beginning on December 2001, assuming that values for the missing Sites 1 and 2 were the same as for December 2002. If this figure is extrapolated to the total area covered by rivers in the United Kingdom (3 x 10^9 m^2; (Palmer and Roy, 2001)), an emission of 1.3 Gg of N_2O is estimated. This value may represent an underestimation of the emission, as the River Itchen does not have a very high load of nutrients if compared to some other UK rivers (i.e. Great Ouse system, for example). This emission is in the same order of magnitude as the N_2O emission from the fuel combustion by manufacturing industries and construction (2.4 Gg N_2O), wastewater handling (3.9 Gg N_2O), and manure management (4.3 Gg N_2O) in the UK, estimated for the year 2003 (Baggott et al., 2005). Regardless of where the N_2O was produced, if in the river or received by run off from the surrounding area, the point of entry of the N_2O to the atmosphere was at the river's surface; reinforcing the idea that rivers are a potentially significant source of N_2O.

3.4. Applying the model to the Itchen Estuary

Nitrous oxide concentrations in the estuarine water were generally higher in the upper estuary, and decreased with increasing salinity (see previous Chapter). The magnitude of variation between maximum and minimum concentrations was not as large as in the river (average concentration in the upper estuary = 22 nM and average concentration in the lower estuary = 10 nM). For this reason, N_2O fluxes from water to air were calculated for the estuary as a whole, using the average N_2O concentration in the estuarine water (Table 3-3).

3.4.1. N_2O fluxes from the estuarine surface waters

The highest nitrous oxide flux from the estuary was for the survey in Nov/02 (864 nmol N_2O $m^{-2}h^{-1}$). This result was expected as the average excess of N_2O and the wind speed were high in November/02 (Table 3-3).

Similar reasons for the higher fluxes in the river (discussed in section 3.3.2) apply for the estuary, these being the combination of high average N_2O excess and thin interface films.

The effect of water temperature on the N_2O excess in the water, also discussed in Section 3.3.2, can be seen in Table 3.3. The similar N_2O concentrations observed for example in Nov01/Dec01 and Apr02/Dec02, correspond to different N_2O excesses in the water.

A comparison with five other estuaries (Table 3-4) confirms that the N_2O flux from the Itchen Estuary is relatively low, but it is important to keep in mind that the flux from other estuaries were not estimated using the same data density and same spatial and temporal extrapolations.

Survey	N_2O conc. in water	N_2O excess in water	Water temp.	Wind speed	Thickness of the film	N_2O water-air flux
Nov/01	16.0	6.5	9.3	2.9	165	214
Dec/01	16.1	4.1	4.2	3.0	160	119
Jan/02	14.2	3.0	9.1	4.9	102	160
Feb/02	13.4	2.8	8.7	7.3	70	220
Apr/02	15.6	6.5	13.3	4.3	115	348
May/02	17.2	8.4	14.2	5.5	91	584
June/02	16.7	9.7	16.6	4.5	110	595
July/02	14.7	7.8	17.7	5.0	100	545
Aug/02	19.4	10.9	19.3	3.2	151	525
Sept/02	18.3	9.2	15.0	3.2	151	393
Oct/02	18.5	8.3	11.8	4.3	115	428
Nov/02	25.5	15.8	10.4	4.8	104	864
Dec/02	15.1	3.3	8.0	4.4	113	153

Table 3-3. N_2O concentration in estuarine water (nmol N_2O l^{-1}); N_2O excess in water (nmol N_2O l^{-1}); Water temperature (°C); Wind speed (m s^{-1}); Thickness of the film (µm); N_2O flux (nmol N_2O m^{-2} h^{-1}).

Estuary	N_2O sat. %	N_2O Flux	No of surveys	Date	Author
Itchen	52-679	0.40	13	Nov/01-Dec/02	This work
Tamar	100-330	0.41	4	Aug,Oct/88; May/89; June/90	(Law et al., 1992)
Colne	50-450	27.9	6	Feb/93 - Mar/94	(Robinson et al., 1998)
Humber	200-4000	1800	6	Nov/95 - Dec/96	(Barnes and Owens, 1998)
Alsea	90-239	0.38	3	July-Sept/79	(De Angelis and Gordon, 1985)
Hudson	117-700	0.23	7	Mar-Sept/78	(Cole and Caraco, 2001)

Table 3-4. N_2O water-air flux (μmol N_2O m^{-2} h^{-1}) in a number of temperate estuaries.

3.4.2. N_2O emissions from the estuary

Because of its small area and relatively low flux, the Itchen Estuary has a low N_2O emission of about 470 kg $year^{-1}$ (Table 3-5). As for the river, this estimation was made using the twelve monthly surveys starting in December 2001.

Survey	N_2O Emission
Nov/01	20
Dec/01	11
Jan/02	15
Feb/02	21
Apr/02	33
May/02	55
June/02	57
July/02	52
Aug/02	50
Sept/02	37
Oct/02	41
Nov/02	82
Dec/02	15

Table 3-5. Nitrous oxide emissions (kg N_2O $year^{-1}$) from the Itchen Estuary. Total area of the estuary = 3 x 10^3 m^2.

Considering that the total estuarine area of the United Kingdom is about 2.8 x 10^9 m^2 (Buck and Davidson, 1993), and assuming that all UK estuaries have a similar N_2O flux, a total emission of 0.44 Gigagrams per year can be estimated. Again, this emission should represent the lower limit, as the Itchen Estuary is known to have a lower nutrient load than other Uk estuaries

(e.g. Humber). This emission is in the same order of magnitude as the N_2O emission from waste incineration (0.16 Gg N_2O), fugitive emissions from fuels (0.13 Gg N_2O), and fuel combustion (by sectors other than transport and energy, manufacturing and construction industries; 0.69 Gg N_2O) in the UK, estimated for the year 2003 (Baggott et al., 2005).

Similarly to the River Itchen, regardless of whether the N_2O was produced within the estuary or introduced to it, the point of entry of this N_2O to the atmosphere was at the estuary's surface, and its magnitude suggests that estuaries are a potentially significant source of N_2O.

3.5. Comparing emissions: River Itchen against the Itchen Estuary

Comparing the estimated emissions from River Itchen and Itchen Estuary (respectively 262 and 470 kg of N_2O per year), it is clear that the Itchen Estuary emits more nitrous oxide to the atmosphere in a year than the River Itchen, despite the variations seen on a monthly basis (Figure 3-2).

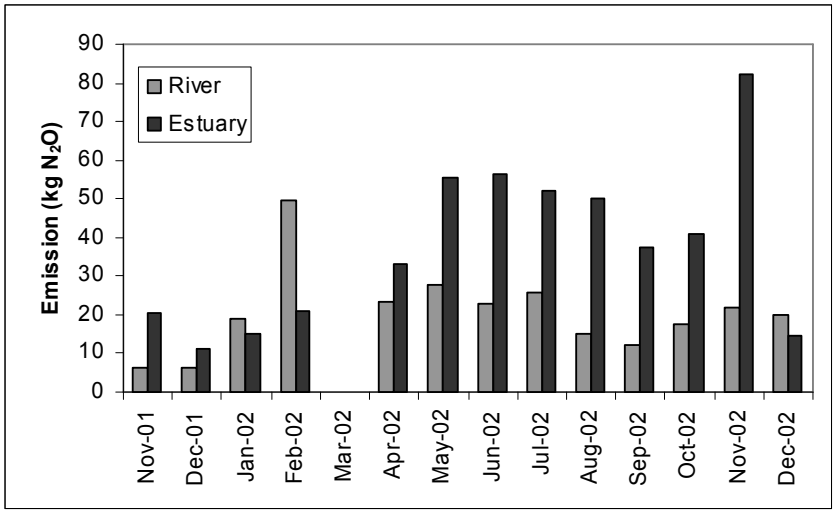

Figure 3-2. Total N_2O emission from River Itchen and Itchen Estuary (kg N_2O $year^{-1}$) for every month sampled.

On the other hand, if the same emissions are expressed by unit of area, then the river emissions will be higher for every month sampled (Figure 3-3). This means that on a per unit basis, the River Itchen emits more nitrous

oxide from its water surface to the atmosphere than the Itchen Estuary, suggesting that rivers can be larger sources of N_2O than estuaries. Therefore, the estimates of the annual N_2O emission from the Itchen Estuary are higher than the emission from the River Itchen mainly because the estuarine area is larger than the river area.

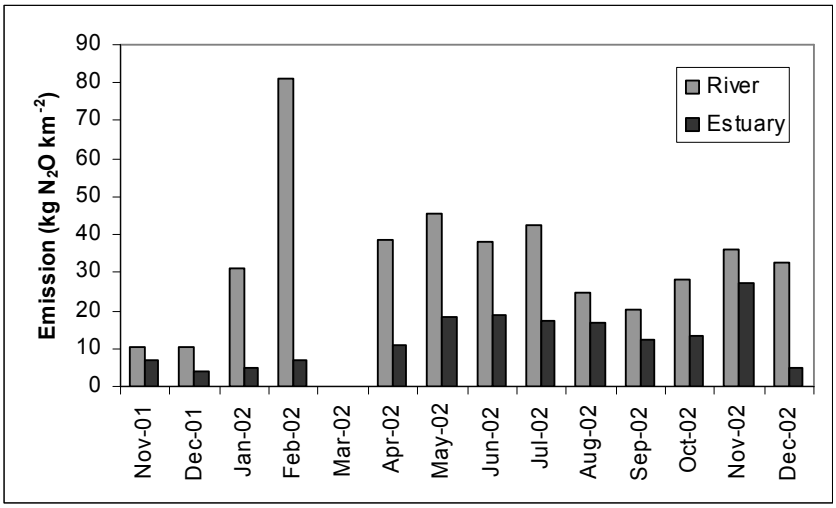

Figure 3-3. Emission of N_2O per unit of area (kg N_2O km^{-2}) from the River Itchen and Itchen Estuary.

This is a very interesting finding and particularly important when values are extrapolated to a national or global picture. Models of the global distribution of nitrous oxide production (Seitzinger and Kroeze, 1998) also show that rivers are quantitatively larger sources of N_2O than estuaries. Unfortunately, rivers (and especially non-tidal rivers) are also the least well studied with respect to nitrous oxide production, making it difficult to compare different river systems.

3.6. Extrapolating the Itchen area to a global picture

Due to the low N_2O flux estimated relative to other estuaries, extrapolation of the N_2O emission from the Itchen estuary to a global scale will give an indication of the lower limit of the total estuarine N_2O emission. Based on the area of the Itchen (3×10^3 m^2), relative to the total area covered by

estuaries globally (1.4 x 10^{12} m^2), the total estuarine emission was estimated to be 0.22Tg N_2O $year^{-1}$.

This estimate is low compared to the total estuarine N_2O emission of 3.7 to 5.7 Tg $year^{-1}$, as reported by Bange et al. (1996); but fits well to the reported total estuarine N_2O emission of 0.11 to 1.1 Tg $year^{-1}$ by Seitzinger and Kroeze (1998).

Similar extrapolation for the global riverine area is not possible by the same methodology, as there is no information available on the global area covered by rivers. It is important to note that global area estimations carry large uncertainties and such estimates should be considered with caution due to enormous variations in temperature and detailed biogeochemistry of these systems.

3.7. Summary

1. Nitrous oxide fluxes were much higher for the river source area than for the remaining area of the river, highlighting the significance of groundwater fed rivers as a source of N_2O, and also the importance of spatial variability.

2. Maximum N_2O fluxes from both, the river and the estuary, occurred when the combination of high average N_2O excess and thin interface film was observed. This confirms that the model used to calculate the fluxes in this study is highly dependent on the wind speed.

3. Extrapolation of the annual emission of N_2O estimated for the River Itchen, to the total area covered by rivers in the United Kingdom, gives the same magnitude values as for the 2003 N_2O emissions estimates from the fuel combustion by manufacturing industries and construction, wastewater handling and manure management in the UK.

4. Extrapolation of the annual emission of N_2O estimated for the Itchen Estuary, to the total area covered by estuaries in the United Kingdom, gives the same magnitude values as for the 2003 N_2O emissions estimates from the fugitive emissions from fuels, waste incineration, and fuel combustion by

sectors other than transport and energy, manufacturing and construction industries in the UK.

5. Regardless of whether the nitrous oxide is produced in or introduced to these water systems, emissions of nitrous oxide from the River Itchen and Itchen Estuary were significant and support the view that rivers and estuaries as potentially significant sources of N_2O.

6. A comparison between the River Itchen and the Itchen Estuary emissions shows that the Itchen Estuary exports more nitrous oxide to the atmosphere than the River Itchen.

7. Comparison between the River Itchen and the Itchen Estuary emissions per unit of area shows that the River Itchen represents a larger source of nitrous oxide to the atmosphere than the Itchen Estuary, suggesting the importance of groundwater fed rivers to emissions of N_2O to the atmosphere.

Chapter 4

SEDIMENT INCUBATION STUDIES OF NITROUS OXIDE PRODUCTION AND RELEASE

4.1. Introduction

The precise mechanisms of N_2O production in nearshore waters are still unclear but generally, benthic denitrification is considered to be the primary estuarine source of nitrous oxide (Delwiche, 1981; Law et al., 1991; Nedwell and Trimmer, 1996; Robinson et al., 1998). Denitrification and nitrification in the water column are also reported (Billen et al., 1985; Butler et al., 1987; Law et al., 1992; Robinson et al., 1998), but are generally considered as a secondary source of nitrous oxide in estuaries.

This chapter presents the description and discussion of core incubation studies undertaken with the objective of better understanding the nitrous oxide production in estuaries, and where the major processes take place.

The incubation experiments were done with sediment collected in the Itchen estuary, making this study closely related to previous chapters by adding to the discussion of what processes are taking place and comparing fluxes estimated from *in situ* data in the water column with incubation data.

4.2. Testing the method with an intact sediment core

The first step in this incubation study was an exercise to test the designed experiment and equipment, checking for possible mechanical problems and making improvements. This exercise was done using an intact sediment core collected in August/03, on an inter-tidal mudflat close to the railway bridge in the Itchen Estuary (Figure 2-3), during low tide.

The sediment core (about 15 cm deep) was collected using a plexiglass cylinder (9 cm internal diameter, 40 cm height), which was carefully removed by hand, sealed with a rubber bung and immediately transported to the laboratory. The core was taken at low tide but was not air exposed, as there was about 2 cm of overlying water. Once back in the laboratory, the cylinder was filled to the rim with water collected *in situ*, and allowed to re-equilibrate overnight.

The objective of this exercise was to test the equipment for any possible malfunctioning that could occur as a result of the long time (six hours) running the experiment (like the warming up of the stirring equipment, for example); and also to make the decisions of what temperature and sub-sampling method should be used in the final incubation experiments. There was no preliminary intention to use the data obtained during this exercise to interpret the processes happening in the estuary.

4.2.1. Methods

Four preliminary sediment incubations were done using two different temperatures and sub-sampling methods with the same core in order to reduce variability between cores. A control core with *in situ* water but no sediment was also incubated, with the objective of checking whether biological activity in the water column would cause any changes in the concentrations of nitrous oxide. All incubations were done in the dark to avoid biofilm development on the core sides. Furthermore, a study in the Colne Estuary showed no significant differences between the rates of N_2O production under dark and light conditions (Dong et al., 2002).

Once in the laboratory, the plexiglass cylinder was kept vertical, and secured by a frame especially built for these experiments (Figure 4-1). This frame had a fixed plastic boot on its PVC base, to keep the core from sliding sideways. In the PVC top plate of the frame there was a threaded hole holding the threaded rod used to push the top piston downwards as water was removed during the experiment. Also attached to the frame was the motor responsible for rotating the stirring mechanism (Figure 4-1).

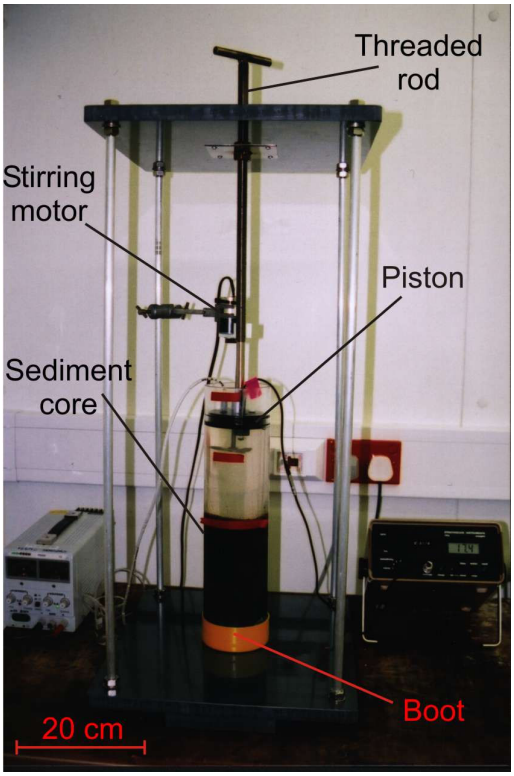

Figure 4-1. Picture of the sediment core on the incubation frame.

At the start of the experiment (time zero), water samples were taken with a plastic syringe for analyses of initial concentration of N_2O and dissolved nutrients. All the analyses were performed according to the methods described in Chapter 2. The core was then sealed with an air-tight PVC piston.

The piston had two luer fittings, a place for the oxygen probe and a place for the stirring rod (Figure 4-2). The luers allowed water samples to be taken or added to the cylinder during the experiment without the need to open it.

The oxygen electrode attached to the core top was a Clark-type polarographic electrode, with a 22 micron diameter platinum cathode and silver/silver chloride anode, connected by a buffered potassium chloride electrolyte solution (Strathkelvin Instruments). Large tip diameter electrodes like this (tip diameter larger than 5 micron) are known to be affected by stirring. In order to obtain reliable oxygen values the stirrer was switched off at each sub-sampling time and oxygen readings taken when values had stabilised. This type of electrode is also affected by temperature variations and needs to be used at a controlled temperature. Calibration of the electrode was done at the beginning and at the end of each experiment, by taking triplicate water samples and measuring oxygen concentrations by Winkler titration (Strickland and Parsons, 1972).

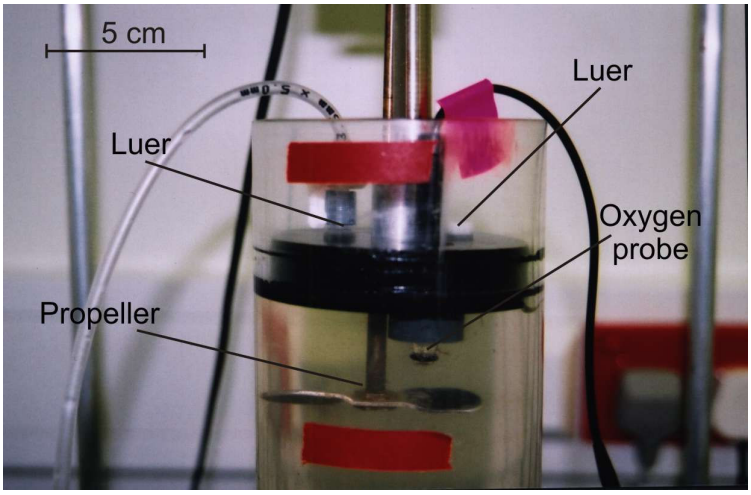

Figure 4-2. Picture of the piston and its attachments.

The propeller attached to the stirring rod was kept about 10 cm above the sediment and was set to 50 rpm. Previous tests proved this rotation to be sufficient to fully mix a water column as deep as 20 cm without disturbing the surface of the bottom sediment. Stirring was used in all incubation experiments to avoid the build up of diffusive concentration gradient that could affect the solute fluxes from sediment to water and all associated processes.

Two different sub-sampling methods (*V* and *F*) were tested. In method *V* the sample would run out by one of the luers, through PVC tubing, once the piston was slid downwards, pushed by the threaded bar. As a result, the

volume of overlying water was changed at every sampling time. In method *F*, a syringe containing 70ml of water of known nutrients and N_2O concentration was connected to one of the luers. At the sampling time, this water was injected into the cylinder, pushing a sample of the water column out, through the PVC tubing connected to the second luer. As a result, the volume of overlying water was kept constant.

In both methods, sub-samples were taken every two hours over six hour experiments (four samples in total). As the same sediment core was used in all incubations during this exercise, the overlying water was siphoned out after each incubation experiment, replaced with *in situ* water (which was kept under aeration in the laboratory) and the core allowed to re-equilibrate overnight.

All the experiments took place in a controlled temperature room, and each incubation setting (Table 4-1) was repeated to test the reproducibility of the method.

Incubation number	Core	Temperature (°C)	Sampling method
S20V	Sediment and water	20	Method *V* - changing volume
S20F	Sediment and water	20	Method *F* - fixed volume
S10V	Sediment and water	10	Method *V* - changing volume
S10F	Sediment and water	10	Method *F* - fixed volume
W20V	Water	20	Method *V* - changing volume

Table 4-1. Conditions used on each incubation. The incubation number represents these conditions as follows: first digit indicates if sediment and water (S) or only water (W) was incubated; next digits indicate the temperature, 10°C or 20°C (10 or 20), in which the incubation system was maintained; and the final digit indicates the sub-sampling method used, if changing volume (V) or fixed volume (F).

The sediment core was also incubated at two different temperatures, 10°C and 20°C. The rationale for testing the incubations at different temperatures is that the production of nitrous oxide is biologically driven, and consequently is likely to vary according to the temperature. The temperatures used in these tests were chosen based on previous studies in the area of Southampton Water (Collins and Ansell, 2000), showing that temperatures generally vary annually from 5°C to 24°C. Additionally, biological processes typically double with a 10°C increase in temperature within a certain range (Grant, 1986), and therefore a significant difference in system response was expected between these two temperatures.

4.2.2. Results and discussion

Overall, the sediment incubations showed an increase in the nitrous oxide concentration in the water column within the 6 hour experiment (Figure 4-3), with the exception of the control "core" (containing only water), which presented very little variation. This suggests that most changes in nitrous oxide concentration in the sediment incubations were due to benthic activity. Insignificant nitrous oxide production in the water column was also found in the Tamar estuary (Law et al., 1992), and no evidence of nitrous oxide production within the water column was reported in the Colne estuary and the Swale-Ouse river system (Dong et al., 2002; Garcia-Ruiz et al., 1999; Ogilvie et al., 1997).

With respect to the sub-sampling methods used, method *V* showed better comparability between replicates than method *F.* Figure 4-4 shows more clearly that in the incubations using sub-sampling method *V* it is possible to detect a higher increase in nitrous oxide concentrations within time, in experiments conducted at 20°C than at 10°C. In contrast, the same is not observed in incubations using sub-sampling method *F*. A probable cause could be that the water sub-sampled by method *F* may be a mixture of the incubated water and the water introduced into the cylinder during the sub-sampling process. This would lower the N_2O concentration in the sub-sample

as the water introduced into the cylinder had a N_2O concentration similar to the water incubated at T0.

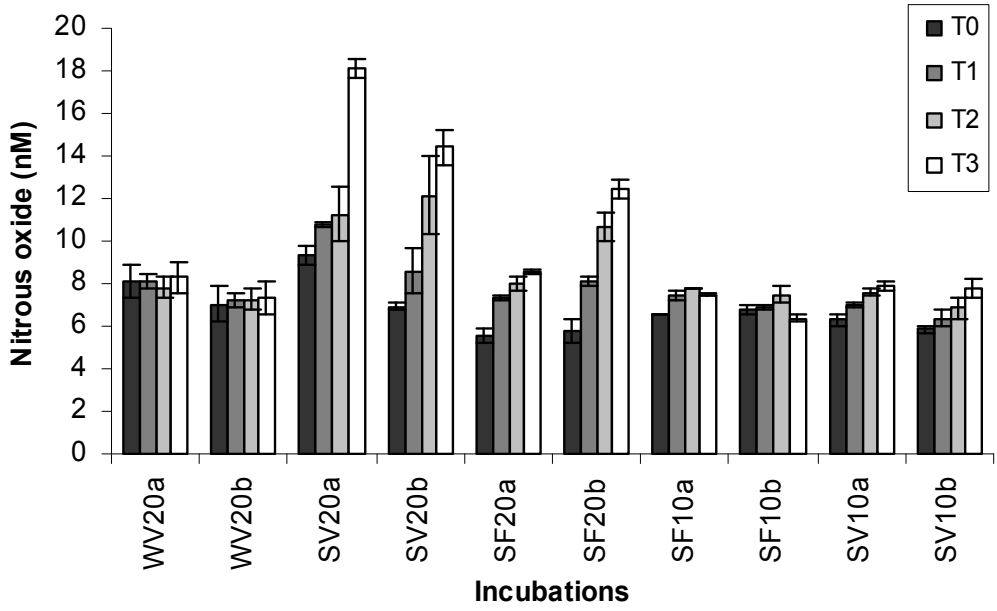

Figure 4-3. Changes in nitrous oxide concentration (nM) at each incubation experiment. Letters a and b at the end of each incubation number represent replicates. T0 indicates concentration at the beginning of the incubation; T1, within 2 hours; T2, 4 hours and T3, 6 hours.

Biological activity, mainly by Polychaetes and small mussels, was observed (but not quantified) in the top layer of the sediment during all incubations done at 20°C. Polychaetes were dead within two days from the beginning of the incubation experiments, but the small mussels were still active at the end of all experiments. These observations suggest that bioturbation could also have affected the reproducibility of the incubations as the fluxes from sediment to water may vary according to the macrobenthos activity. Many studies (Gilbert et al., 1998; Meyer et al., 2001; Svensson et al., 2000) have shown that bioturbation by benthic macrofauna can drastically affect the total area of the sediment/water interface and thereby significantly stimulate *in situ* sediment denitrification. In addition, patches of lighter colour sediment were observed within some areas of this very dark coloured sediment core. This observation indicates the existence of more oxygenated

micro-zones at depth, probably caused by biological and physical disturbance, and thus making the sediment core heterogeneous.

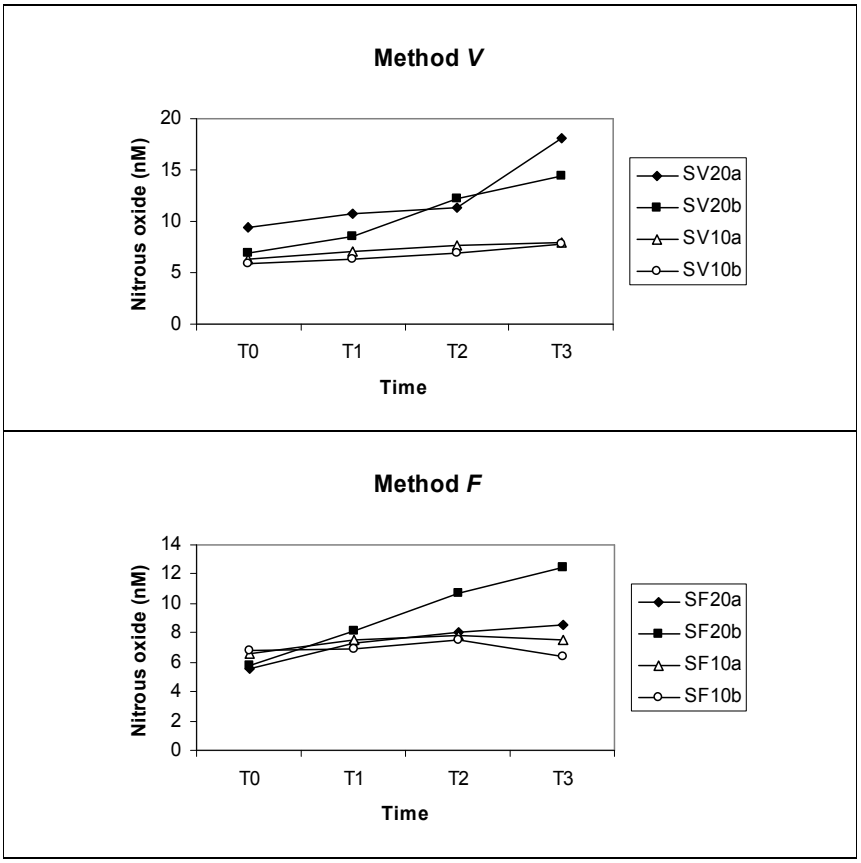

Figure 4-4. Nitrous oxide concentrations in water sub-sampled by methods *V* and *F*. Black filled markers represent incubations at 20°C and open marks, 10°C.

Higher nitrous oxide production was observed in incubations conducted at 20°C compared to 10°C. This was expected and it is in accordance with other authors, who show that lowering incubation temperatures from 22 to 4°C resulted in about 77% decrease in the N_2O production rates (Pfenning and McMahon, 1997).

Concentrations of nitrite, nitrate and oxygen were also measured during this preliminary experiment. Taking incubations SV20A and SV20B as an example (Appendix P), it is possible to observe that the concentrations of these variables differ at the beginning of each experiment (time 0). This is due to different concentrations in the *in situ* water, which was collected at the beginning of each incubation.

Nitrous oxide showed an inverse relationship with oxygen, nitrate and nitrite concentrations, suggesting that denitrification is taking place. As the control "core" (experiment containing only water) did not show any significant change in the nitrous oxide concentration during the six hours experiment, it is reasonable to assume that the denitrification process in incubations SV20A and SV20B is taking place in the sediment.

Significant correlations were also found between nitrous oxide, nitrate, nitrite and oxygen (Table 4-2). This is in agreement with a previous chapter (chapter 2), where nitrous oxide was measured in the surface waters of the Itchen Estuary.

	SV20A	SV20B
Nitrate	r=-0.88, P=0.05	r=-0.99, P=0.001
Nitrite	r=-0.93, P=0.02	r=-0.96, P=0.04
Oxygen	r=-0.86, P=0.06	r=-0.98, P=0.004

Table 4-2. Correlations between Nitrous oxide, nitrate, nitrite and oxygen, using data from incubation experiments SV20A and SV20B.

The correlations above further support the idea of denitrification as a source of nitrous oxide in the incubated cores. Denitrification as a source of nitrous oxide in sediment core incubations was also detected by other authors (Dong et al., 2006; Laverman et al., 2007; Trimmer et al., 2006). Trimmer et al. (2006), when studying the limits of a methodology for measuring anammox and denitrification in intact cores, found denitrification being the only significant source of $^{15}N\text{-}N_2O$ from $^{15}NO_3^-$.

4.2.3. Summary

Sub-sampling method *F* was shown to be disadvantageous and a more complex flow through system would be needed to guarantee that only incubation water would be sampled. Method *V* showed good reproducibility and practicality, and should be used in the final experiments.

Bioturbation and physical disturbance observed in the experiments indicate that the heterogeneity of the sediment core could cause variations in the intensity of the nitrous oxide production process, and also make it difficult to compare two or more intact cores. Therefore, the use of homogeneous sediment cores would be advantageous as more detailed information on the nitrous oxide production is intended to be achieved.

Incubations at 20°C showed an increase of nitrous oxide concentrations in the water column relative to 10°C after a period of 6 hours, suggesting that incubations at this temperature will offer a higher probability of finding measurable nitrous oxide concentrations in the sediment porewater.

4.3. Incubating the homogeneous sieved sediment core

In order to eliminate the effect of bioturbation in the sediment cores to be incubated and maximise the chances of good reproducibility, the sediment used in these final incubations was initially sieved, thus eliminating any macro-organisms and large particles. This procedure is believed to be appropriate as the main objective of these incubations is not to try to reproduce the *in situ* production of N_2O in the Itchen Estuary, but to get a better understanding of the process producing nitrous oxide.

4.3.1. Preparation of the homogeneous sediment

Two buckets of sediment were collected from the Itchen Estuary, in the same location as described for the previous experiments, and brought to the laboratory for wet sieving. A 500 μm mesh sieve was used to separate macro-organisms and large particles from the sediment to be used in the incubations.

After sieving, the sediment was left undisturbed in the controlled temperature laboratory (at 20°C) for a week. This time was necessary to

enable the very fine suspended particles to settle and excess overlying water to evaporate, leaving only about 2 cm of water on the sediment surface.

The sieved sediment was then divided into six plexiglass cylinders. The reason for using six cylinders is that some of the analyses were destructive to the sediment core, so six cores were needed to allow replication. Each cylinder was filled up to 20 cm with sediment and 10 cm of overlying *in situ* water. These sediment cores were then allowed to re-equilibrate over 18 days to have their redox layer re-established. This was observed to occur by the development of a dark coloured layer in the subsurface of the sediment, and was stable after the 18 days.

Once the redox layer was re-established, the sieved sediment cores were considered ready to start the measurements that were performed in the water column, sediment and porewater (Table 4-3). This type of approach has been successfully used by Soares (1998).

4.3.2. Physical and chemical characteristics of the sediment

The organic carbon and total nitrogen content of the sediment were analysed in sediment samples taken from cores A and C. Ten samples were taken from each core, one at every 0.5 cm depth interval (up to 5 cm depht). Samples were dried at 60°C, ground to homogenise the sample, and treated with hydrochloric acid to eliminate carbonates. After successive washing to remove the acid, samples were dried to constant weight and sub-samples were taken for organic carbon and total nitrogen analysis following a modified version of the Verardo et al. (1990) method. A Carlo Erba elemental analyser, calibrated with sulphanilamide as a standard (51.78% C, 20.14% N), was used for these analyses.

Measurements		Cores					
		A	B	C	D	E	F
Sediment	Organic carbon and total nitrogen content	T		T			
	Porosity			T			T
Water column	N_2O and nutrient analysis	T	T				
	O_2 measurements	T	T				
Porewater	Nutrient analysis	T	T				
	N_2O analysis				T	T	
	N_2O and O_2 electrochemical measurements				T	T	

Table 4-3. Measurements done in the sieved sediment cores.

Porosity analyses were done using the top 5 cm of cores C and F. These cores were sub-sectioned and a known volume of sediment collected at every 0.5 cm depth interval. Each sediment section was then weighed and dried at 60°C to a constant weight. The sediment porosity (*n*) was calculated using the following equation (Bennett and Lambert, 1971):

$$n = \frac{W_w}{V_{ws}} \times 100$$

Where: W_w = weight loss on drying (g);

V_{ws} = volume of wet sediment (cm^3)

4.3.2.1. Results and discussion

The organic carbon and total nitrogen content of sieved sediment samples (% dry weight) are shown in Figure 4-5. Vertical distribution of organic carbon and total nitrogen contents of the upper 5 cm sediment did not show a significant variation. The average organic C and total nitrogen content were 3% and 0.4%, respectively (standard deviation = 0.4 and 0.05) for both cores.

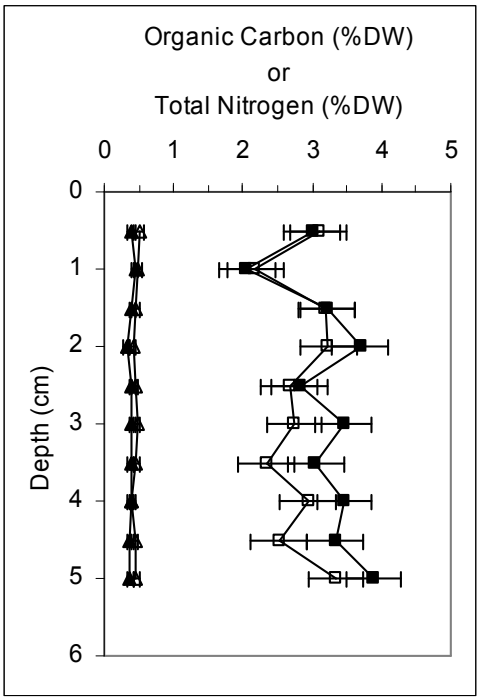

Figure 4-5. Vertical distribution of organic carbon (square symbols) and total nitrogen (triangle symbols) in the sediment cores A (open symbols) and C (filled symbols). Concentrations represent % of dry weight. Bars show ± 1 standard deviation (n=10).

This result was expected as the sediment incubated in these cores was intentionally homogenised. Interesting though, is the fact that this behaviour is also observed in intact cores. Denis and Grenz (2003) found no vertical variation in organic carbon and total nitrogen contents of surficial sediments from the Gulf of Lions (Mediterranean) up to 7 cm depth.

The C/N ratios of the Itchen sediment ranged from 5:1 to 12:1. Overall, C/N ratios were slightly lower for core A than core B, as concentrations of organic carbon and total nitrogen in the sediment were also lower (Table 4-4). The C/N ratio obtained suggests that the organic material in the estuarine sediment is relatively new. The average C/N ratio of plankton is 7:1 (Redfield et al., 1963), and tends to increase with time, after deposition. Consequently, sediments that contain organic material with C/N ratios close to 7:1 indicate recent deposition (Byers et al., 1978), as would be expected from a surficial estuarine sediment.

Depth (cm)	Organic Carbon (% DW)		Total Nitrogen (% DW)		C/N ratio	
	Core A	Core C	Core A	Core C	Core A	Core C
0 – 0.5	3.09	3	0.51	0.38	6	8
0.5 – 1.0	2.18	2.06	0.48	0.44	5	5
1.0 – 1.5	3.2	3.22	0.45	0.4	7	8
1.5 – 2.0	3.23	3.69	0.41	0.32	8	12
2.0 – 2.5	2.67	2.82	0.44	0.4	6	7
2.5 – 3.0	2.74	3.45	0.47	0.4	6	9
3.0 – 3.5	2.34	3.05	0.46	0.38	5	8
3.5 – 4.0	2.94	3.46	0.4	0.38	7	9
4.0 – 4.5	2.52	3.33	0.44	0.37	6	9
4.5 – 5.0	3.34	3.88	0.46	0.37	7	11
Mean	2.8	3.2	0.5	0.4	6	9

Table 4-4. Averaged measurements from replicates of organic carbon and total nitrogen in percentage of dry weight (%DW) in the top 5 cm of sediment from cores A and C.

Vertical variation in the porosity of the top 5 cm of sediment from cores C and F showed relatively small variation with depth (Figure 4-6). Porosity values ranged from 69% and 61% (cores C and F respectively) in the upper 0.5 cm, to 94% and 95% (at 2.5 cm depth for core A, and 3.5 cm for core F).

The differences observed between the porosity profiles of cores A and C are likely to be the result of practical difficulties during the vertical sectioning of the cores. Porewater is likely to drain from the upper-most section, thereby resulting in an underestimate of porosity. This may be the reason for the lower porosity values observed at the top 0.5 cm of sediment. Porewater also adheres to any utensils (e.g. spatulas) used during the sectioning, further compromising the porosity measurement, especially when handling thin depth intervals like 0.5 cm.

An average porosity of 74% and 85% (for cores C and F, respectively) may be assumed for the top 5 cm depth of sediment. This is consistent with porosity values (ranging from 76% to 90%) for surface sediment (grain size < 500 μm) from many coastal areas (Denis and Grenz, 2003; Traykovski and Geyer, 2004).

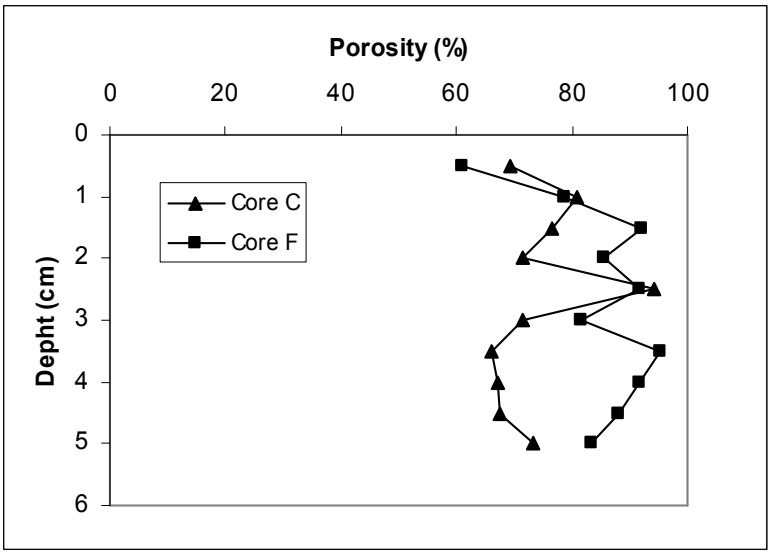

Figure 4-6. Porosity (%) profile for cores C and F.

4.3.3. Water column measurements, analyses and fluxes

The six hour incubation experiments previously described in this chapter (section 4.2) were now repeated using the sieved sediment cores A and B. These two cores were incubated exactly under the same conditions with the objective of replicating the experiment, and thus checking the consistency of the data acquired.

Water sub-samples taken from the closed experimental system were analysed for nutrients and nitrous oxide concentrations. Oxygen was measured at every sampling time. All details pertinent to analytical and incubation methods can be found in Chapters 2 and 4 (section 4.2), respectively.

Fluxes from sediment to overlying water were determined by applying the change in overlying water concentrations within time to the volume of overlying water in the incubation core. Corrections were made for the volume change in the water column at every sampling time, despite the small sample volume (0.09 litres) compared to the overlying water volume (1.24 litres).

4.3.3.1. Results and discussion

Oxygen concentrations ranged from 100 to 180 μM (i.e. from 42 to 77% saturation) and generally decreased with time in both cores A and B (Figure 4-7). Oxygen fluxes ranged from -1100 to -3000 $\mu mol.m^{-2}.h^{-1}$ (negative fluxes mean oxygen going into the sediment) and generally decreased within time, in both cores A and B (r^2=0.99 and 0.97, respectively; Figure 4-7). These oxygen uptakes from the water column were expected as the cores were incubated in the dark and reflect bacterial respiration in the sediment. Similar incubations done with intact cores collected from the River Colne estuary have shown fluxes of the same magnitude (Dong et al., 2000).

Average nitrate concentrations in the water column were 76 μM (± 1.6) for core A and 59 μM (± 0.4) for core B (Figure 4-7). Core A also had higher concentrations than core B for nitrite (80 μM ± 2.5 and 34 μM ± 0.8, respectively), ammonium (37 μM ± 2.5 and 11 μM ± 1.5, respectively), phosphate (14 μM ± 1.8 and 4 μM ± 0.3, respectively) and silicate (82 μM ± 2.6 and 63 μM ± 1.6, respectively). This behaviour suggests that core A was biologically more active than core B. A possible reason is that core A was poured first when the six cores were prepared. In this way core A may have higher quantities of lower density organic particles than the other cores, and so it could be more bio-reactive.

Changes in the nitrate concentrations in core A linearly increased within time (r^2=0.90). The same did not happen in core B, where a decrease in the nitrate concentration was observed in the last sample taken (at time = 6 hours). Also in core B, the change in nitrate concentration between T1 and T2 (i.e., between 2 and 4 hours of incubation) was lower than the analytical variability, and thus interpreted as zero flux. Nitrate fluxes ranged from 0.2 to 0.03 $mmol.m^{-2}.h^{-1}$ in core A, and from 0.06 to -0.07 $mmol.m^{-2}.h^{-1}$ in core B. Fluxes were generally directed from the sediment to the overlying water and decreased within time in both cores (Figure 4-7). However, for both nitrate and nitrite the overall changes observed were small over the time period examined.

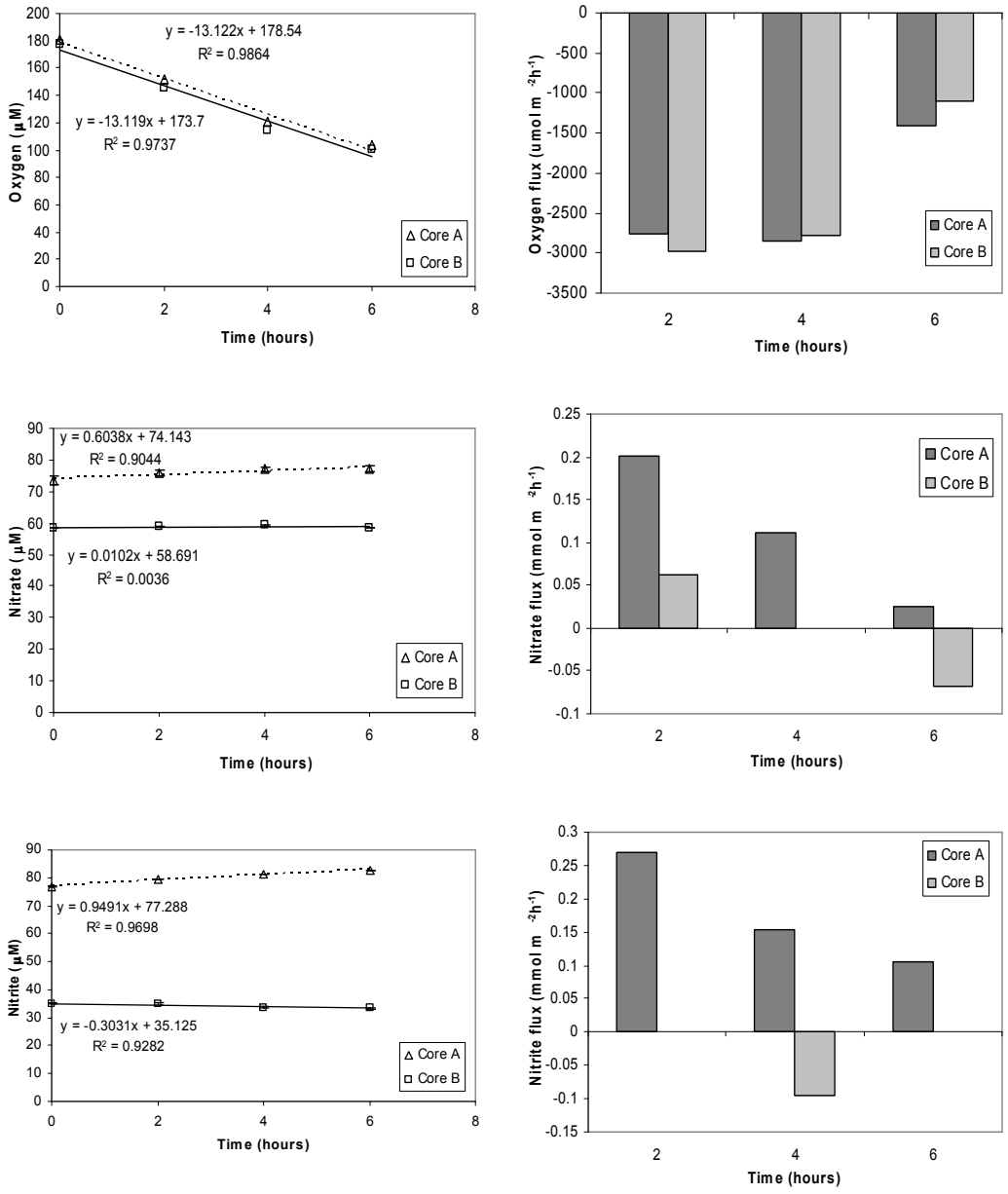

Figure 4-7. Oxygen (top), nitrate (middle) and nitrite (bottom) changes in the overlying water of cores A and B during the six hour incubation experiment (left side panels). Error bars show ± 1 standard deviation (n=3). Linear regressions ($P<0.05$) are also shown. Oxygen (top), nitrate (middle) and nitrite (bottom) fluxes measured in cores A and B during the six hour incubation experiment (right side panels).

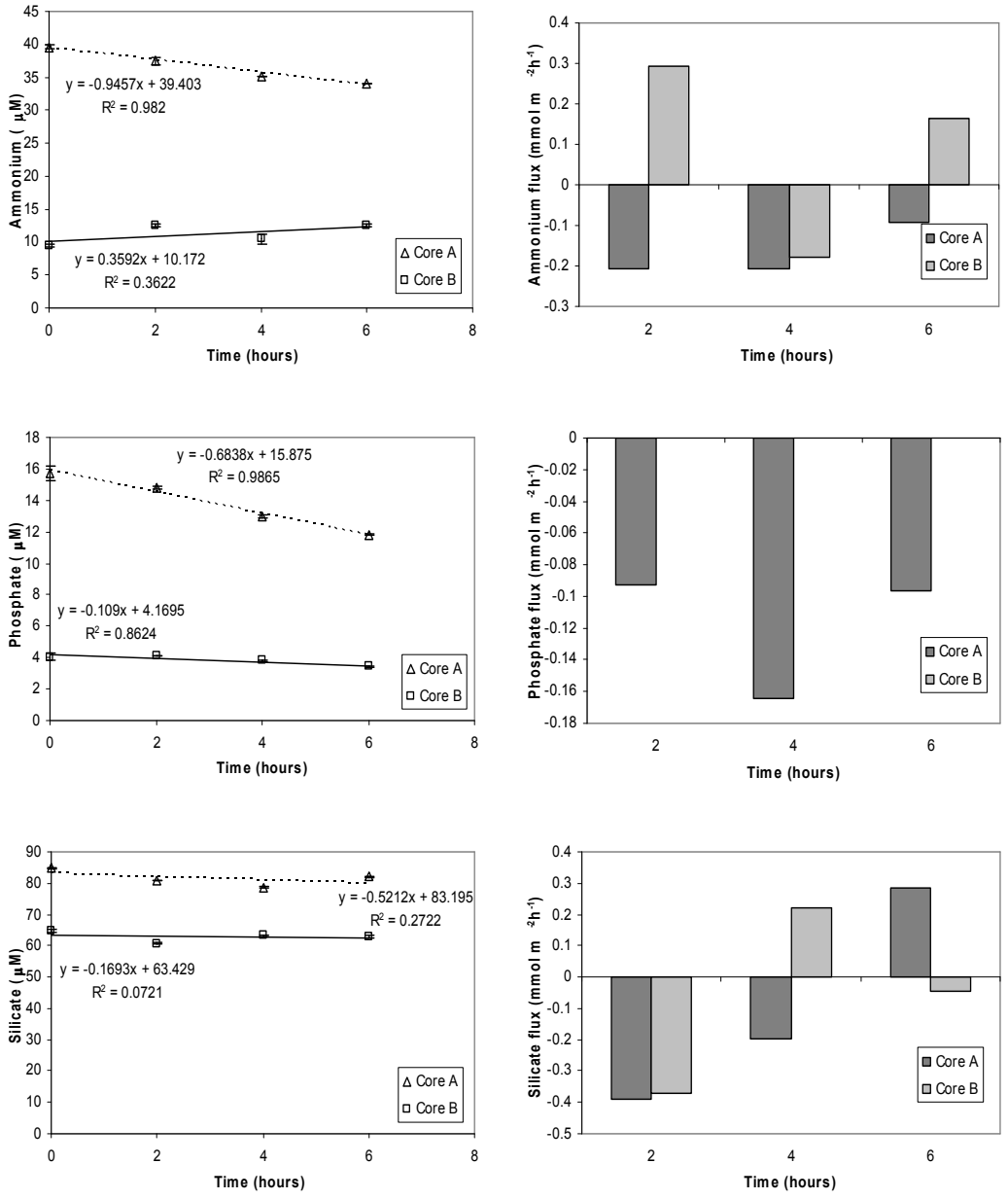

Figure 4-7 (continued). Ammonium (top), phosphate (middle) and silicate (bottom) changes in the overlying water of cores A and B during the six hour incubation experiment (left side panels). Error bars show ± 1 standard deviation (n=3). Linear regressions (P<0.05) are also shown. Ammonium (top), phosphate (middle) and silicate (bottom) fluxes measured in cores A and B during the six hour incubation experiment (right side panels).

Nitrite concentrations increased with time in core A (r^2=0.97) and decreased in core B (r^2=0.93). As a result, nitrite fluxes had opposite directions in core A (from sediment to water) and core B (from water to

sediment). Fluxes also decreased within time in core A, ranging from 0.3 to 0.1 mmol.m^{-2}.h^{-1} . In core B, changes in nitrite concentrations between T0 and T1, and T2 and T3 were too small to be measured, and zero flux was assumed. The only flux calculated for core B was -0.1 mmol.m^{-2}.h^{-1} , between 2 and 4 hours of incubation (Figure 4-7).

Concentrations of ammonium in the water column decreased linearly with time in core A (r^2=0.98), but not in core B (r^2=0.36). Ammonium fluxes in core A were from water to sediment, and decreased within time, from -0.2 to -0.1 mmol.m^{-2}.h^{-1}. A different behaviour was observed in core B (Figure 4-7), with positive fluxes within two and six hours of incubation, and a negative flux within 4 hours of incubation (fluxes ranging from 0.3 to -0.2 mmol.m^{-2}.h^{-1}).

Phosphate concentrations decreased with time in cores A and B. Changes in concentration in core B were too small to be considered, and zero flux was assumed for the six hour incubation period. Phosphate fluxes in core A were from water to sediment, increasing within time during the first four hours and than decreasing. Core A fluxes ranged from -0.2 to -0.1 mmol.m^{-2}.h^{-1} (Figure 4-7).

Concentrations of silicate in the overlying water generally decreased with time in both cores, but not linearly (r^2=0.27 and 0.07 for cores A and B, respectively). Silicate fluxes directed towards the sediment decreased withi time in core A during the first four hours, and then reversed towards the water. Fluxes ranged from -0.4 to 0.3 mmol.m^{-2}.h^{-1}. In core B, silicate fluxes were negative during the first two hours, becoming positive in the next two hours and negative again in the last two hours of the incubation experiment. Fluxes ranged from -0.4 to 0.2 mmol.m^{-2}.h^{-1} in core B (Figure 4-7).

Nitrous oxide concentrations in the overlying water from core A (average = 4700 nM $\pm$ 540) were very much higher than from core B (average = 23 nM $\pm$ 3; Figure 4-8). Nitrous oxide fluxes in core A ranged from -4 to -81 μmol.m^{-2}.h^{-1} and were always from the water to the sediment (Figure 4-8). In core B, nitrous oxide flux was positive in the first two hours (2.6 μmol.m^{-2}.h^{-1}) decreasing to -0.4 μmol.m^{-2}.h^{-1} in the following four hours.

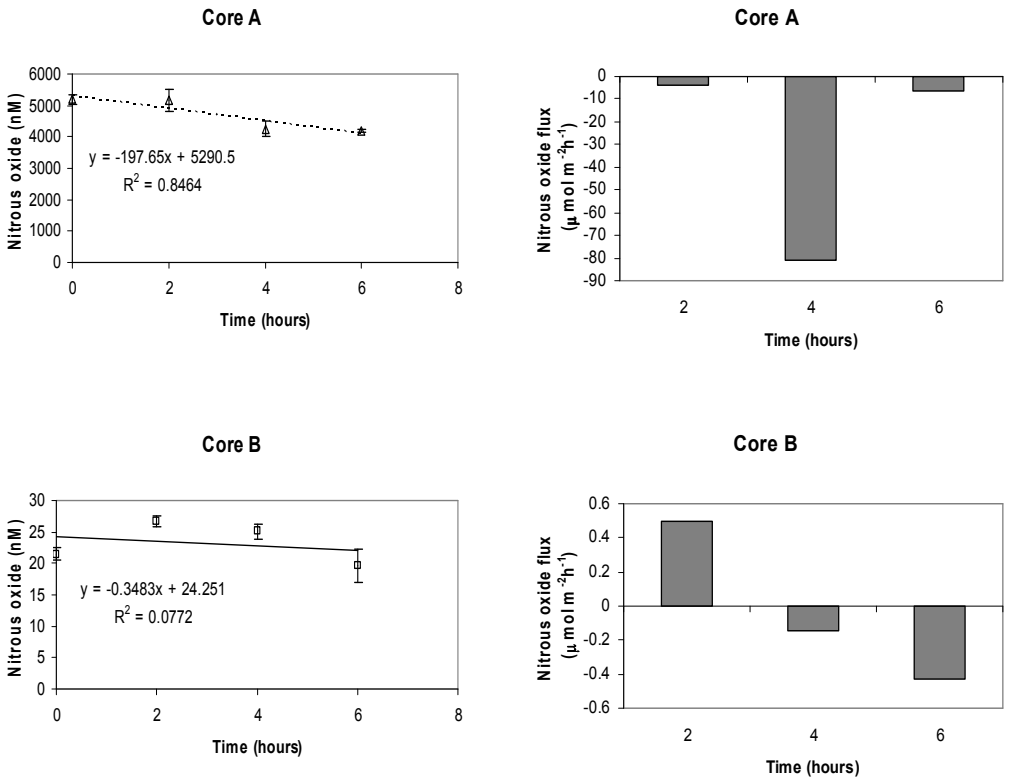

Figure 4-8. Nitrous oxide changes in the overlying water of cores A (top) and B (bottom), during the six hour incubation experiment (left side panels). Error bars show ± 1 standard deviation (n=3). Nitrous oxide fluxes measured in cores A (top) and B (bottom) during the six hour incubation experiment (right side panels).

There was a positive correlation between nitrous oxide flux and phosphate flux in both cores A and B (r=0.99). In core B, nitrous oxide flux also showed a positive correlation with nitrate (r=0.92) and a negative correlation with oxygen (r=-0.79) and silicate (r=-0.70).

The fact that these incubation experiments were totally closed may have affected the dynamics of the solute fluxes between water and sediment, as they needed to adjust for changes in the overlying water volume and concentrations. Fluxes calculated for the two hour intervals between T1-T2 and T2–T3 (between 2 and 4, and 4 and 6 hours, respectively) may not show a totally re-established new dynamic of solute fluxes between water and sediment. The fluxes calculated for the first two hours of this incubation experiments (T0-T1) possibly are the most realistic of the three fluxes calculated.

4.3.4. Pore water

After the six hour incubation experiment cores A and B were opened and, after about two hours, sectioned for porewater extraction. Water column measurements were made before the core was extruded for slicing. The ten sections (each of them 0.5 cm deep) were sliced in a nitrogen filled glove bag, containing Anaerocult-A (Merck) sachets, which were used to remove any residual oxygen. Core sections were transferred into 250 ml tubes under nitrogen and centrifuged for 30 minutes at 3000 rpm in a refrigerated centrifuge at 20°C. The supernatant was taken up in plastic syringes and filtered through 0.45 μm filters under nitrogen. Samples were used for nutrient analyses.

Electrochemical measurements of oxygen and nitrous oxide in the porewater (cores D and E) were made using microsensors (OX500 and N2O25, respectively, both from Unisense). These sensors are Clark-type electrodes with a built-in reference and guard cathode. The outer tip diameter of the oxygen sensor was about 50 μm ($40 < \phi < 60$), and the nitrous oxide sensor was about 35 μm ($20 < \phi < 50$). The electrodes were connected to a high sensitivity picoammeter and inserted into the sediment by a hand driven manipulator. Oxygen and nitrous oxide concentrations were measured at 1 mm depth interval until 5 mm, and then at 5 mm depth interval until 50 mm depth. Calibration of the oxygen electrode was performed using sodium ascorbate to obtain an oxygen free solution, and well aerated water (obtained by vigorous bubbling during 5 minutes) to obtain a solution 100% saturated with oxygen. Calibration of the nitrous oxide sensor was made in a calibration chamber, bubbling N_2O in the water during 5 minutes, to obtain a solution 99% saturated with N_2O; and bubbling nitrogen to obtain a nitrous oxide free solution. Both sensors respond linearly, and, consequently, a 2 point calibration is suggested by the manufacturer. The nitrous oxide sensor has a detection limit of 0.1 μM.

After the electrochemical measurements, cores D and E were sub-sampled using a mini-core, made out of a cut off syringe. The sub-sampled

core (5 cm long and internal diameter of 1.2 cm) was extruded at 5 mm depth intervals into vials containing 10 ml of high purity water, and immediately closed with a butyl rubber stopper. The vials were shaken to make a slurry, and nitrous oxide was measured in the headspace as previously described in Chapter 2. High purity water and air samples were also analysed to permit the back-calculation of nitrous oxide concentration in the porewater.

4.3.4.1. Results and discussion

Two profiles of oxygen concentration were measured in each core (D and E) and had good reproducibility. Oxygen concentrations in the water column (5 mm above the sediment) were 174 and 168 μM in cores D and E, respectively. Oxygen concentrations in the porewater were exhausted within the top 2 millimetres (Figure 4-9).

Nutrient analyses were made using cores A and B, as previously mentioned. Nutrient concentrations presented here are different from previous values (showed in Section 4.3.3.1) as they represent the situation about two hours after T3 in the incubation experiment.

Nitrate concentrations in the water column (5 mm above the sediment surface) were 104 μM (± 0.8) and 54 μM (± 0.3) in cores A and B, respectively. Concentrations then dropped dramatically in the porewater samples from the top 5 mm depth, and were generally kept lower than 20 μM in the porewater from deeper sediments, in both cores (Figure 4-10).

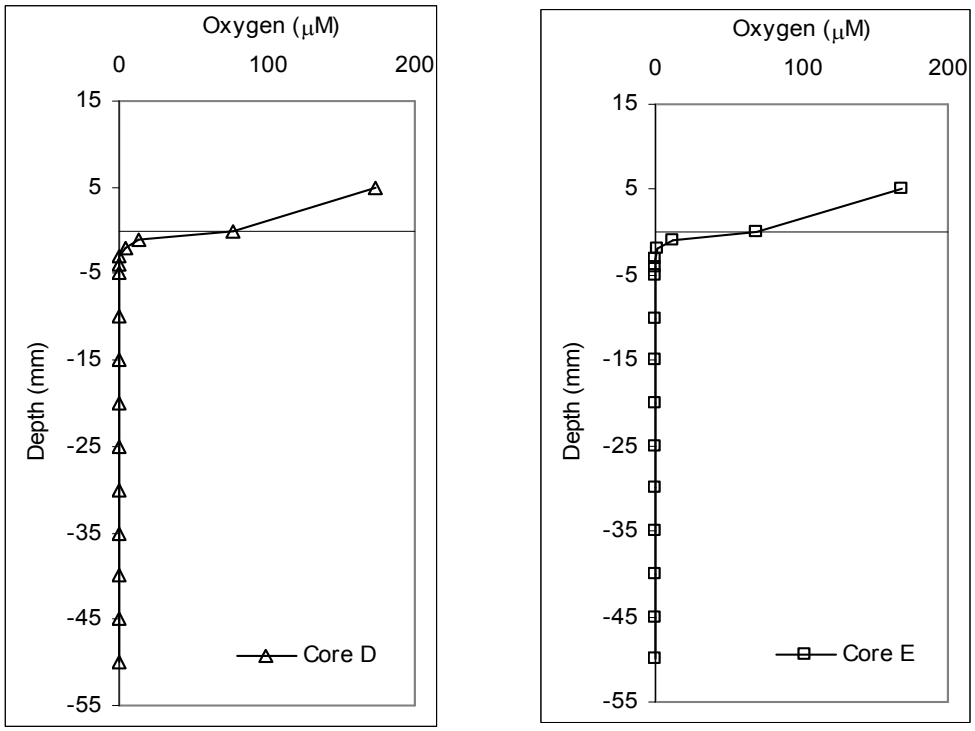

Figure 4-9. Oxygen profiles in cores D and E. Values are average of 2 measurements.

Similar behaviour was observed in the nitrite profile. Concentrations of nitrite in the water column were 164 μM (± 0.4) and 28 μM (± 0.2) in cores A and B, respectively. Concentrations decreased sharply in the porewater samples from the top 5 mm depth, and were generally lower than 10 μM in the porewater from deeper sediments, in both cores (Figure 4-10).

Opposite behaviour was observed in the ammonium profiles, which showed undetectable concentrations in the water column and increasing porewater concentrations within depth. Porewater concentrations of ammonium in core A ranged from 150 to 1300 μM; and in core B, from 50 to 960 μM (Figure 4-10).

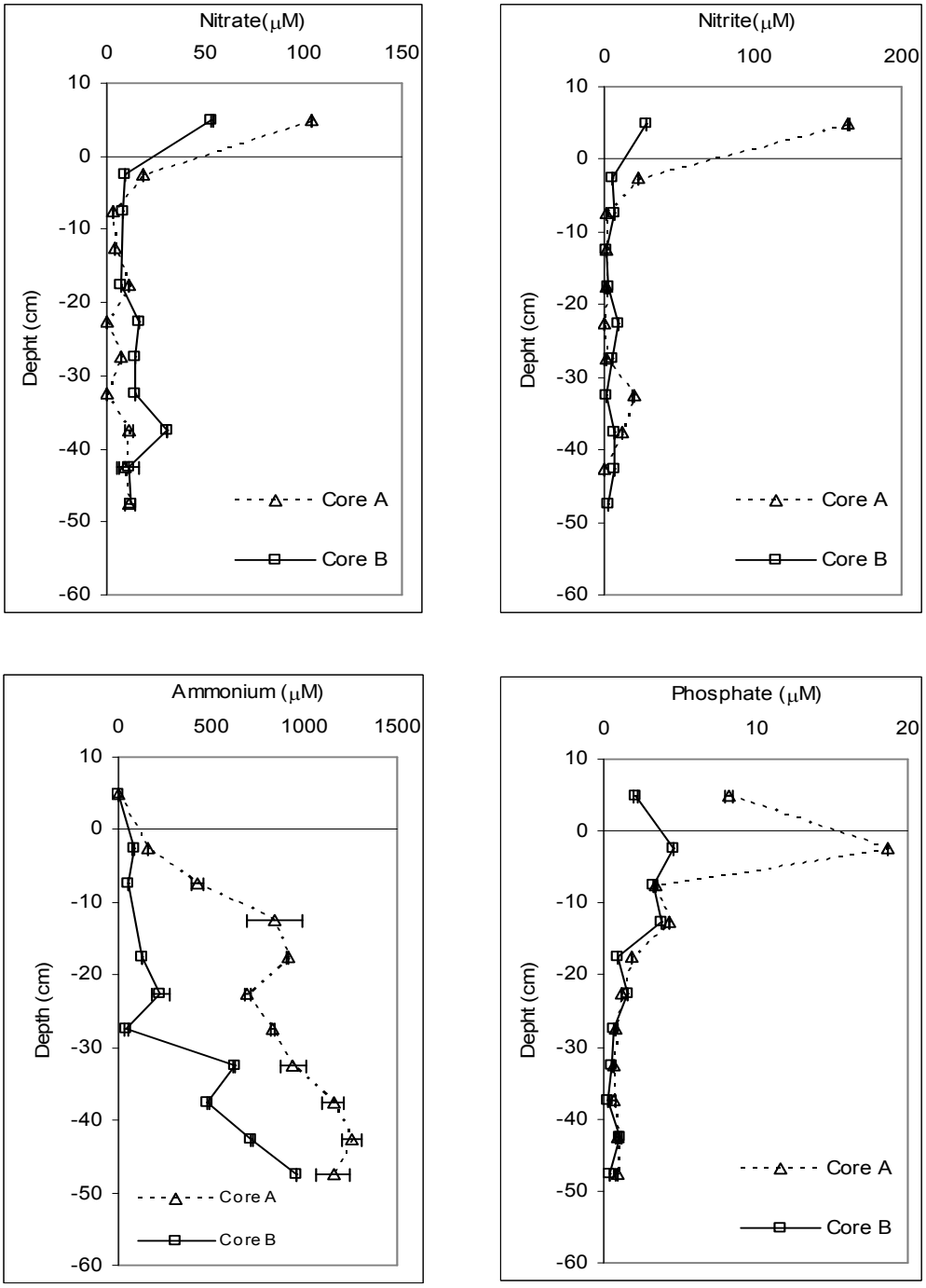

Figure 4-10. Nutrient concentrations in the porewater of cores A and B. Error bars show ± 1 standard deviation (n=3).

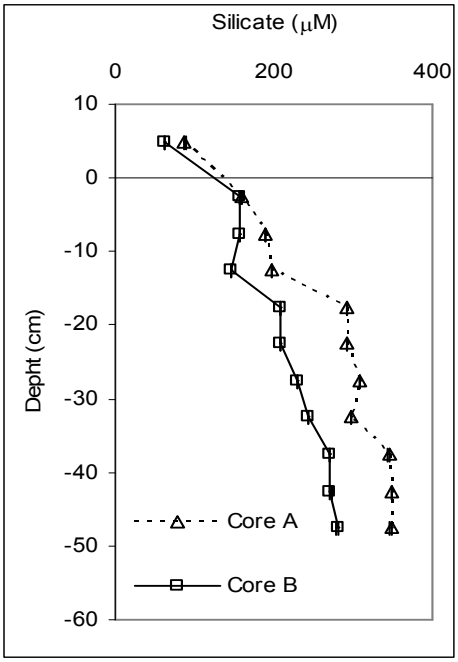

Figure 4-10 (continued). Nutrient concentrations in the porewater of cores A and B. Error bars show ± 1 standard deviation (n=3).

The nitrate and nitrite concentration gradients across the sediment-water interface indicated net removal of these nutrients from the water column into the sediment. This flux was already observed in core B (besides fluxes were small; Section 4.3.3.1) and it was expected, as estuarine sediments are generally reported as sinks for nitrate (Jorgensen and Sorensen, 1985).

In addition, the decreasing nitrate and nitrite concentrations within the top 7 mm of sediment indicate two possibilities: the diffusion of the higher concentrations from the water column into the sediment, and also the removal of nitrate and nitrite below the oxic zone (below the top 2 mm, as indicated by the oxygen profile).

The sequential occurrence of redox processes in sediments supports the argument of nitrate and nitrite removal below the oxic zone. In anoxic conditions, any oxidised molecule, such as nitrate and nitrite, may be utilised as alternative electron acceptors to oxygen, and reduced by anaerobic bacteria. The sequence of electron acceptors used in organic matter decomposition (O_2, NO_3^- + NO_2^-, Mn, Fe, SO_4^{-2}) can be explained by the

decreasing Gibbs Free Energy (Table 4-5) involved in these sequential redox reactions (Stumm and Morgan, 1996).

Reaction	Free energy changes ($kJ.mol^{-1}$ CH_2O)
$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-475
$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3 + CO_2 + 3H_2O$	-448
$CH_2O + 3CO_2 + H_2O + 2MnO_2 \rightarrow Mn^{2+} + 4HCO_3^-$	-349
$CH_2O + 7CO_2 + 4Fe(OH)_3 \rightarrow 4Fe^{2+} + 8HCO_3^- + 3H_2O$	-114
$CH_2O + SO_4^{2-} \rightarrow 2H_2S + 2HCO_3^-$	-77

Table 4-5. Sequence of electron acceptors used in the organic matter decomposition and corresponding free energy changes (adapted from Berner, 1981).

Nitrification is also possible in the oxic sediment layer, but it was not possible to measure peaks of nitrate or nitrite within the top 2 mm of oxic sediment, as the resolution for nutrient data was 5 mm.

The increase in ammonium concentration with depth is consistent with the anoxic conditions of the sediment. The lack of dissolved oxygen makes it impossible for it to be oxidized to nitrite or nitrate, resulting in the build up of the concentrations observed. In addition, a linear correlation between the ammonium concentration profile and depth was observed (r^2=0.85 and 0.79, for cores A and B, respectively). Blackburn and Blackburn (1993) found that if the organic content of sediment was mixed evenly with depth, their modelled mineralization generated linear ammonium concentration profiles. The organic content of these homogenized sediment cores are reasonably evenly distributed with depth, as shown by the organic carbon and total nitrogen contents of the sediment cores A and C.

Phosphate concentrations in the overlying water of cores A and B were 8 and 2 μM (±0.2), respectively. A peak of phosphate was observed in the porewater samples from the top 5 mm of sediment. This peak can also be explained by the lack of oxygen. Phosphate, when in oxic conditions, is

strongly adsorbed on ferric oxides. Upon removal of oxygen and reduction of iron, the phosphate is liberated to solution, increasing the concentration (Berner, 1980).

Concentrations of silicate in the overlying water were 88 and 63 μM (±0.2) in cores A and B, respectively. Porewater concentrations increased with sediment depth

Nitrous oxide concentrations in the porewater were measured by two different methods: direct electrochemical measurements in the sediment cores D and E; and by the sectioning of a sub-core sampled from cores D and E, with subsequent nitrous oxide analysis by gas chromatography (as described in Section 4.3.4).

The electrochemical measurements were attempted in two different points within the surface area of each core, and in only one case in core E (Figure 4-11) were there measurable concentrations of nitrous oxide. Nitrous oxide concentrations in the porewater measured by this method ranged from 170 to 340 nM, with the higher concentrations at the surface, and decreasing with increasing sediment depth. Measurements were made at 1 mm sediment depth intervals and concentrations became lower than the detection limit (100nM) at 6 mm depth. Nitrous oxide concentration was also measured in the overlying water, 5 mm above the sediment, and was 140 nM.

Nitrous oxide concentrations in the porewater measured by the headspace analysis of the sediment slurry showed a slightly higher concentration within the top 5 mm of sediment in core D (370 nM) than core E. Concentrations then decreased with sediment depth until about 20 mm where the lowest porewater concentration was measured (8 nM). Porewater from sediment deeper than 20 mm had concentrations ranging from 100 to 150 nM. Nitrous oxide concentration in the overlying water 10 mm above the sediment was 10 nM (Figure 4-11). However, the general pattern of N_2O concentrations from electrochemical and headspace gas chromatography analyses are very similar.

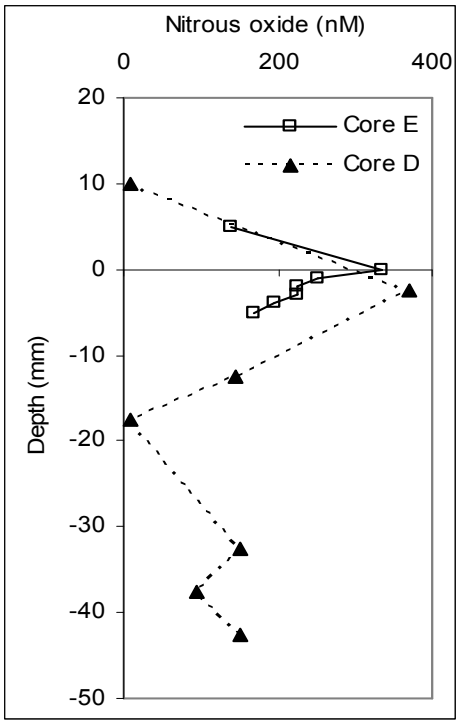

Figure 4-11. Nitrous oxide concentrations in the porewater of sediment cores D and E. Open markers indicate electrochemical measurements. Filled markers indicate headspace analysis by chromatography.

The fact that the sub-sampled core E did not reproduce the profile shown in core D is probably related to problems within the sub-sampling method and heterogeneity in the N_2O content of the sediment (as evident from the electrochemical measurements). Very small volumes of sediment are handled during this kind of sub-sampling, making it difficult to get precise and equal sample portions. Adding to that, the nitrous oxide concentrations in the slurry (which was made with this sediment plus ultra high purity water) was, in some samples, too close or lower than the detection limit, invalidating some of the data that could be used to get a better resolution of profile D.

Both measurements show that the concentration of nitrous oxide in the overlying water is lower than in the top 5 mm of sediment porewater. This concentration gradient suggests that the nitrous oxide was produced into the surface layers of sediment, diffused to the overlying water column and then was lost to the atmosphere. This result emphasises results found elsewhere, that sediments are important sources of nitrous oxide (Delwiche, 1981; Law et al., 1991; Robinson et al., 1998).

Nitrous oxide concentration profiles measured by both methods show decreasing concentrations with increasing sediment depth. The higher nitrous oxide concentrations within the top 5 mm of the sediment porewater coincide with the higher concentrations of oxygen (in the top 2 mm), and nitrate and nitrite. This suggests denitrification as the main process producing the nitrous oxide within the anoxic layer (between 2 and 5 mm of sediment depth, approximately), as nitrate and nitrite concentrations are shown to be decreasing within this sediment depth.

The high concentration of nitrous oxide in the top 2 mm of sediment may also be explained by the production of this gas by denitrification. Although denitrification is usually considered to be a strictly anaerobic process, it has been established that a number of denitrifying bacteria strains denitrify while simultaneously respiring oxygen (Jorgensen et al., 1984; Robertson and Kuenen, 1990).

It has been shown that increased concentrations of nitrate and nitrite are associated with elevated concentrations of nitrous oxide (Dong et al., 2002; Law et al., 1991; Usui et al., 2001), this may be due not only to the higher concentrations of these nutrients, but also to an inhibition of the nitrous oxide reductase caused by higher concentrations of nitrate (Blackmer and Bremner, 1978).

The combination of low oxygen conditions and high concentrations of nitrate and nitrite in the surface sediment layer seems to be controlling the production of nitrous oxide in these sediment cores. As similar conditions are not uncommon in estuaries, this illustrates the importance of estuaries as potential sources of nitrous oxide to the atmosphere.

4.5. Summary

1. The objective of producing six homogeneous sediment cores with good reproducibility was partially achieved, and most of the observed differences in the results of measurements were generally related to the sub-sampling methods used.

2. Organic carbon and total nitrogen contents of the sediment did not vary significantly with depth and indicate a relatively low organic matter content. The C/N ratio obtained suggests that the organic contents of the sediments are relatively new.

3. Porosity of the sediment did not present substantial variation with sediment depth, supporting the homogeneity of the sediment cores, and was in the expected range for surface sediment with grain size lower than 500 μm.

4. The nutrient concentrations in the overlying water column from sediment core A suggested that this core was biologically more active than core B. This was observed at the beginning of the six hour incubation experiment (time zero), indicating that the biologically led processes taking place during the 18 days prior to that (time that the sediment cores were left undisturbed to re-establish the redox conditions) were happening with different intensities in cores A and B.

5. Results from the six hour incubation experiments show relatively small fluxes and great variability of fluxes calculated for each two hour interval. Fluxes calculated for the first two hours of incubation are possibly the most representative of real fluxes.

6. Nitrous oxide fluxes from the sediment to the overlying water were calculated for core B and showed a positive correlation with nitrate and negative correlation with oxygen. This result indicates nitrous oxide production within the sediment (there was no detectable nitrous oxide production in the water only control core) and suggests that denitrification could be the process producing it.

7. Measurements done in the porewater showed that oxygen is completely exhausted within the top 2 mm of sediment, and nitrate and nitrite are significantly reduced in sediment deeper than about 7 mm. This indicates that the sediment is fully anoxic from that point.

8. Electrochemical measurements of nitrous oxide showed similar concentrations in the top 5 mm of sediment to as measured by headspace analysis.

9. Nitrous oxide concentrations were higher in the porewater of the top 5 mm of sediment, and were higher than the concentrations in the overlying water, again suggesting a nitrous oxide flux from the sediment to the water column.

10. The occurrence of higher concentrations of nitrous oxide in the same sediment layer as the consumption of nitrate and nitrite suggests that denitrification is the process producing nitrous oxide in the surface sediment of these cores.

11. Anaerobic and aerobic denitrification are suggested as nitrous oxide has been observed in both, the oxic and anoxic layers of sediment.

Chapter 5

CONCLUSIONS AND FUTURE WORK

5.1. Conclusions

Nitrous oxide concentrations in the River Itchen and Itchen Estuary waters were generally higher than the saturation concentration during the thirteen months sampled, indicating emission of nitrous oxide from the water to the atmosphere in all surveys.

Concentrations of N_2O in the river water were highest at the source, followed by a decrease in concentration within 7 km downstream. It appears the groundwater feeding the river has very high concentrations of nitrous oxide. The decrease downstream indicates rapid degassing and mixing with water containing less nitrous oxide. The data suggests the importance of the groundwater as a local source of nitrous oxide to the atmosphere.

Nitrous oxide concentrations in the estuarine water were higher in the upper estuary and decreased downstream. The high concentrations measured in samples collected close to the sewage outlet suggested input or production in this area. Production of nitrous oxide within the estuary was also suggested as concentrations in the water with salinity ranging from 10 to 28 (approximately) were higher than predicted by a theoretical dilution line.

River water was consistently supersaturated with N_2O, with values ranging from 104 to 2800%. Estuarine waters within a salinity range 0 to 28 had an average saturation of 171%. The supersaturation measured in this study in the River Itchen and the Itchen Estuary further contributes to the argument that rivers and estuaries are sources of nitrous oxide to the atmosphere.

As the Itchen Estuary is representative of temperate climate systems, we might expect that based on the data at different temperatures as presented by this work, for tropical estuaries to produce more and polar estuaries to produce less nitrous oxide than the Itchen. Additionally, on the possibility of an increase in the global temperature, presumably more nitrous oxide would be produced across the globe, thus providing a positive feedback loop and enhancing the global warming.

Estimation of nitrous oxide fluxes from the River Itchen was much higher for the river source area than for the remaining area of the river. This highlights the significance of groundwater fed rivers as a source of N_2O, and also the importance of spatial variability.

Comparison between the River Itchen and the Itchen Estuary emissions per unit of area showed that the River Itchen represents a larger source of nitrous oxide to the atmosphere than the Itchen Estuary on a per m^2 basis. However, a comparison between the River Itchen and the Itchen Estuary emissions showed that the Itchen Estuary exports more nitrous oxide to the atmosphere than the River Itchen, because of its larger surface area.

Extrapolation of the annual emission of N_2O estimated for the River Itchen, to the total area covered by rivers in the United Kingdom, gave the same magnitude of values as for the 2003 N_2O emissions estimates from the fuel combustion by manufacturing industries and construction, wastewater handling and manure management in the UK.

Extrapolation of the annual emission of N_2O estimated for the Itchen Estuary, to the total area covered by estuaries in the United Kingdom, gave the same magnitude of values as for the 2003 N_2O emissions estimates from the fugitive emissions from fuels, waste incineration, and fuel combustion by

sectors other than transport and energy, manufacturing and construction industries in the UK.

The methodology used to obtain reproducible homogeneous sediment cores must be revised as the reproducibility of the cores was only partially achieved.

Nitrous oxide fluxes between the sediment-water interface were calculated for the incubation experiments done with homogenised sediment cores. N_2O fluxes showed a positive correlation with nitrate and negative correlation with oxygen.

Measurements done in the porewater from the homogenised sediment cores showed that nitrous oxide concentrations were higher in the top 5 mm of sediment than in the overlying water. This finding suggests a nitrous oxide flux from the sediment to the water column.

Nitrate and nitrite analyses in the porewater indicated that these nutrients were consumed within the top 7 mm of sediment. The occurrence of high concentrations of nitrous oxide in the same sediment layer as the consumption of nitrate and nitrite suggests that denitrification is the process producing nitrous oxide in the surface sediment of these cores.

Aerobic and anaerobic bacterial denitrification are the most likely mechanisms for the production of nitrous oxide, as this gas was observed in both the top of the sediment, where oxygen from the overlying water column was still available, and deeper in the sediment (top 7 mm), where oxygen was scarce or totally absent.

5.2. Future work

5.2.1. Role of groundwater nitrous oxide in the global budget

The high concentrations of nitrous oxide observed in the River Itchen suggests that groundwater fed rivers are important sources of nitrous oxide and should be investigated further. The fact that higher N_2O concentrations were found in the source of the river than downstream highlights the potential

importance of the groundwater as a source of nitrous oxide to the atmosphere. Nitrous oxide should be analysed in water from wells and springs in different areas to investigate the influence of the activities in the catchment area on the formation of N_2O in the groundwater. An important point when studying these water systems is the use of a detailed sampling strategy, as degassing is quite rapid and can significantly decrease high nitrous oxide concentrations in just a few kilometres from the source.

5.2.2. Improving our knowledge of nitrous oxide fluxes from estuaries

Sediment cores could also be taken from different points in the estuary to investigate how nitrous oxide fluxes from the sediment to the water vary along the estuary. A more detailed study, like a cross section, is suggested for the upper estuary. This could help to understand if the high N_2O concentrations in that area are a result of higher fluxes from the sediment or just the input from the treated sewage discharged.

The incubation experiments done in this study were a very good approach to obtaining detailed data on the nitrous oxide concentrations in porewater, and fluxes of the gas from the sediment. Modifications to the incubation system, as indicated in Chapter 4, are suggested for further investigations.

5.2.3. Modelling of nitrous oxide production and the nitrogen cycle in sediments

The data obtained with the homogenised sediment incubations will be used in a modelling exercise, with the objective of developing a diagenetic model of nitrous oxide production. This model will investigate the role of oxygen controlling the proportioning between the production of nitrous oxide and dinitrogen gas. The aim is to calibrate the model against, primarily, oxygen and N_2O fluxes but also against nitrate and ammonium fluxes. In doing so, model validation will be achieved against porewater profiles of

nitrate, nitrous oxide and ammonium. Once this is achieved, the effect of changing the oxygen concentration in the overlying water column on N_2O fluxes will be investigated.

The modelling exercise will build up on the Kelly-Gerreyn model that is described in Kelly-Gerreyn et al., (1999).

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Appendices

Appendix A - Nutrients and nitrous oxide data in the River Itchen from November 2001 to December 2002.

Appendix B - Nutrients and nitrous oxide data in the Itchen Estuary from November 2001 to December 2002.

Appendix C - Distances between the source of the River Itchen and each sample site.

Appendix D - Range of standards used in the nutrient analyses.

Appendix E - Standard curves for nutrient analyses.

Appendix F - Nitrous oxide calibration curve.

Appendix G - Ammonium concentration in the River Itchen (full data set).

Appendix H- Correlations between nitrous oxide and nitrate concentrations in the surface water from the River Itchen.

Appendix I - Correlations between nitrous oxide and nitrite concentrations in the surface water from the River Itchen.

Appendix J - Correlations between nitrous oxide and ammonium concentrations in the surface water from the River Itchen.

Appendix K - Ammonium concentration in the Itchen Estuary (full data set).

Appendix L - Nitrous oxide concentrations in the Itchen Estuary from November 2001 to December 2002 (showing the theoretical dilution line.

Appendix M - Average N_2O concentrations and fluxes for every month studied. N_2O concentrations (nmol N_2O l^{-1}), N_2O fluxes (nmol N_2O m^{-2} h^{-1}),). * No data available as sites 1 and 2 were included in the sampling scheme only from January onwards.

Appendix N - Comparison of N_2O emissions from the River Itchen (kg $month^{-1}$) estimated by: adding the emissions calculated separately for each segment of the river (Total emission); and by using the average flux to calculate the emission for the full length of the river (Whole river). * No data available as sites 1 and 2 were included in the sampling scheme only from January onwards.

Appendix O - Wind speed data (knots) for each month sampled (time in hours). Doted line indicates the average wind for the month. Arrow shows the wind at the sampling time.

Appendix P - Concentrations of nitrate, nitrite, oxygen and nitrous oxide in the preliminary incubations SV20A and SV20B.

Appendix A

Nutrients and nitrous oxide data in the River Itchen from November 2001 to December 2002.

Nov-01

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	175.42	0.40	425.32	22.17	9.87	0.10	4.44	0.05	5.85	0.29	15.63
15	183.61	0.30	446.95	1.88	4.97	0.30	1.79	0.02	1.45	0.14	16.21
14	172.97	0.20	458.33	0.94	4.94	0.05	2.16	0.00	1.34	0.14	19.80
13	172.24	0.15	466.83	0.40	4.38	0.05	1.97	0.01	1.65	0.00	28.07
12	187.92	0.64	461.41	0.84	3.36	0.00	3.39	0.03	2.31	0.07	27.25
11	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
10	175.95	0.54	473.10	0.74	3.26	0.05	3.77	0.02	2.21	0.22	29.55
9	183.47	0.00	466.73	0.64	3.57	0.00	4.85	0.01	0.39	0.08	19.40
8	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
7	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
6	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
5	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
4	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
3	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
2	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
1	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN

Dec-01

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	201.43	0.15	453.39	1.88	8.61	0.99	0.66	0.00	0.39	0.19	15.91
15	202.69	0.15	443.03	0.89	5.15	0.14	2.78	0.00	1.73	0.13	16.73
14	195.48	0.25	448.32	0.74	5.25	0.00	3.37	0.02	0.94	0.59	20.49
13	184.35	0.35	450.10	0.30	5.13	0.04	2.77	0.03	0.66	0.33	29.40
12	201.32	0.20	443.87	1.48	4.20	0.07	2.13	0.01	0.62	0.39	26.66
11	178.96	0.25	441.18	0.15	1.13	0.11	1.66	0.01	1.45	1.06	27.38
10	188.09	0.40	444.19	2.43	3.63	0.11	2.87	0.02	1.09	0.79	29.29
9	188.44	0.10	454.30	0.10	6.03	0.04	0.75	0.00	0.33	0.00	22.86
8	173.36	3.12	444.85	0.49	2.18	0.00	2.07	0.00	6.21	0.07	27.37
7	172.76	0.49	438.20	0.20	1.34	0.03	1.33	0.41	0.53	0.00	25.68
6	175.53	0.45	444.43	0.59	2.98	0.03	2.22	0.02	15.75	0.08	35.32
5	176.79	0.25	436.94	0.69	2.07	0.01	1.43	0.01	1.45	0.00	28.94
4	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
3	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
2	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
1	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN

Jan-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	182.35	0.40	436.42	0.84	8.65	0.25	3.91	0.01	0.36	0.00	16.41
15	179.55	0.20	427.21	1.48	4.78	0.04	1.96	0.00	0.51	0.07	15.75
14	180.11	0.40	439.39	0.10	4.68	0.04	2.34	0.01	1.56	0.14	20.03
13	179.03	0.05	437.43	1.09	4.50	0.00	2.19	0.01	0.61	0.07	33.53
12	179.31	0.25	436.24	0.20	2.91	0.05	2.50	0.01	0.66	0.14	27.68
11	177.84	0.35	436.94	0.49	2.94	0.08	2.57	0.01	0.41	0.07	30.15
10	188.62	0.25	440.86	0.99	3.06	0.03	2.54	0.00	0.31	0.07	34.41
9	189.21	0.10	439.29	0.25	3.59	0.00	2.92	0.00	0.86	0.00	18.94
8	177.31	1.09	443.91	0.05	2.13	0.01	2.16	0.01	0.61	0.21	32.53
7	172.06	0.00	428.86	1.34	2.19	0.00	2.20	0.01	0.56	0.00	28.30
6	179.94	0.15	434.88	2.13	0.92	0.03	2.00	0.00	NaN	NaN	42.17
5	177.38	0.49	427.46	1.24	1.80	0.02	1.45	0.01	3.44	0.00	32.56
4	160.83	0.15	450.45	0.30	1.15	0.01	0.44	0.01	0.34	0.00	25.12
3	157.19	0.64	453.67	0.10	1.23	0.00	0.31	0.02	0.29	0.06	43.43
2	134.26	0.79	510.51	0.10	0.23	0.04	0.29	0.00	0.34	0.00	97.53
1	133.32	0.15	525.75	0.70	0.03	0.02	0.17	0.00	0.43	0.26	146.73

Feb-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	187.78	0.05	454.58	14.95	12.64	0.35	3.07	0.04	2.99	0.15	24.77
15	188.76	0.15	457.00	8.27	6.20	0.25	1.90	0.05	0.51	0.07	26.46
14	186.94	0.05	458.92	3.17	5.53	0.00	1.94	0.00	3.04	0.37	32.74
13	186.13	0.49	468.27	14.11	5.15	0.05	1.91	0.09	2.89	0.00	41.42
12	188.51	0.20	469.32	0.15	4.62	0.10	2.02	0.01	0.51	0.22	42.19
11	198.14	0.45	462.56	1.78	4.62	0.00	2.17	0.01	1.72	0.00	40.78
10	181.90	0.54	458.36	1.78	4.31	0.05	2.15	0.01	0.77	0.30	50.30
9	179.41	0.10	454.48	0.05	4.34	0.10	2.32	0.01	0.88	0.30	31.74
8	184.03	0.10	470.75	10.20	3.29	0.10	1.83	0.01	5.53	0.00	41.80
7	181.58	0.10	467.64	10.05	3.47	0.35	1.76	0.04	1.51	0.00	40.54
6	180.01	1.04	461.34	0.45	4.41	0.00	1.75	0.00	0.98	0.00	63.90
5	174.02	0.59	464.35	5.49	2.73	0.10	1.21	0.01	4.63	0.52	67.46
4	159.92	0.05	480.45	7.77	1.65	0.05	0.35	0.00	2.36	0.00	33.13
3	162.16	0.45	480.90	0.40	1.54	0.00	0.28	0.01	0.07	0.00	52.71
2	159.01	1.53	508.31	5.49	0.77	0.00	0.23	0.01	0.31	0.35	101.80
1	144.06	0.20	574.25	1.93	0.70	0.00	0.10	0.01	7.48	0.00	249.05

Apr-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	82.78	0.35	450.38	7.33	5.85	0.25	0.77	0.00	3.57	0.08	11.68
15	86.91	0.15	435.61	1.98	2.21	0.11	0.30	0.01	0.72	0.08	17.05
14	106.12	0.00	426.65	2.38	2.19	0.00	0.42	0.00	0.99	0.47	20.68
13	92.51	0.05	441.84	0.69	2.42	0.01	0.40	0.00	1.38	0.23	30.84
12	100.35	0.15	436.31	3.17	1.69	0.03	0.66	0.00	0.61	0.08	30.93
11	83.20	0.25	450.28	11.53	1.79	0.01	0.76	0.01	1.21	0.16	25.34
10	105.07	0.30	448.32	1.53	1.65	0.00	0.78	0.00	0.94	0.08	28.72
9	80.43	0.20	432.32	0.40	1.90	0.02	1.26	0.01	0.50	0.08	27.78
8	98.63	0.30	442.61	3.66	0.79	0.06	0.61	0.00	1.05	0.08	29.98
7	98.84	0.40	433.02	6.14	0.86	0.00	0.59	0.00	0.44	0.00	29.37
6	118.90	0.35	434.70	0.49	0.98	0.01	0.58	0.00	10.46	0.46	42.00
5	117.71	0.05	424.52	2.03	1.25	0.01	0.50	0.01	1.21	0.16	41.36
4	119.21	0.20	458.40	2.33	0.21	0.11	0.14	0.00	0.61	0.08	27.55
3	157.99	0.49	474.74	0.30	0.73	0.06	0.06	0.00	0.07	0.00	39.65
2	151.17	0.15	511.70	7.92	0.30	0.06	0.00	0.00	0.07	0.00	82.96
1	164.15	0.10	546.04	5.79	0.33	0.01	0.00	0.00	0.07	0.00	330.92

May-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	169.41	0.93	414.65	1.93	7.66	0.38	3.37	0.04	6.45	0.00	15.12
15	160.86	0.00	415.07	0.74	3.27	0.08	1.49	0.01	0.52	0.08	18.77
14	170.72	0.93	420.11	0.05	3.36	0.04	1.74	0.08	1.23	0.15	23.23
13	170.06	0.00	421.75	0.10	3.29	0.01	1.71	0.01	1.34	0.00	32.20
12	165.46	0.93	423.36	0.30	3.06	0.02	3.01	0.02	0.47	0.00	29.90
11	169.41	0.93	414.19	0.99	3.01	0.01	3.43	0.02	2.59	0.08	29.62
10	160.86	0.00	424.48	1.68	3.00	0.01	3.66	0.03	2.31	0.00	26.17
9	166.12	0.00	418.43	0.64	2.37	0.01	4.22	0.01	3.67	0.23	23.75
8	159.55	0.00	419.20	0.64	1.62	0.04	2.47	0.01	4.27	0.15	28.39
7	152.98	0.00	411.15	0.45	1.68	0.01	2.30	0.01	3.24	0.54	27.64
6	152.98	0.00	412.09	1.68	2.24	0.16	2.17	0.01	15.71	0.00	37.31
5	152.32	0.93	402.50	0.99	1.89	0.00	1.73	0.01	3.71	0.07	44.87
4	151.01	0.93	429.66	0.49	0.55	0.16	0.51	0.01	1.17	0.07	25.63
3	147.72	0.00	438.10	0.64	0.33	0.10	0.31	0.02	0.22	0.14	49.58
2	141.15	0.00	461.27	0.15	0.21	0.02	0.00	0.00	0.27	0.21	103.69
1	139.84	0.00	470.44	0.74	0.20	0.00	0.12	0.01	0.37	0.21	188.76

Jun-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	165.38	0.74	424.73	1.83	9.19	0.93	3.62	0.01	4.56	0.00	14.79
15	161.88	0.64	405.20	0.35	3.74	0.31	0.93	0.01	1.69	0.35	16.36
14	165.59	0.25	409.61	0.05	3.65	0.01	1.35	0.01	1.44	0.00	21.79
13	159.88	1.19	412.55	0.94	3.80	0.04	1.29	0.01	1.81	0.18	27.22
12	165.62	0.40	416.26	4.01	4.40	0.01	2.77	0.01	1.50	0.09	29.68
11	163.94	0.10	416.19	0.25	4.00	0.04	3.63	0.00	2.69	0.18	28.89
10	161.98	0.30	418.92	0.35	3.96	0.01	4.48	0.01	3.12	0.09	32.50
9	158.69	0.00	417.52	3.81	4.02	0.07	5.29	0.04	4.81	0.00	23.63
8	168.11	0.05	425.81	0.59	2.54	0.07	3.61	0.01	5.69	0.00	30.44
7	155.12	1.39	408.98	4.50	3.19	0.23	3.20	0.02	6.06	0.00	28.58
6	155.79	0.15	404.32	0.79	3.06	0.04	2.39	0.01	4.31	0.53	31.59
5	154.32	0.15	380.17	0.20	3.52	0.02	2.58	0.04	4.81	0.18	45.70
4	148.79	0.05	439.57	0.64	0.71	0.27	0.49	0.01	1.56	0.18	27.44
3	146.20	0.05	450.87	0.49	0.71	0.05	0.25	0.02	1.31	0.18	50.11
2	137.24	0.25	512.72	0.84	0.30	0.07	0.00	0.00	1.01	0.08	115.94
1	146.83	0.15	477.44	0.54	0.02	0.03	0.00	0.00	0.84	0.00	186.49

Jul-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	174.23	0.59	414.44	1.73	6.69	0.45	3.07	0.00	3.99	0.08	14.11
15	159.04	1.09	392.91	3.27	3.85	0.49	0.90	0.01	0.93	0.07	17.60
14	167.69	0.45	400.72	2.62	3.52	0.04	0.90	0.00	0.25	0.00	21.97
13	166.25	1.68	402.22	1.29	3.36	0.01	0.93	0.02	0.25	0.00	30.09
12	169.65	0.25	396.87	0.15	3.19	0.05	1.77	0.01	0.04	0.00	32.72
11	169.05	0.20	401.70	0.35	3.43	0.00	2.23	0.01	0.15	0.00	31.80
10	157.89	0.35	406.00	1.19	3.75	0.05	2.53	0.00	0.67	0.00	36.26
9	157.15	0.00	391.27	0.84	3.69	0.01	3.52	0.01	1.57	0.08	30.04
8	161.14	0.49	395.71	0.20	1.71	0.11	4.03	0.03	2.71	0.31	37.17
7	159.18	0.00	389.45	0.64	1.91	0.08	3.77	0.01	4.16	0.00	30.88
6	157.75	0.25	387.42	1.24	1.99	0.02	3.03	0.01	1.09	0.00	30.92
5	152.22	0.74	370.37	0.89	2.86	0.02	4.22	0.01	3.21	0.08	50.60
4	134.86	1.04	419.37	4.55	0.37	0.25	0.47	0.01	0.67	0.00	26.59
3	142.59	0.00	444.08	2.28	0.45	0.02	0.27	0.01	0.61	0.07	51.41
2	135.14	0.15	530.08	1.24	0.10	0.08	0.00	0.00	0.58	0.00	58.44
1	178.22	0.40	340.45	0.74	0.02	0.03	0.00	0.00	0.41	0.08	108.74

Aug-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	164.19	0.64	423.50	5.15	7.07	0.04	0.30	0.01	4.53	0.01	21.46
15	162.79	0.25	404.29	4.21	2.80	0.05	0.03	0.00	1.23	0.18	14.02
14	153.27	0.15	341.18	1.19	2.77	0.08	1.18	0.00	1.04	0.09	17.66
13	169.02	0.05	415.70	0.94	3.22	0.09	1.06	0.01	1.30	0.09	17.47
12	163.07	0.05	405.83	0.35	3.60	0.02	0.03	0.01	1.17	0.09	18.96
11	161.11	0.25	404.01	0.74	3.69	0.01	0.03	0.00	1.49	0.18	17.91
10	162.54	0.00	402.47	0.25	2.88	0.01	0.05	0.00	1.49	0.00	32.25
9	157.85	0.30	389.66	5.49	3.53	0.06	0.12	0.01	2.01	0.18	22.72
8	161.46	0.05	416.12	0.84	0.58	0.14	0.01	0.01	1.62	0.00	25.24
7	165.17	0.35	408.03	1.88	1.56	0.03	0.28	0.00	2.33	0.09	21.70
6	164.22	0.20	403.66	2.92	1.22	0.05	0.30	0.01	1.43	0.27	22.89
5	159.85	0.05	403.97	1.09	1.65	0.01	0.51	0.00	1.88	0.00	43.39
4	147.70	0.10	432.46	4.55	0.16	0.11	0.93	0.00	1.43	0.09	24.65
3	145.95	0.00	446.18	1.58	0.00	0.00	0.07	0.01	1.55	0.27	42.92
2	130.34	0.20	567.14	0.10	0.00	0.00	1.24	0.00	0.98	0.00	42.58
1	139.90	0.15	498.79	1.73	0.05	0.01	0.11	0.01	1.49	0.00	143.88

Sep-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	166.11	0.20	456.44	2.62	11.93	0.02	7.40	0.04	5.93	0.10	17.66
15	161.32	0.15	412.55	3.51	3.50	0.08	1.07	0.01	1.51	0.26	14.65
14	162.19	0.30	412.76	0.74	3.23	0.05	1.35	0.00	1.08	0.17	19.50
13	159.78	0.15	419.58	0.20	3.37	0.01	1.33	0.01	0.96	0.00	29.24
12	163.91	0.15	415.91	6.09	3.29	0.01	1.94	0.00	1.57	0.35	29.04
11	161.28	0.40	410.03	0.25	3.10	0.03	1.85	0.01	0.96	0.34	27.42
10	162.19	0.49	407.75	1.48	2.61	0.02	2.29	0.02	1.02	0.09	26.44
9	159.46	0.00	392.70	1.98	3.43	0.03	4.08	0.01	1.82	0.17	31.87
8	171.15	0.00	431.66	6.09	1.80	0.07	3.43	0.01	3.59	0.00	NaN
7	166.57	0.25	408.03	0.49	1.69	0.01	3.57	0.01	2.69	0.36	29.86
6	163.63	0.25	399.60	0.74	1.22	0.02	2.15	0.01	2.19	0.18	NaN
5	159.85	0.05	387.91	0.74	2.04	0.03	4.57	0.04	2.06	0.00	45.38
4	149.31	0.89	416.61	1.24	0.75	0.04	1.14	0.01	1.69	0.52	NaN
3	153.13	0.74	442.79	2.72	0.83	0.01	1.41	0.01	2.13	0.09	45.13
2	135.49	0.05	575.33	0.59	0.06	0.06	1.28	0.01	1.01	0.09	25.13
1	252.46	0.74	150.96	4.01	0.00	0.00	3.75	0.01	3.26	0.00	89.75

Oct-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	189.35	0.20	444.19	1.04	9.00	0.34	6.46	0.04	1.36	0.00	15.51
15	177.98	0.45	437.89	0.74	4.45	0.22	2.45	0.01	0.21	0.00	17.13
14	182.74	0.05	431.69	0.49	3.96	0.02	2.33	0.01	0.21	0.00	21.32
13	182.67	0.25	436.63	0.74	3.77	0.08	2.22	0.01	0.21	0.00	28.23
12	174.58	0.30	430.99	2.67	4.58	0.01	3.57	0.01	0.31	0.00	NaN
11	174.55	0.05	434.11	0.35	4.39	0.08	4.02	0.02	0.21	0.00	26.66
10	171.54	0.25	425.88	0.10	3.85	0.02	4.01	0.00	0.21	0.00	32.14
9	170.73	0.99	430.19	4.50	3.97	0.01	5.18	0.00	0.36	0.07	22.30
8	175.95	0.15	442.05	0.30	2.26	0.10	5.72	0.02	0.41	0.00	30.14
7	169.54	0.20	427.84	1.19	2.61	0.08	5.46	0.01	1.68	0.00	29.74
6	169.51	0.05	428.51	0.05	3.03	0.01	3.58	0.01	0.41	0.00	27.48
5	168.88	0.05	407.86	0.74	3.64	0.01	4.89	0.01	0.41	0.00	40.72
4	159.25	0.30	430.12	2.03	1.08	0.06	1.94	0.00	0.31	0.00	25.94
3	156.21	0.25	440.13	1.04	1.16	0.00	1.48	0.00	0.83	0.30	44.98
2	125.48	0.64	474.32	1.98	0.25	0.06	1.24	0.01	0.91	0.00	69.61
1	242.87	0.05	90.27	2.52	1.95	0.13	5.61	0.01	9.41	0.16	90.66

Nov-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	188.27	0.54	423.82	2.82	6.86	0.20	4.10	0.04	1.75	0.00	19.65
15	192.64	0.40	426.62	0.15	3.85	0.10	1.53	0.01	0.44	0.07	20.55
14	183.05	0.49	431.20	0.89	3.36	0.00	1.64	0.01	0.91	0.00	25.06
13	190.33	0.30	435.26	0.30	3.05	0.05	1.63	0.01	1.54	0.00	30.99
12	193.20	0.59	437.15	0.40	3.43	0.00	2.38	0.01	1.96	0.00	34.33
11	190.54	0.10	433.23	1.78	2.98	0.05	2.71	0.00	1.12	0.00	33.58
10	180.29	0.35	444.96	1.14	2.91	0.05	2.74	0.01	2.87	0.08	NaN
9	180.29	0.54	451.85	1.58	2.87	0.00	3.29	0.01	2.92	0.00	26.25
8	177.66	0.30	456.23	2.43	2.42	0.05	2.59	0.01	2.98	0.23	33.60
7	172.41	0.49	444.43	0.99	2.45	0.00	2.48	0.00	0.85	0.07	31.75
6	171.22	0.30	446.29	0.64	2.21	0.05	1.73	0.00	4.28	0.08	28.89
5	170.35	0.15	430.15	0.99	2.38	0.00	1.83	0.01	0.59	0.00	43.12
4	158.94	0.25	465.78	1.58	1.12	0.10	0.25	0.00	1.28	1.12	37.99
3	153.48	0.54	468.51	0.10	1.05	0.00	0.02	0.00	0.80	0.00	56.27
2	133.18	0.05	490.11	1.44	0.18	0.05	0.00	0.00	0.70	0.00	90.60
1	135.10	0.20	512.23	0.35	0.07	0.00	0.00	0.00	0.70	0.00	156.81

Dec-02

Sample site	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
16	198.21	0.74	414.16	3.12	6.41	0.35	3.50	0.01	5.90	0.09	23.72
15	193.27	1.09	405.93	1.19	3.29	0.30	2.02	0.00	2.58	0.08	29.84
14	196.21	0.10	413.67	3.32	3.05	0.05	2.18	0.02	3.04	0.08	35.79
13	197.12	0.79	411.57	0.54	2.87	0.00	2.18	0.01	3.10	0.00	20.52
12	200.13	0.10	413.81	0.35	2.73	0.00	2.13	0.01	2.75	0.17	24.69
11	193.80	0.35	408.70	1.04	2.77	0.05	2.30	0.01	3.75	0.75	24.27
10	194.74	0.59	412.37	3.66	2.70	0.05	2.29	0.01	3.93	0.00	26.16
9	191.14	0.45	407.40	1.88	2.66	0.00	2.45	0.02	4.88	0.00	35.54
8	188.27	0.15	406.74	0.35	1.86	0.05	1.70	0.02	3.81	0.00	38.07
7	191.31	0.00	402.57	0.10	1.75	0.00	1.70	0.01	4.76	0.00	32.25
6	184.59	0.00	397.71	0.54	1.51	0.05	1.30	0.02	2.23	0.08	26.96
5	181.76	0.54	365.33	4.26	1.61	0.00	1.30	0.01	3.81	0.00	40.76
4	167.76	0.05	378.60	3.71	1.02	0.05	0.43	0.01	1.99	0.41	34.85
3	160.58	0.10	371.00	3.37	0.70	0.00	0.24	0.00	1.42	0.08	58.38
2	146.83	0.35	382.97	1.78	0.07	0.00	0.15	0.01	1.01	0.00	96.12
1	148.51	0.15	407.16	5.79	0.04	0.05	0.05	0.01	0.73	0.08	219.94

Appendix B

Nutrients and nitrous oxide data in the Itchen Estuary from November 2001 to December 2002.

Nov-01

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
0	183.96	0.89	430.50	2.57	16.66	0.89	4.41	0.05	96.39	0.52	17.03
2	193.76	0.59	376.22	0.45	32.70	0.15	4.27	0.02	200.53	0.00	17.55
4	181.09	0.10	403.90	0.00	14.21	1.09	5.11	0.01	47.05	2.64	21.77
6	176.16	0.45	320.71	0.35	33.40	0.74	4.51	0.01	200.53	0.00	23.98
9	141.86	0.64	346.54	0.45	11.03	0.15	4.74	0.01	37.71	0.00	22.24
12	126.95	0.25	311.78	0.20	9.84	0.15	4.36	0.01	36.18	0.25	21.39
15	110.99	0.84	271.01	0.35	8.68	0.10	3.94	0.02	30.91	0.24	21.09
18	110.32	0.49	225.05	0.59	8.09	0.15	3.46	0.01	28.24	0.00	19.13
20	99.19	0.79	202.55	0.15	7.25	0.05	3.19	0.02	26.70	0.36	18.54
21	75.11	0.20	191.24	0.49	6.83	0.05	3.10	0.00	25.97	0.69	19.30
23	78.79	0.15	162.40	0.30	6.13	0.05	2.80	0.01	24.84	0.00	18.36
26	49.70	0.00	125.09	0.20	4.83	0.10	2.25	0.01	23.55	0.00	15.10
28	38.99	0.00	96.67	0.40	3.71	0.10	1.84	0.01	18.97	0.22	11.88
30	25.62	0.10	65.24	0.30	2.80	0.10	1.32	0.00	18.97	0.22	8.50
32	31.15	0.20	42.63	0.20	1.68	0.10	0.94	0.01	12.45	0.00	9.36
34	5.46	0.20	19.25	0.00	0.88	0.05	0.45	0.01	3.87	0.00	6.67

Dec-01

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
1	182.39	0.35	462.39	2.92	9.52	0.30	3.91	0.01	24.73	0.69	19.84
3	182.56	0.30	436.28	1.83	11.31	0.05	3.91	0.00	40.92	0.51	22.69
4	167.34	0.45	414.47	1.58	14.04	0.05	4.16	0.01	68.89	0.00	24.74
6	158.73	0.35	385.53	1.04	16.91	0.05	4.46	0.01	91.26	0.30	21.35
9	146.69	0.05	358.23	0.05	14.56	0.00	4.05	0.01	82.62	0.30	22.44
11	134.16	0.25	333.20	0.99	12.95	0.10	3.82	0.01	65.24	1.14	21.91
12	123.80	0.05	313.67	0.30	10.89	0.05	3.73	0.01	48.21	0.00	20.36
14	132.48	1.04	287.39	0.64	9.77	0.05	3.48	0.00	45.26	0.52	20.97
16	127.79	0.45	262.99	0.40	9.45	0.00	3.28	0.01	46.73	0.00	21.86
18	92.30	0.05	231.70	0.49	8.30	0.05	3.05	0.01	39.14	0.00	17.86
20	91.07	0.20	212.49	0.64	7.46	0.05	2.83	0.01	36.68	0.49	19.71
22	73.02	0.08	179.51	0.83	6.21	0.13	2.59	0.00	28.50	0.00	13.30
24	61.56	0.17	152.54	0.02	5.42	0.02	2.31	0.00	24.90	0.47	16.93
26	50.67	0.04	125.10	0.17	4.47	0.04	1.98	0.00	24.01	0.00	15.24
28	57.71	0.02	102.38	0.28	3.68	0.02	1.67	0.00	20.71	0.23	14.85
30	29.88	0.09	67.91	0.11	2.61	0.08	1.38	0.01	16.51	0.00	7.14
32	20.09	0.00	45.44	0.16	1.61	0.01	1.09	0.01	11.37	0.21	10.89

Jan-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
1	182.18	0.25	429.38	1.19	9.14	0.05	3.51	0.02	14.51	0.21	15.10
1	181.16	0.00	395.92	0.30	18.20	0.30	4.62	0.02	96.76	0.70	16.61
4	172.24	0.25	377.09	0.59	11.34	0.10	4.03	0.01	42.97	0.51	17.80
5	172.34	0.10	361.24	0.15	10.12	0.05	3.90	0.01	35.29	0.49	13.73
7	150.75	0.05	345.17	0.79	9.87	0.10	3.86	0.01	33.74	0.24	14.51
9	138.53	0.69	319.59	0.25	9.00	0.05	3.64	0.01	31.54	0.48	16.40
13	127.75	0.10	275.14	1.73	7.49	0.00	3.22	0.01	25.60	0.00	15.87
14	125.69	0.15	252.07	0.10	7.25	0.05	3.10	0.01	25.60	0.00	14.92
16	102.41	0.79	233.80	0.59	6.83	0.05	2.84	0.01	24.15	0.23	14.15
18	93.10	0.49	212.66	0.10	6.23	0.10	2.63	0.03	23.18	0.23	14.13
20	83.86	0.00	188.37	0.49	5.60	0.10	2.37	0.01	21.60	0.22	12.68
22	71.84	0.02	154.64	0.23	4.91	0.06	2.02	0.01	19.40	0.22	14.84
24	58.44	0.13	128.79	0.30	4.41	0.04	1.68	0.00	19.09	0.22	10.78
26	61.34	0.15	111.50	0.40	3.89	0.02	1.40	0.03	16.84	0.22	11.23
29	36.29	0.06	75.41	0.32	2.42	0.06	0.99	0.01	11.73	0.21	10.25

Feb-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
0	191.77	0.25	426.34	2.43	6.09	0.20	2.34	0.00	7.42	0.25	14.43
0	186.97	0.69	425.57	0.15	7.11	0.05	2.55	0.01	14.17	0.25	14.85
2	177.63	0.15	395.22	0.49	9.07	0.05	2.66	0.00	46.13	1.26	14.86
5	155.68	0.20	367.96	0.64	8.72	0.05	2.39	0.00	42.40	0.00	14.20
7	156.63	0.25	343.42	0.49	7.74	0.05	2.46	0.01	34.06	0.25	15.66
8	148.68	0.10	328.79	0.69	7.39	0.05	2.38	0.01	33.17	0.50	14.26
10	144.03	0.25	306.78	0.74	6.93	0.10	2.26	0.00	29.62	0.00	14.39
12	119.42	0.40	288.23	0.05	6.44	0.00	2.27	0.01	25.35	0.50	13.32
15	105.42	0.10	251.76	0.74	5.60	0.00	2.09	0.01	22.87	0.50	12.69
18	90.27	0.35	219.52	0.40	4.83	0.00	1.93	0.01	20.20	0.75	13.01
21	77.79	0.42	173.81	1.21	4.40	0.06	1.73	0.00	17.54	0.00	12.05
24	61.11	0.13	138.68	0.15	3.66	0.00	1.44	0.00	14.70	0.00	13.06
26	69.35	0.32	114.92	0.62	3.08	0.02	1.24	0.01	12.57	0.00	11.22
28	42.29	0.06	95.40	0.76	2.51	0.02	1.07	0.01	10.79	0.50	10.79
29	47.91	0.14	68.14	0.24	2.04	0.03	0.83	0.01	9.02	0.00	9.33
32	35.25	0.06	44.64	0.10	1.23	0.04	0.61	0.00	4.82	0.00	8.91

Apr-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
0	82.71	0.25	458.29	4.65	6.76	0.35	1.17	0.01	5.20	0.89	14.75
2	141.16	0.35	237.23	0.89	76.88	2.43	2.48	0.01	983.22	6.70	22.24
3	77.00	0.00	422.35	2.52	10.71	2.57	1.19	0.01	19.41	4.68	16.25
7	70.14	0.00	386.33	1.29	10.40	0.05	1.46	0.00	24.40	0.48	19.27
4	74.66	0.25	411.50	3.71	10.71	0.10	1.51	0.01	22.38	0.47	17.48
9	63.14	0.10	349.62	1.73	9.03	0.00	1.29	0.01	22.05	0.00	18.31
18	42.95	0.05	227.71	2.18	6.34	0.15	0.89	0.00	18.23	0.23	17.05
11	61.67	0.10	318.19	1.34	9.52	0.20	1.19	0.00	21.55	0.24	19.21
13	55.97	0.05	291.48	0.99	7.88	0.05	1.06	0.01	22.89	0.24	17.54
16	47.67	0.20	260.19	1.88	7.53	0.15	0.95	0.01	20.55	0.24	18.17
20	37.31	0.00	203.35	0.10	5.74	0.10	0.75	0.01	20.55	1.18	13.44
22	32.67	0.00	165.83	0.28	4.70	0.15	0.62	0.01	19.55	0.70	11.68
24	25.73	0.15	140.93	0.87	4.08	0.00	0.52	0.00	18.40	0.00	13.58
27	17.21	0.02	100.37	0.02	3.36	0.04	0.36	0.00	22.38	0.00	10.28
30	11.30	0.11	71.19	0.04	2.57	0.06	0.26	0.00	15.40	0.30	5.76

May-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
0	159.05	0.19	384.48	1.73	8.68	0.40	5.62	0.06	32.32	0.80	17.58
0	172.64	0.42	315.98	0.49	40.78	1.04	24.15	0.04	448.78	4.68	36.40
2	150.01	0.23	359.59	0.20	9.80	0.79	5.90	0.01	41.64	1.53	20.34
5	148.89	0.14	342.83	0.35	7.84	0.10	7.19	0.02	29.81	0.39	19.89
8	132.37	0.23	304.47	0.74	6.51	0.00	6.73	0.02	25.76	0.37	19.58
6	135.76	0.09	330.09	0.94	7.18	0.05	7.02	0.01	27.36	0.38	21.36
16	84.08	0.05	217.32	1.83	5.15	0.05	3.60	0.01	23.68	0.36	19.10
10	115.50	0.09	284.59	0.05	6.23	0.10	4.48	0.00	24.45	0.73	19.82
14	92.96	0.05	236.01	0.25	5.39	0.00	3.80	0.01	21.90	0.00	17.62
18	76.15	0.19	197.09	0.54	4.45	0.05	3.26	0.01	24.58	0.55	17.09
20	67.49	0.05	175.21	0.49	3.50	0.20	3.26	0.03	23.93	0.00	15.89
22	62.09	0.14	141.80	0.57	4.59	0.13	3.00	0.00	21.90	0.71	14.01
25	39.74	0.00	108.98	0.53	2.93	0.06	1.87	0.01	16.33	0.33	11.36
28	25.34	0.02	78.06	0.64	1.83	0.04	1.35	0.01	9.38	0.20	10.53
30	13.72	0.01	46.78	0.01	0.96	0.02	0.75	0.53	6.35	0.19	8.39
32	7.27	0.01	33.43	0.04	0.58	0.01	0.57	0.40	2.57	0.19	7.60

Jun-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
0	172.56	0.18	436.37	0.28	8.13	0.40	3.96	0.02	6.30	0.59	16.22
4	194.48	0.11	114.75	2.55	133.29	0.43	29.26	0.04	314.15	4.31	53.78
2	176.84	0.05	396.97	0.46	13.96	5.23	4.07	0.08	12.53	0.00	17.12
9	130.80	0.41	306.72	0.56	11.01	0.22	4.04	0.03	38.52	0.35	20.79
4	172.40	0.14	370.20	3.15	14.03	0.28	5.19	0.01	61.85	0.42	21.33
11	135.50	0.50	284.27	0.28	10.43	0.13	3.98	0.00	41.75	0.71	21.81
7	151.98	0.68	326.22	2.78	10.99	0.15	4.41	0.01	44.82	0.00	21.09
15	98.76	2.00	235.77	0.56	7.40	0.20	2.76	0.00	19.63	0.00	14.44
17	90.29	0.05	215.48	0.74	6.28	0.13	2.62	0.02	17.39	0.00	16.26
19	73.90	0.00	182.04	0.65	3.56	0.42	2.25	0.01	14.04	0.24	12.60
22	60.57	0.04	150.78	0.44	4.39	0.11	1.92	0.01	11.39	0.23	14.70
24	51.67	0.25	134.09	1.75	3.94	0.01	1.74	0.01	9.66	0.00	11.66
26	35.69	0.10	95.60	0.56	3.41	0.09	1.29	0.00	9.36	0.00	6.25
29	18.02	0.02	62.72	0.57	2.08	0.86	0.89	0.03	4.28	0.00	6.52
30	12.21	0.07	44.22	0.37	0.93	0.08	0.68	0.01	13.94	0.71	6.00
32	6.50	0.04	27.46	0.14	0.30	0.06	0.45	0.00	1.50	0.30	6.11

Jul-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
1	164.28	0.14	424.33	4.54	12.41	0.52	3.41	0.00	2.00	1.45	14.99
5	196.50	2.55	103.58	3.50	108.89	0.43	3.71	0.00	298.90	0.00	27.12
3	155.17	0.55	383.56	2.96	18.41	1.43	3.33	0.01	27.92	0.00	12.75
5	146.32	0.23	354.69	0.09	19.90	0.01	3.37	0.01	47.50	0.26	12.18
6	140.36	0.00	322.30	0.37	24.15	0.00	3.22	0.01	73.37	0.27	15.31
8	129.12	0.05	311.37	0.28	17.40	0.34	3.23	0.01	41.02	1.04	15.27
11	114.47	0.18	279.16	0.09	13.56	0.04	2.89	0.01	25.95	0.76	20.13
12	109.42	0.23	270.72	0.37	12.77	0.10	2.79	0.01	22.38	0.75	18.66
14	100.82	1.18	248.99	2.22	24.81	0.33	2.57	0.01	21.32	0.75	17.15
16	92.29	0.14	224.38	0.19	24.36	0.02	2.45	0.01	20.08	0.50	16.48
20	74.31	0.04	177.73	1.19	22.11	0.07	2.02	0.01	17.78	0.25	15.95
19	83.41	0.02	199.05	0.32	22.61	0.01	2.18	0.00	18.31	0.00	16.29
24	56.46	0.23	129.80	0.75	19.90	0.10	1.62	0.01	15.32	0.25	14.37
22	69.28	0.14	153.83	0.32	20.63	0.05	1.85	0.03	15.50	0.00	15.67
26	45.20	0.02	104.07	0.08	18.45	0.10	1.39	0.00	24.71	0.00	14.69
28	36.84	0.04	76.92	0.38	16.93	0.09	1.15	0.00	5.60	0.32	6.25
30	28.90	0.03	54.20	0.32	15.60	0.10	0.92	0.01	2.27	0.63	6.69
33	14.97	0.04	20.15	0.28	14.79	0.11	0.55	0.01	0.00	0.00	4.48

Aug-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
0	181.48	0.74	420.28	3.46	5.74	0.40	6.66	0.03	16.02	0.21	16.17
1	171.82	0.05	676.03	17.77	26.23	1.73	4.92	0.08	439.73	15.33	24.10
3	164.99	0.00	374.33	3.81	16.77	0.74	6.00	0.84	192.68	0.00	21.53
4	153.62	0.15	386.82	3.46	14.35	0.10	NaN	0.06	192.68	0.00	20.91
7	156.45	0.10	346.47	2.23	12.50	0.15	5.06	0.11	79.49	0.93	21.17
9	140.28	0.20	354.87	0.05	11.73	0.05	6.83	0.02	65.72	0.58	22.27
13	113.75	0.79	268.91	1.83	9.07	0.05	4.31	0.02	44.67	1.04	22.48
11	124.95	0.20	304.43	0.49	9.31	0.00	4.62	0.00	48.77	0.00	23.04
18	90.23	0.00	219.07	1.63	6.93	0.10	5.78	0.03	30.89	0.48	22.32
16	102.31	0.05	278.67	4.55	8.19	0.00	6.38	0.03	36.75	0.00	22.88
20	83.62	0.45	199.61	5.00	4.24	0.35	3.21	0.00	25.42	0.23	21.85
23	63.62	0.23	155.34	0.93	4.46	0.11	2.87	0.02	19.54	0.44	19.72
25	54.72	0.17	126.02	1.42	4.40	0.02	4.38	0.01	16.78	0.00	16.24
28	43.91	0.02	92.49	1.87	2.54	0.06	1.69	0.01	13.19	0.00	14.64
31	27.27	0.06	47.61	0.45	1.82	0.04	4.95	0.01	17.94	0.00	10.81
32	24.37	0.06	35.69	0.23	0.76	0.05	1.35	0.00	8.99	0.25	10.22

Sep-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
0	175.56	0.20	461.34	2.72	10.15	0.30	8.75	0.09	6.41	0.37	10.59
NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN
3	179.55	0.30	415.10	1.19	15.19	2.08	7.71	0.01	42.13	1.83	19.83
5	177.38	0.10	319.24	1.93	32.94	0.00	6.31	0.02	461.03	11.87	20.48
7	180.64	0.05	279.41	5.00	35.99	0.86	5.61	0.03	436.26	94.57	21.15
9	158.03	0.45	236.15	2.62	42.94	3.73	5.03	0.01	419.21	0.00	22.79
18	92.86	0.54	225.79	0.25	10.85	0.89	5.13	0.01	41.22	3.12	22.20
12	120.96	0.20	308.74	0.74	11.73	0.05	6.72	0.01	45.30	0.00	22.97
16	106.37	0.05	263.27	0.49	9.70	0.05	5.87	0.01	35.27	0.75	21.20
20	80.47	0.15	207.41	3.96	7.32	0.05	4.72	0.01	35.44	0.00	22.13
13	117.25	0.10	299.08	1.44	10.12	0.05	6.48	0.00	36.15	0.00	20.63
22	70.70	0.06	171.21	0.98	6.30	0.08	4.14	0.01	44.92	0.53	15.88
24	59.85	0.04	140.73	1.10	6.17	0.02	3.56	0.01	28.58	0.47	14.29
26	53.21	0.11	121.61	0.23	5.82	0.04	3.27	0.03	27.25	0.00	17.47
28	39.62	0.19	89.27	1.21	3.59	0.11	2.56	0.01	19.31	0.00	15.33
30	29.06	0.13	63.15	0.86	2.68	0.15	1.93	0.01	15.26	0.21	8.14
33	15.57	0.01	29.69	0.45	0.78	0.07	1.15	0.00	9.50	0.20	NaN

Oct-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
32	94.17	0.13	111.69	0.68	4.02	0.04	1.15	0.00	4.33	0.25	8.15
29	51.66	0.04	70.04	0.06	6.18	0.08	2.62	0.02	52.71	0.77	10.94
27	46.20	0.00	94.82	0.23	3.93	0.08	2.90	0.01	20.42	0.91	12.23
25	58.67	0.06	129.09	1.78	5.19	0.04	3.72	0.06	22.14	0.31	NaN
23	73.82	0.23	159.02	0.53	6.21	0.04	NaN	0.08	13.79	0.28	15.31
19	88.03	0.35	204.30	7.18	7.67	0.35	5.27	0.03	30.25	0.33	19.82
21	81.41	0.10	185.22	0.10	6.65	0.00	4.92	0.01	27.26	0.00	18.33
7	146.37	0.20	362.46	2.38	12.57	0.25	8.35	0.02	36.22	0.00	21.86
18	97.86	0.40	229.25	3.17	7.60	0.54	7.14	0.03	26.81	0.00	19.67
9	137.17	0.05	338.63	1.04	11.41	0.30	7.78	0.01	32.37	0.00	21.55
12	122.36	0.10	303.17	3.46	10.57	0.00	7.06	0.03	29.56	0.00	20.68
15	110.15	0.15	261.84	1.34	9.07	0.05	6.46	0.02	30.49	1.98	19.96
5	153.62	0.54	381.22	0.40	14.81	0.25	9.48	0.01	50.55	0.00	22.04
4	163.00	0.15	412.13	2.82	18.31	0.54	11.29	0.06	65.09	0.00	23.27
1	175.00	0.00	431.31	3.91	24.00	0.54	15.83	0.02	86.91	0.00	23.61
0	175.11	0.25	456.61	1.78	12.53	0.30	7.85	0.01	5.35	0.00	18.52
1	174.27	0.35	450.94	4.75	17.68	0.05	12.57	0.03	52.12	0.74	20.72

Nov-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
1	198.17	0.40	348.11	2.08	6.16	0.30	3.17	0.01	6.01	0.22	21.68
5	174.27	0.35	244.41	0.35	42.95	1.34	25.68	0.08	269.92	0.00	78.55
3	175.32	0.15	303.87	0.99	19.32	0.69	10.64	0.03	85.43	0.40	44.68
5	167.83	0.15	286.16	0.99	20.65	0.10	11.56	0.01	97.43	0.00	46.25
7	156.91	0.25	286.97	1.04	7.91	0.49	7.92	5.26	20.36	1.06	25.57
9	157.33	0.25	260.37	0.15	6.90	0.05	3.97	0.11	19.80	0.26	20.94
11	131.95	0.40	241.01	0.10	6.55	0.05	3.80	0.01	16.86	0.26	20.25
13	120.96	0.10	224.04	0.74	5.67	0.10	3.43	0.02	16.14	0.25	21.35
18	92.68	0.20	165.80	0.15	4.48	0.00	2.76	0.00	14.01	0.25	16.78
15	104.86	0.30	193.41	1.68	4.87	0.05	3.06	0.01	16.68	0.00	18.18
21	77.12	0.06	137.04	0.34	3.75	0.17	2.28	0.01	11.76	0.00	17.06
23	70.43	0.15	120.84	0.00	3.44	0.02	2.12	0.01	12.10	0.00	16.31
25	53.63	0.02	91.52	0.02	2.99	0.02	1.75	0.01	11.93	0.24	12.80
27	43.07	0.15	70.70	0.19	2.51	0.02	1.42	0.01	10.74	0.00	11.38
29	33.03	0.04	53.22	0.13	1.65	0.04	1.00	0.05	7.37	0.00	10.58

Dec-02

Salinity	Silicate (μM)	Stdev	Nitrate (μM)	Stdev	Phosphate (μM)	Stdev	Nitrite (μM)	Stdev	Ammonium (μM)	Stdev	Nitrous oxide (nM)
1	192.50	0.40	310.87	5.35	4.80	0.35	3.07	0.01	89.70	0.00	17.67
4	178.15	0.59	280.74	3.42	5.18	0.10	2.96	0.01	33.95	2.23	18.05
5	193.69	0.89	59.15	4.65	25.69	2.38	3.31	0.01	22.55	1.13	22.42
5	182.95	0.15	241.78	0.59	10.68	3.22	3.12	0.01	23.51	0.23	16.16
13	131.60	0.49	181.13	5.00	6.16	0.40	2.79	0.00	20.32	0.22	14.72
8	166.04	0.10	207.24	2.62	6.83	0.05	3.14	0.01	20.64	0.22	13.01
16	114.80	0.79	146.90	3.91	4.87	0.15	2.55	0.02	18.76	0.22	14.79
14	123.87	0.25	152.39	2.77	5.25	0.00	2.70	0.03	22.38	0.00	17.12
18	101.12	0.05	117.64	2.43	4.34	0.00	2.35	0.02	19.38	0.22	15.83
11	140.53	0.05	157.78	1.88	5.81	0.10	2.99	0.01	16.15	0.00	16.13
20	91.98	0.10	96.15	1.93	3.92	0.10	2.18	0.00	15.10	0.21	14.37
22	76.25	0.36	75.14	0.87	3.68	0.11	1.91	0.00	13.46	0.42	13.32
24	66.93	0.08	62.94	1.40	3.36	0.04	1.75	0.01	11.27	0.20	13.99
26	58.41	0.04	51.09	0.85	2.88	0.00	1.61	0.01	9.98	0.81	12.72
28	47.94	0.21	38.55	1.02	2.31	0.04	1.38	0.00	6.35	0.00	11.12
29	34.80	0.04	26.46	0.72	1.58	0.06	1.11	0.00	8.15	0.20	9.73

Appendix C

Distances between the source of the River Itchen and each sample site.

Sample site	Distance from the source (km)
1	Source of the River Itchen
2	0.5
3	2.8
4	5.5
5	7.5
6	10.8
7	12.8
8	14.5
9	19.2
10	20.8
11	22
12	23.2
13	25.8
14	27
15	30
16	34.2

Appendix D

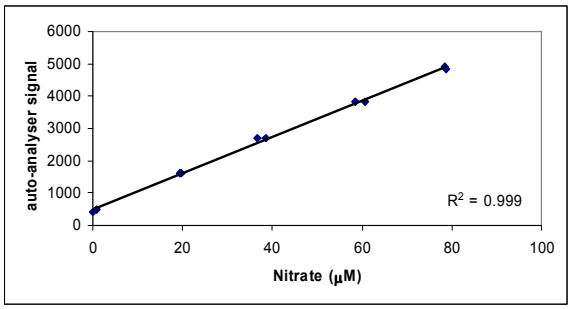
Range of standards used in the nutrient analyses.

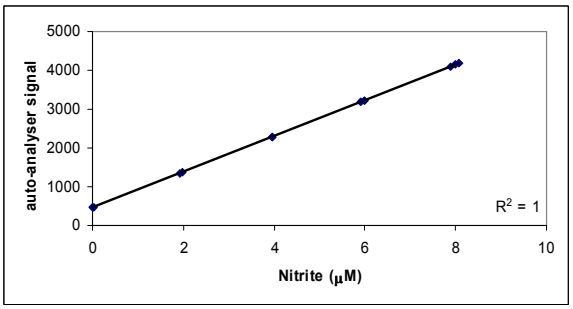
NO_3 (μM)	PO_4 (μM)	Si (μM)	NO_2 (μM)
20	0.5	10	2
40	1	20	4
60	2	30	6
80	3	40	8

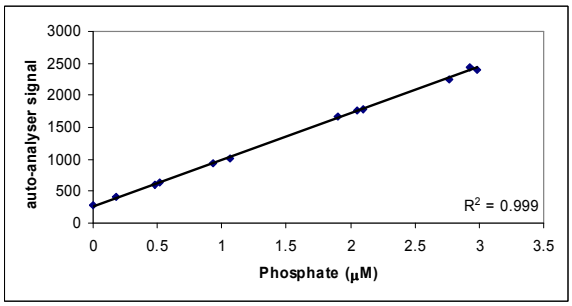
NH_4 (μM)	
River	**Estuary**
5	20
10	40
15	60
20	80

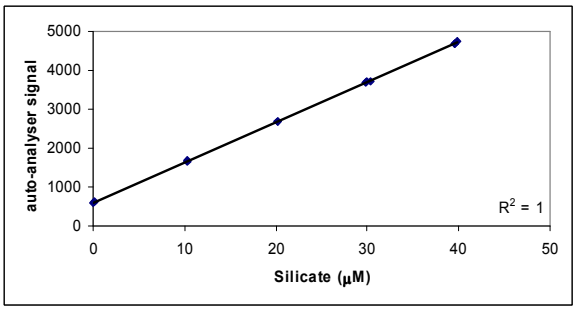
Appendix E

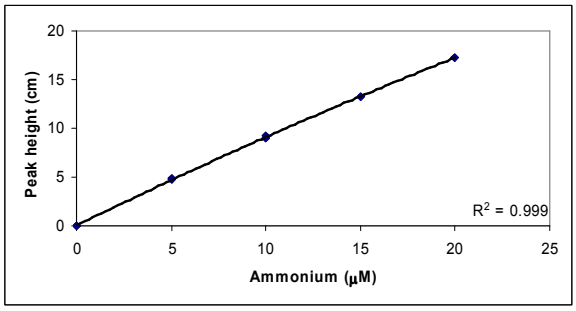
Examples of standard curves for nutrient analyses.

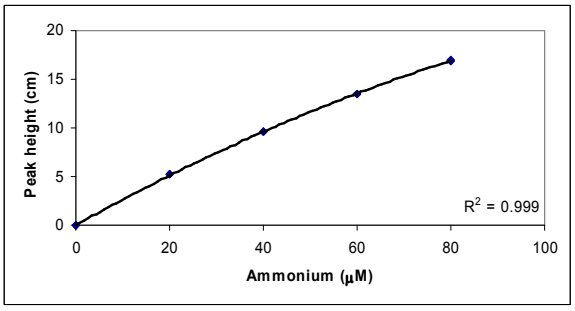






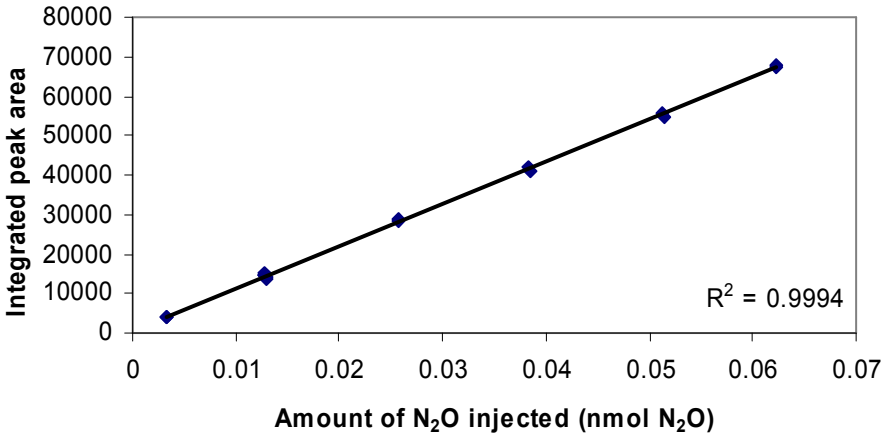






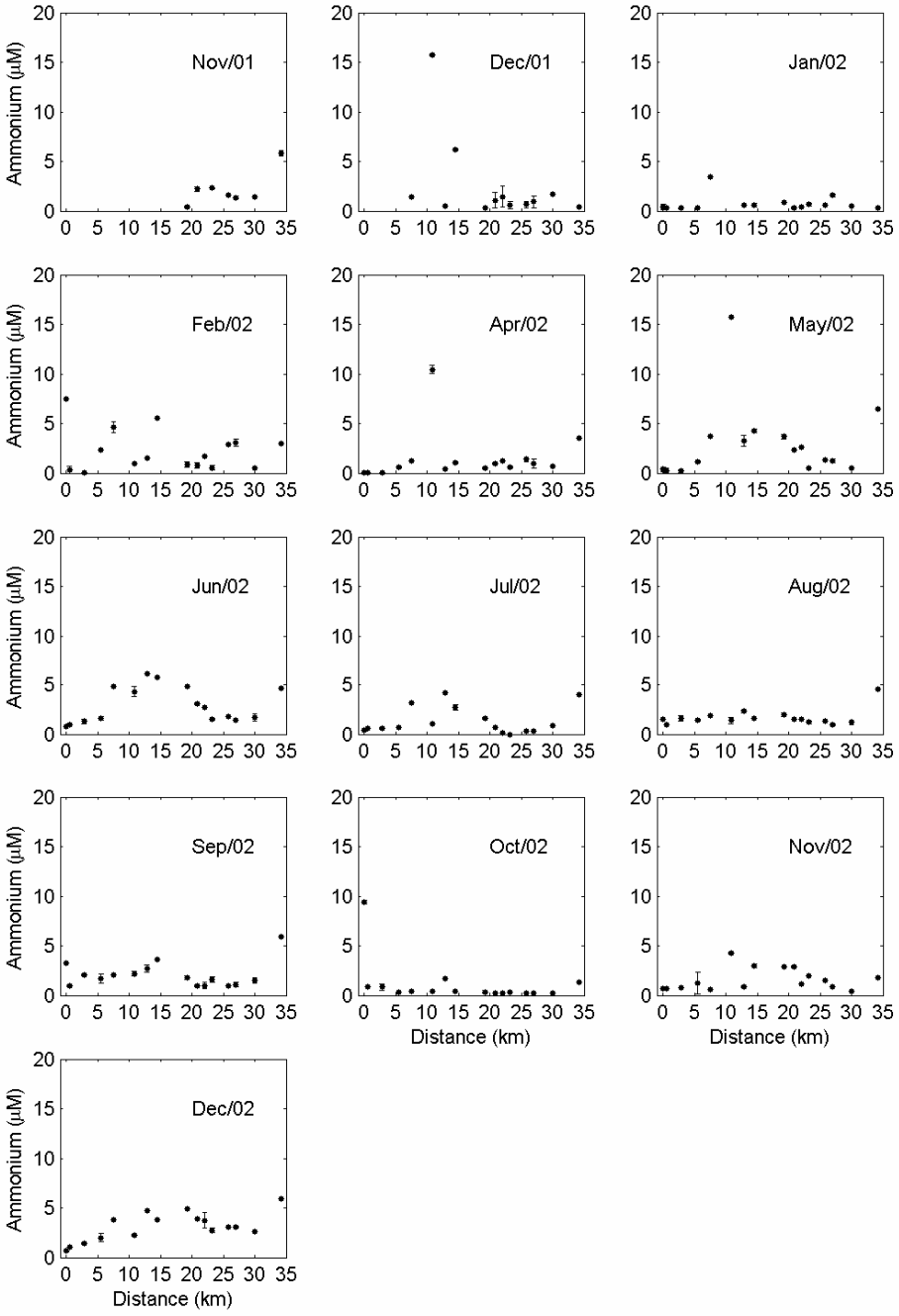
Appendix F

Nitrous oxide calibration curve.



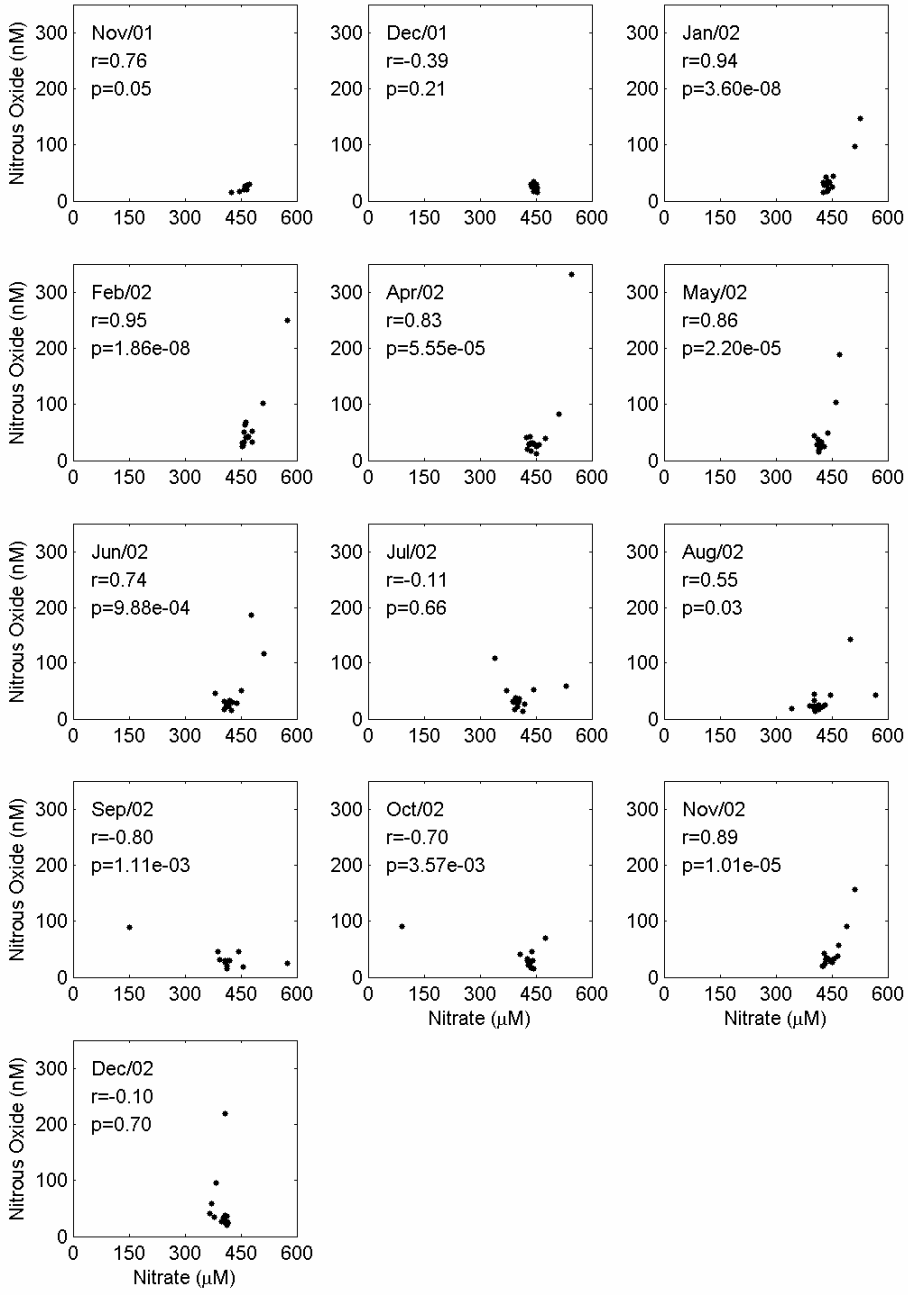
Appendix G

Ammonium concentration in the River Itchen (full data set). Error bars show ± 1 standard deviation (n=3).



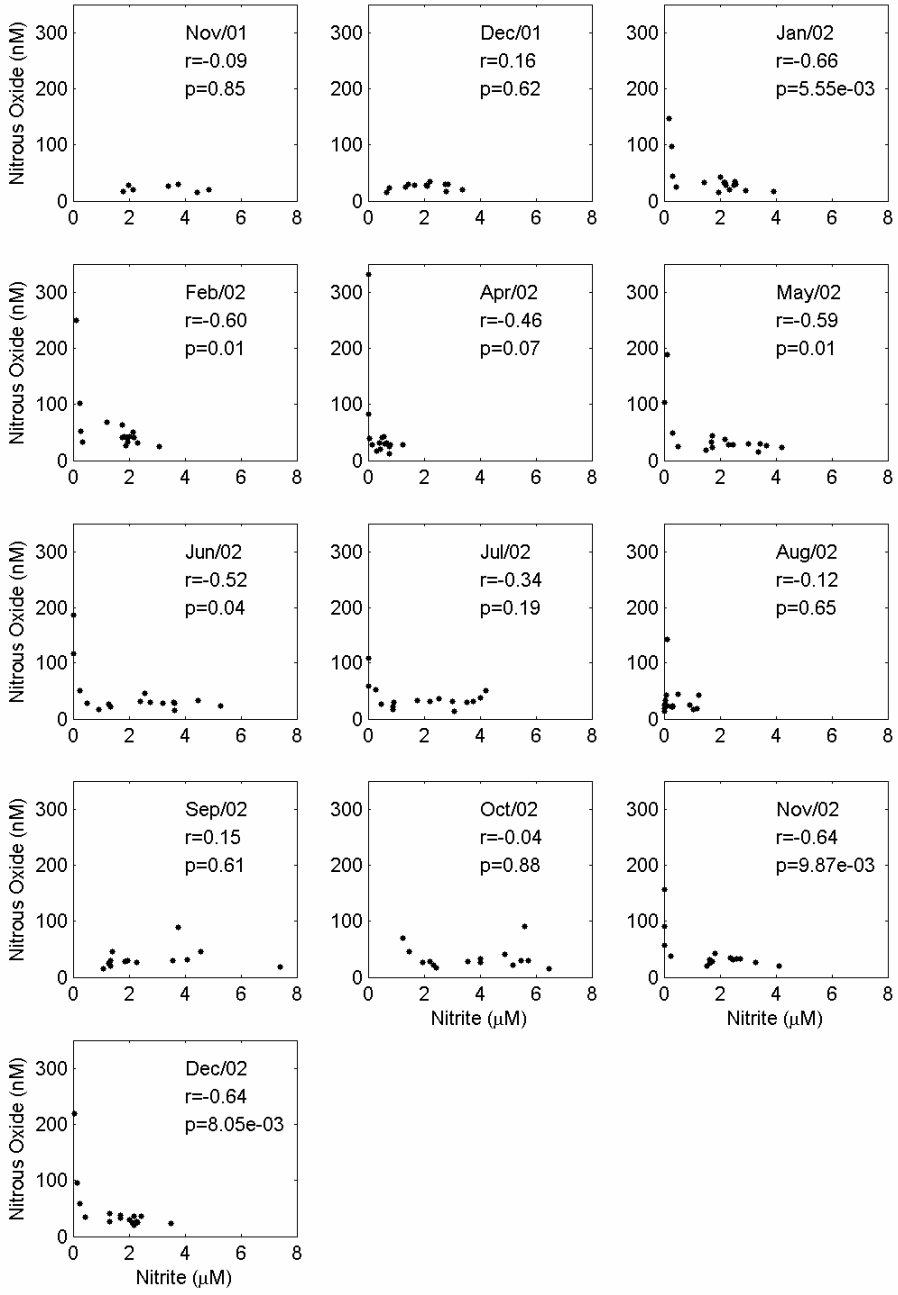
Appendix H

Correlations between nitrous oxide and nitrate concentrations in the surface water from the River Itchen.



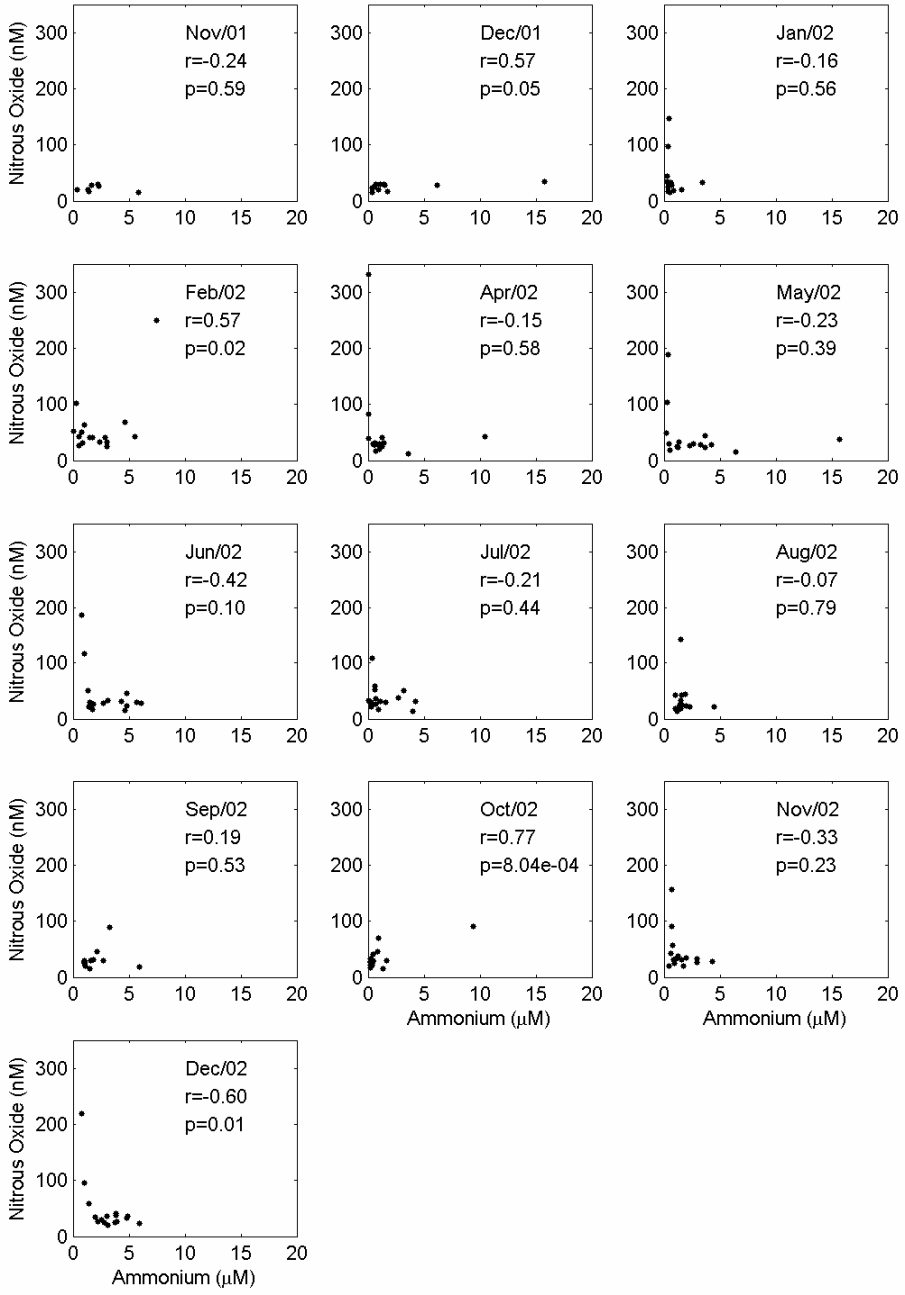
Appendix I

Correlations between nitrous oxide and nitrite concentrations in the surface water from the River Itchen.



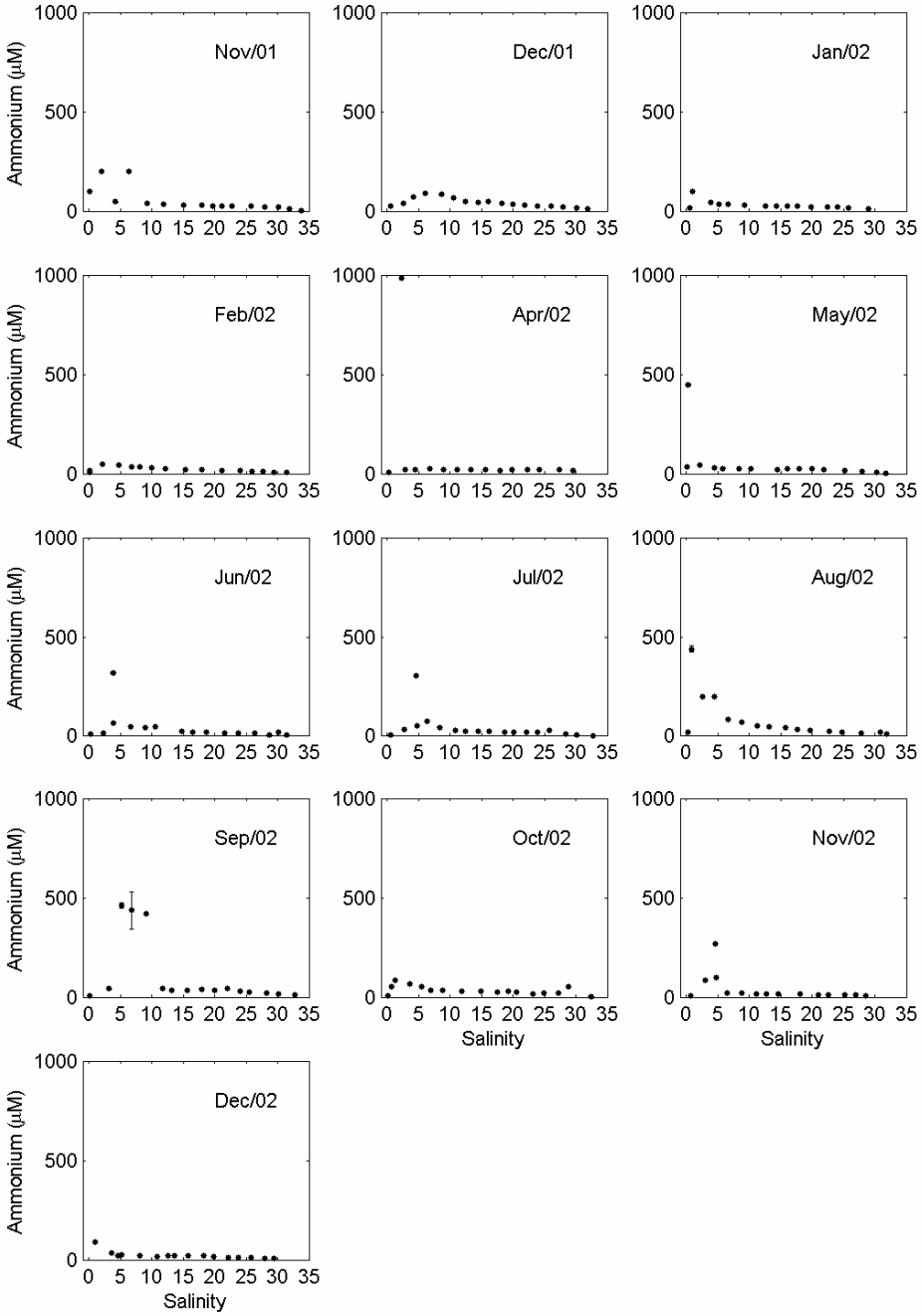
Appendix J

Correlations between nitrous oxide and ammonium concentrations in the surface water from the River Itchen.



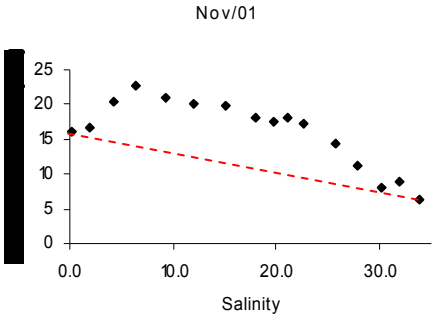
Appendix K

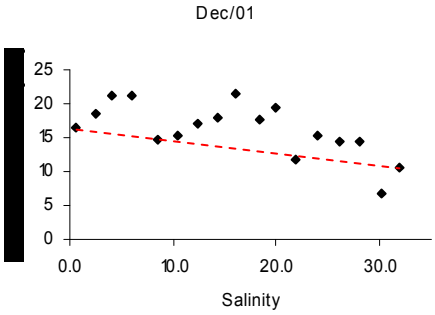
Ammonium concentration in the Itchen Estuary (full data set). Error bars show ± 1 standard deviation (n=3).

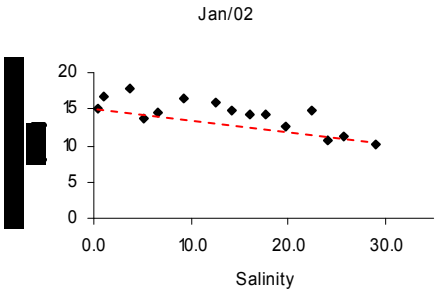


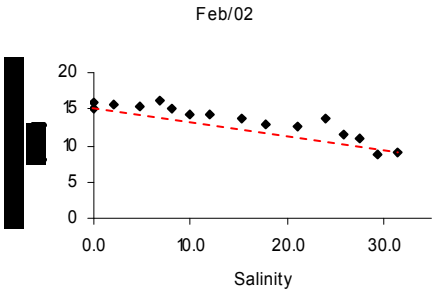
Appendix L

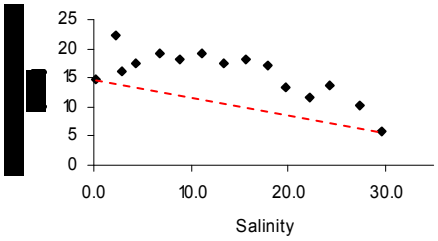
Nitrous oxide concentrations in the Itchen Estuary from November 2001 to December 2002. The red dotted line represents the theoretical dilution line.

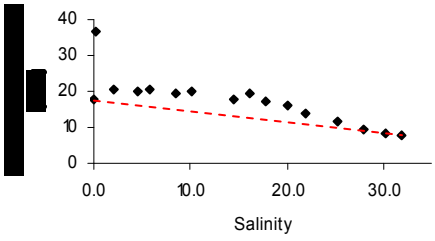




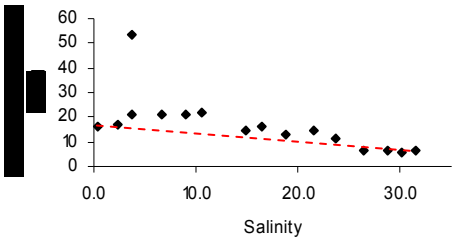




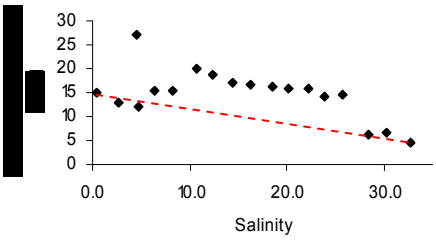




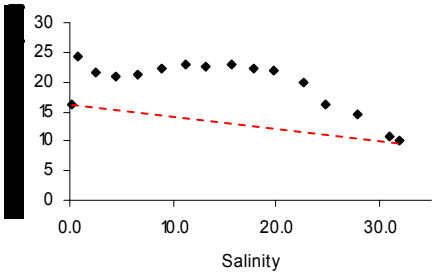
Jun/02



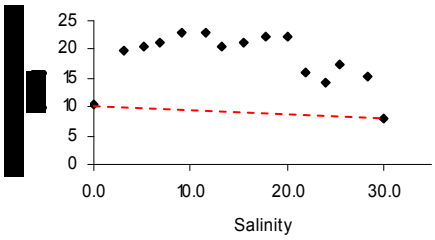
July/02



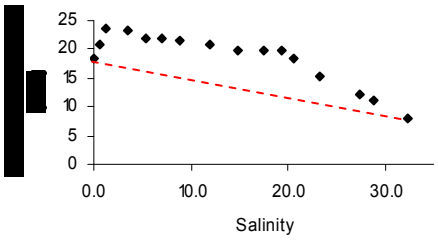
Aug/02



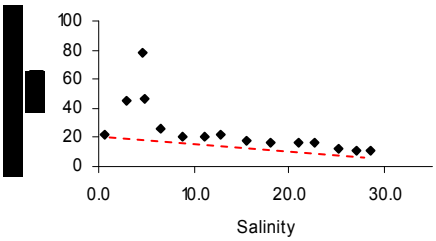
Sept/02



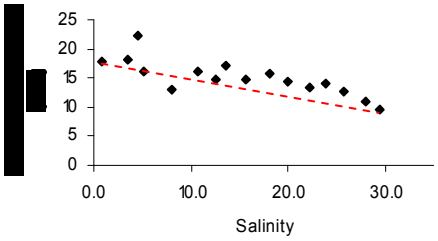
Oct/02



Nov/02



Dec/02



Appendix M

Average N_2O concentrations and fluxes for every month studied. N_2O concentrations (nmol N_2O l^{-1}), N_2O fluxes (nmol N_2O m^{-2} h^{-1}),). * No data available as sites 1 and 2 were included in the sampling scheme only from January onwards.

Survey	Average N_2O concentration			N_2O Flux		
	Sites 1 and 2	Sites 3 to 16	Sites 1 to 16	Sites 1 and 2	Sites 3 to 16	Sites 1 to 16
Nov/01	*	22	22	*	335	335
Dec/01	*	26	26	*	342	342
Jan/02	122	29	40	5989	857	1499
Feb/02	175	42	59	12938	2318	3688
Apr/02	207	29	51	10349	994	2240
May/02	146	29	44	8947	1258	2261
Jun/02	151	29	44	8009	1032	1904
July/02	84	32	38	4546	1268	1511
Aug/02	93	24	33	3490	714	1066
Sep/02	57	29	33	1978	605	777
Oct/02	80	29	35	3545	832	1194
Nov/02	124	32	45	5856	1029	1673
Dec/02	158	32	48	6905	885	1637

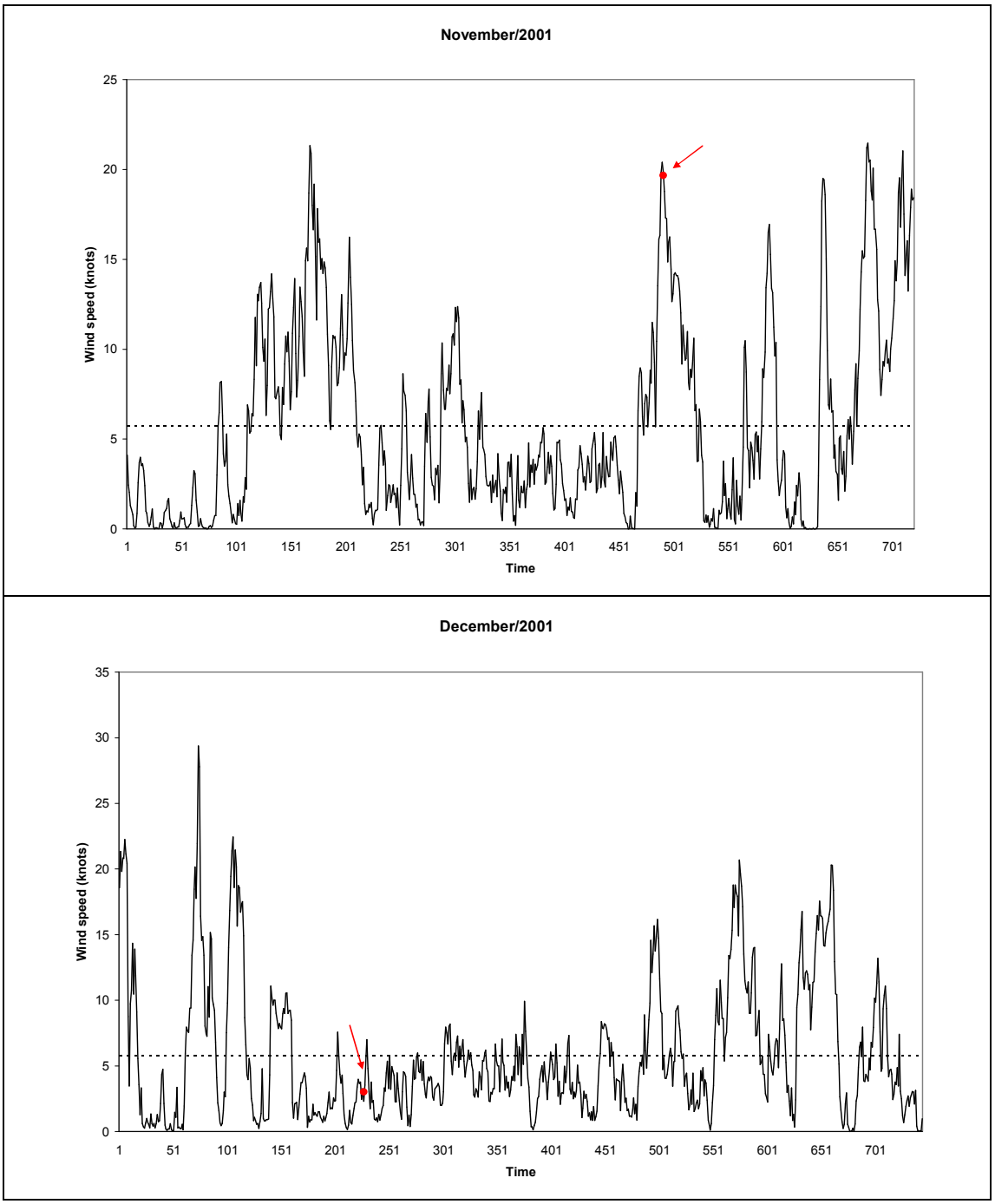
Appendix N

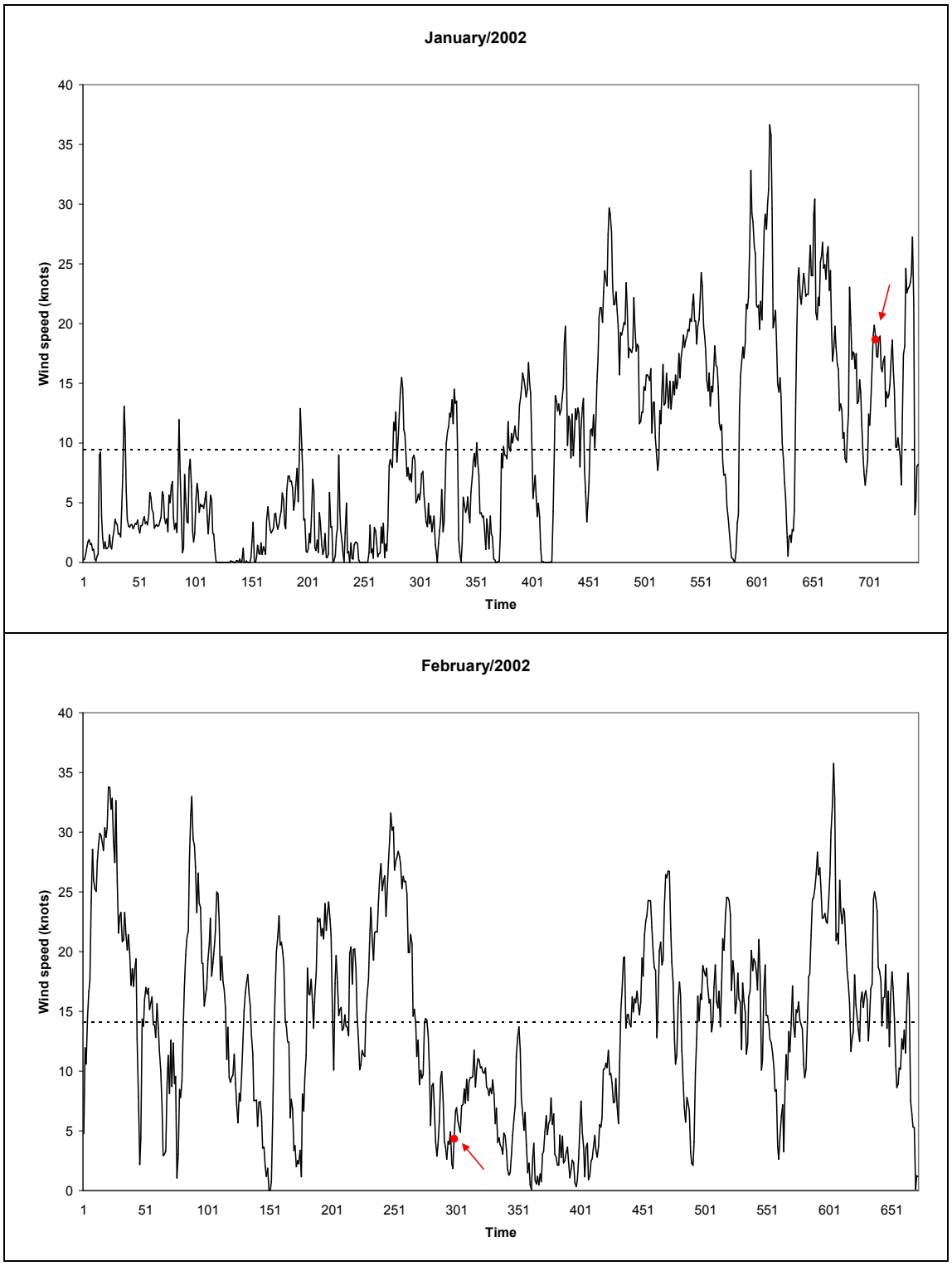
Comparison of N_2O emissions from the River Itchen (kg $month^{-1}$) estimated by: adding the emissions calculated separately for each segment of the river (Total emission); and by using the average flux to calculate the emission for the full length of the river (Whole river). * No data available as sites 1 and 2 were included in the sampling scheme only from January onwards.

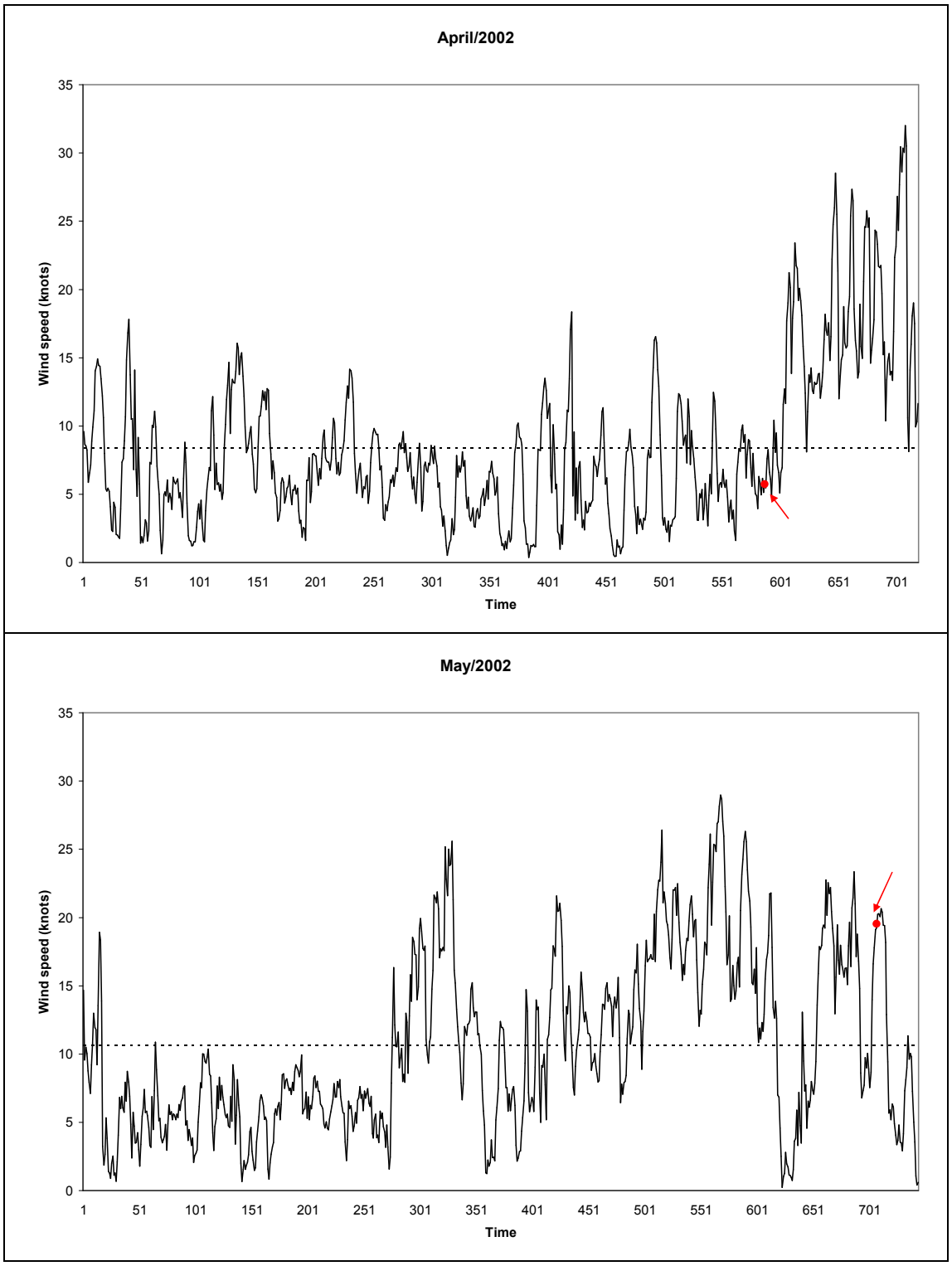
Survey	N_2O emission			N_2O emission
	Sites 1 to 3 Area= 0.01 km^2	Sites 3 to 16 Area=0.6km^2	Total emission for the river	Whole river (using average flux for the full length of the river)
Nov/01	*	6	6	6
Dec/01	*	6	6	6
Jan/02	3	16	19	29
Feb/02	6	44	50	71
Apr/02	5	19	23	43
May/02	4	24	28	44
Jun/02	4	20	23	37
July/02	2	24	26	29
Aug/02	2	14	15	21
Sep/02	1	11	12	15
Oct/02	2	16	17	23
Nov/02	3	19	22	32
Dec/02	3	17	20	32

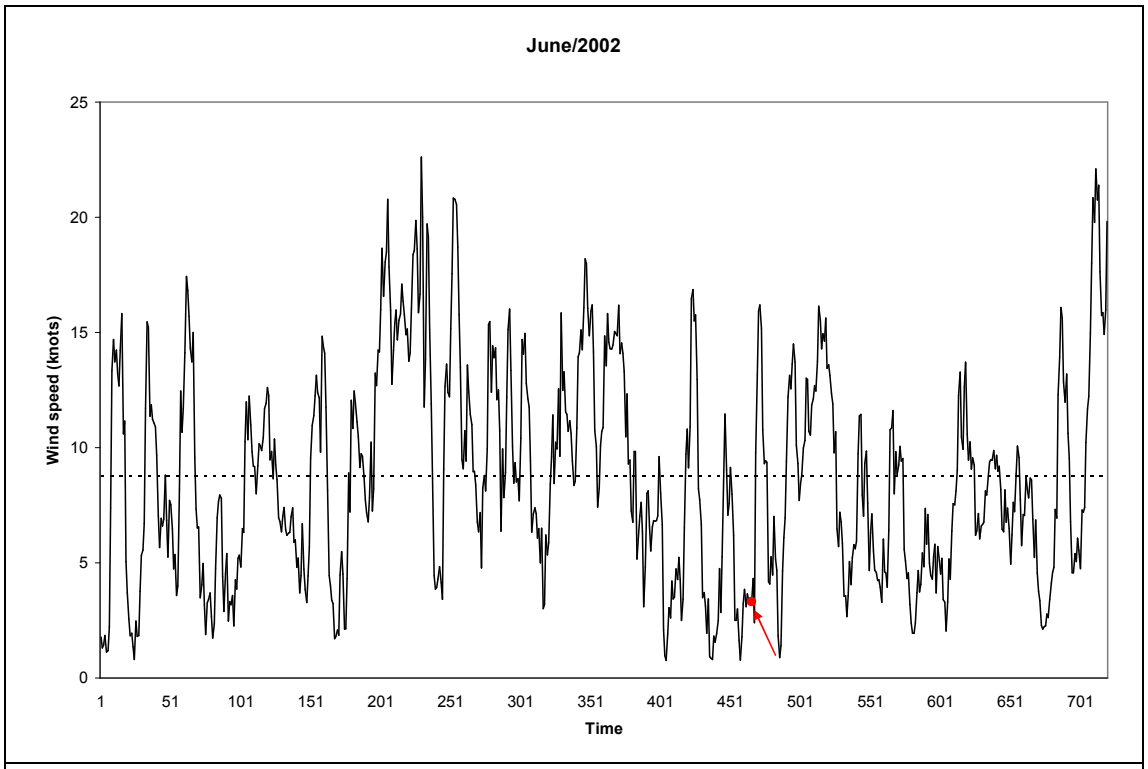
Appendix O

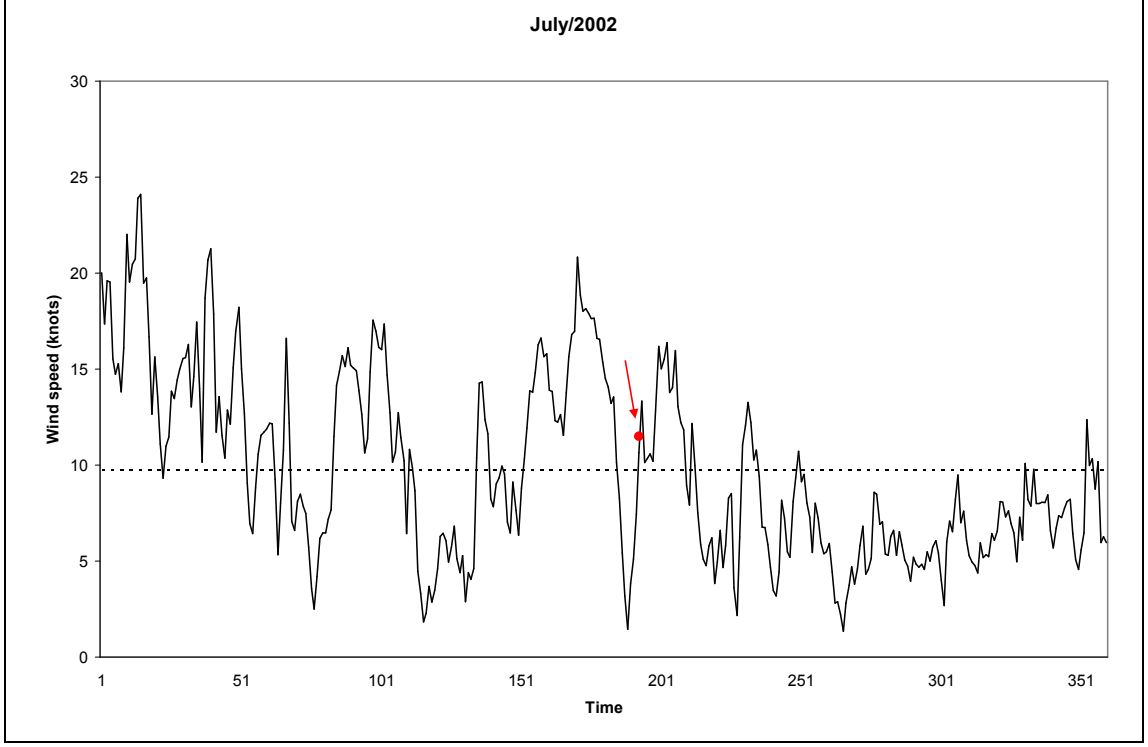
Wind speed data (knots) for each month sampled (time in hours). Doted line indicates the average wind for the month. Arrow shows the wind at the sampling time.

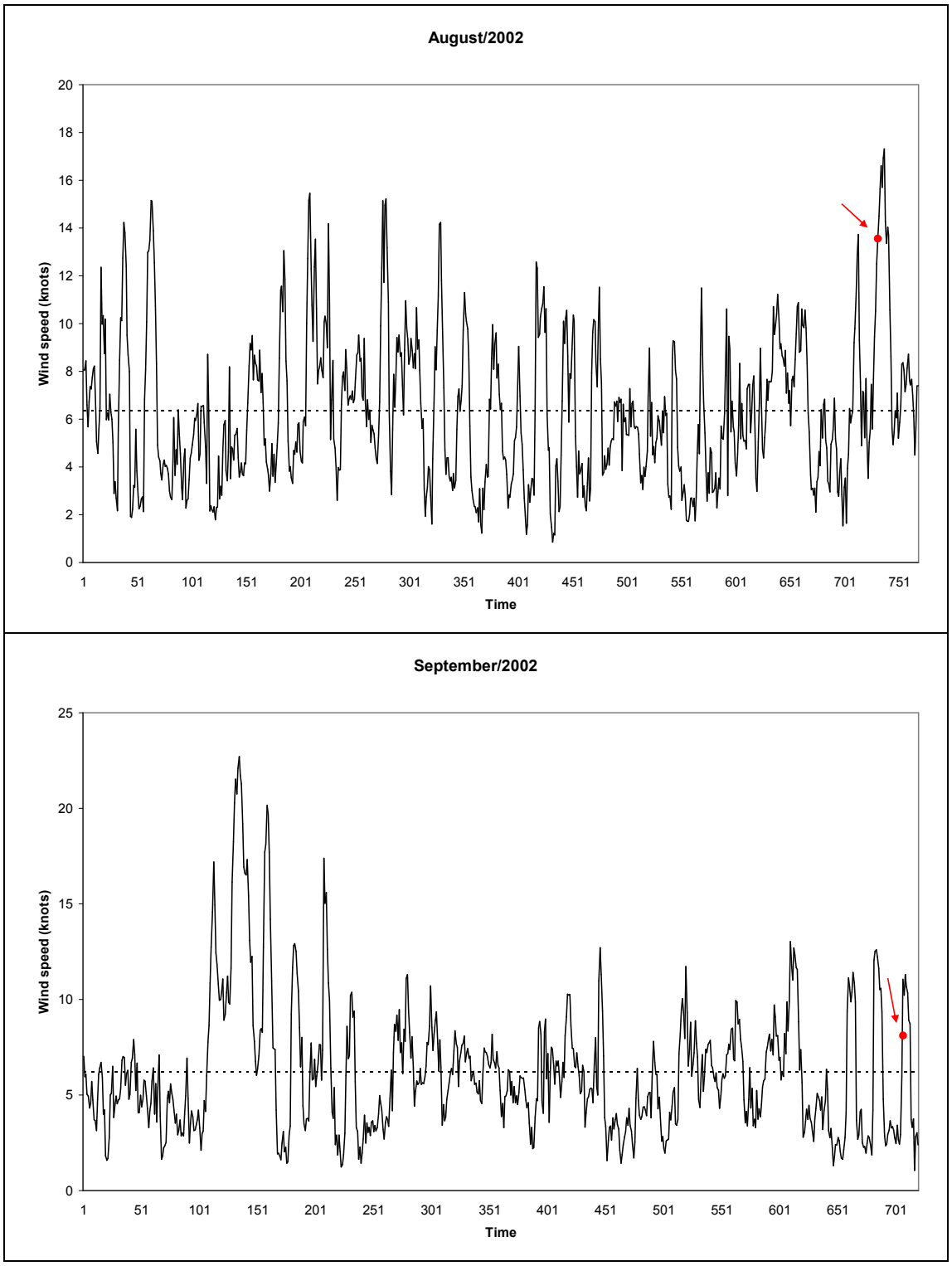


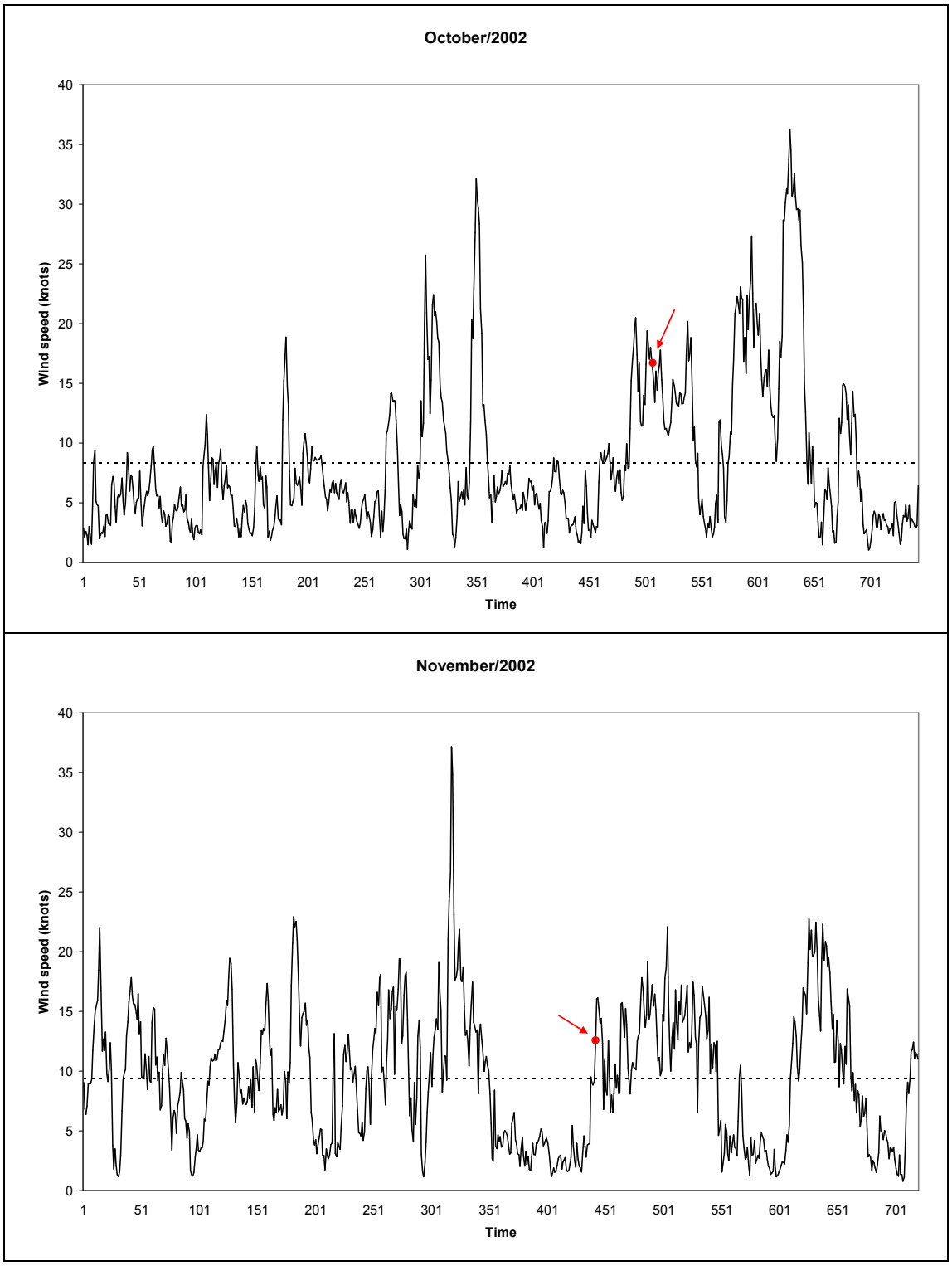

January/2002
Wind speed (knots)
0
5
10
15
20
25
30
35
40
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701
Time
February/2002
Wind speed (knots)
0
5
10
15
20
25
30
35
40
1
51
101
151
201
251
301
351
401
451
501
551
601
651
Time

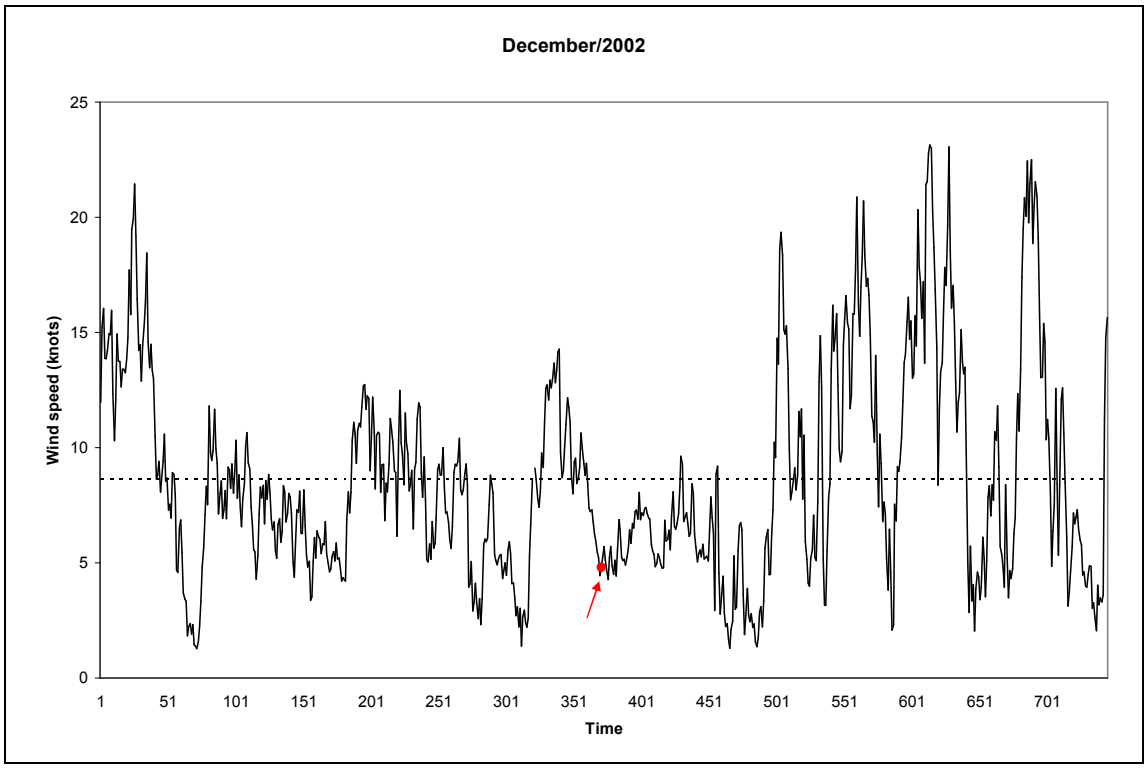

April/2002
Wind speed (knots)
0
5
10
15
20
25
30
35
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701
Time
May/2002
Wind speed (knots)
0
5
10
15
20
25
30
35
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701
Time


June/2002
Wind speed (knots)
0
5
10
15
20
25
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701
Time


July/2002
Wind speed (knots)
0
5
10
15
20
25
30
1
51
101
151
201
251
301
351
Time


August/2002
Wind speed (knots)
Time
0
2
4
6
8
10
12
14
16
18
20
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701
751
September/2002
Wind speed (knots)
Time
0
5
10
15
20
25
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701


October/2002
Wind speed (knots)
0
5
10
15
20
25
30
35
40
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701
Time
November/2002
Wind speed (knots)
0
5
10
15
20
25
30
35
40
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701
Time


December/2002
Wind speed (knots)
25
20
15
10
5
0
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701
Time

Appendix P

Concentrations of nitrate, nitrite, oxygen and nitrous oxide in the preliminary incubations SV20A and SV20B.

Incubation	Time (h)	Nitrate (μM)	Nitrite (μM)	Nitrous oxide (nM)	Oxygen (%sat)
SV20A	0	29.63	4.22	8.82	73.88
	2	23.99	3.71	10.60	46.23
	4	20.05	3.30	11.27	23.12
	6	16.36	2.74	17.73	6.39
SV20B	0	47.90	8.44	6.91	60.00
	2	41.33	7.51	8.60	34.89
	4	33.64	6.78	12.16	13.06
	6	NAN	NAN	14.42	-0.26