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STUDIES OF HYDROXYLAMINE IN THE MARINE ENVIRONMENT

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Abstract approved: _____
Dr. Louis Gordon

Hydroxylamine is a constituent of the nitrogen cycle which has been shown to be an intermediate in both nitrification and nitrate reduction in several in-vitro studies. However, there have been no investigations into the concentrations and distributions of hydroxylamine in the marine environment. The purpose of this work was to fill this gap by developing an analytical procedure for the detection of hydroxylamine in this kind of environment, and then investigate hydroxylamine distributions in marine systems.

The analytical procedure developed involves the oxidation of hydroxylamine to nitrous oxide using ferric ions, immediately after sampling, and subsequent detection by electron capture gas chromatography. The method is linear from 1.2 to 560 nM and has a relative standard deviation of 4% at the 12.5 nM level. The limit of detection as calculated from the precision of the blanks at the 95% confidence limit was found to be 0.6 nM. The procedure requires relatively small sample size, and permits the final measurement to be delayed for up to two weeks. Ammonium and sulfide ions do not interfere.

This method has been used to investigate hydroxylamine

distributions in oxygenated and oxygen-poor marine environments: Saanich Inlet, an intermittently anoxic fjord on Vancouver Island, British Columbia; Yaquina Bay, a coastal plain estuary on the Oregon coast; and a station 14 miles just off the continental shelf break off the Oregon coast.

The results showed production of hydroxylamine in well oxygenated subsurface waters where nitrification takes place, and in deep oxygen depleted waters where microbially mediated reduction of nitrate is likely to be occurring. Production of hydroxylamine was also observed near the commercial oyster beds in Yaquina Bay.

STUDIES OF HYDROXYLAMINE IN THE MARINE ENVIRONMENT

by

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"I think what you felt was just Wonder, not knowledge or knowing. You felt that knowledge was not as important as your sense of Wonder, which was a great feeling without reservation, without obligation, without accounting of yourself. From Wonder must come Realization, because in your making you have gone through every law of nature. It is part of you."

Louis I. Kahn

...but a thesis is a part of you that includes many people. It is imposible to describe what was involved in bringing it about; it took two special years full of wonder, frustrations and happiness, but mos of all full of the true sense of frienship.

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STUDIES OF HYDROXYLAMINE IN THE MARINE ENVIRONMENT

INTRODUCTION

The nitrogen cycle is a series of biologically mediated oxidation reduction reactions. The study of this cycle and the role of some of its components in the marine environment has been an area of intensive research (PAYNE, 1981). Although it is generally accepted that hydroxylamine is the intermediate in the formal oxidation state of -1, in both the oxidation of ammonia and the reduction of nitrate, there have been no investigations of the role of hydroxylamine in the marine nitrogen cycle. Few measurements have been reported in aquatic systems (TANAKA, 1953; FIADEIRO et al., 1967; KOYAMA and TOMINO, 1967; PITWELL, 1975). The reasons are that hydroxylamine is very unstable in oxygenated waters and that it occurs at very low concentrations. In general, the methods available until now were applicable only when the analysis could be carried out immediately after sampling. Therefore, the first objective of this study was to develop a suitable method of analysis for hydroxylamine capable of detecting the low levels found in the marine environment. The resulting procedure involves the immediate oxidation of hydroxylamine to nitrous oxide using ferric ions. The nitrous oxide produced is subsequently measured using electron capture gas chromatography. The method is linear in the range tested, from 1.2 to 560 nM hydroxylamine, with a relative standard deviation of 4% at the 12.5 nM level.

The second objective was to observe distributions of hydroxylamine in environments covering a wide range of salinity and oxygen levels.

Environments of varying dissoved oxygen concentrations would provide clues to the processes controlling hydroxylamine levels in natural systems. Also, since it was expected that hydroxylamine would be found in areas of high bacterial activity, coastal and estuarine environments were selected.

The method developed in the first part of this study, was used to measure hydroxylamine in the water columns off the Oregon coast, a coastal plain estuary and an intermittently anoxic fjord. It was found to vary systematically with other nitrogen species, thus reflecting its involvement in the nitrogen cycle. Two main hypotheses based on the distributions of hydroxylamine and other nitrogen species emerged: Hydroxylamine appears in the water column as an intermediate in nitrification in the subsurface oxygenated waters and in the reduction of nitrate to ammonia in the low oxygen deep waters.

Chemical Properties of Hydroxylamine Solutions

Hydroxylamine in aqueous solution will decompose quite readily when in contact with atmospheric oxygen. ANDERSON (1964) showed that millimolar concentrations of hydroxylamine are stable for only 60 minutes at pH 7.7 in the presence of air. The decomposition products include molecular nitrogen, nitrous oxide and nitrite; the proportion of each product is determined by the conditions of the reaction.

Trace quantities of certain metal ions seem to catalyze the autoxidation of hydroxylamine solution (MOEWS and AUDRIETH, 1959; ANDERSON, 1964; JAMES, 1942) while addition of metal sequestering agents results in an enhanced stability of hydroxylamine solutions.

Low pHs appear to stabilize aqueous solutions of hydroxylamine. ANDERSON (1964) reports solutions to be stable for several hours when the pH was kept at 4.0. Autoxidation of hydroxylamine at pH 11, in the presence of trace quantities of copper, has been shown to result in an 80% conversion to nitrite in oxygen saturated solutions (HUGHES and NICKLIN, 1971). At pH 7 to 8, the product of the copper catalyzed autoxidation is reported to be nitrous oxide in oxygen saturated solutions (HUGHES and NICKLIN, 1970 a,b). Insufficient oxygen favors the production of nitrous oxide relative to nitrite in the autoxidation of hydroxylamine (HUGHES and NICKLIN, 1971).

Reduction potentials for nitrogen species in aqueous solutions are illustrated in Figure 1 (LATIMER, 1952). Investigators have been interested in the redox chemistry of hydroxylamine since the end of the last century. A review of the literature shows many studies where the ultimate products of the redox reactions of hydroxylamine have been elucidated, but the mechanisms of their formation have not usually been reported. The main problem is that few of the reactions proceed quantitatively in one direction, most of them giving variable proportions of several products.

Hydroxylamine and its salts have usually been measured by methods based on oxidation-reduction reactions, either in basic or acidic solutions, using volumetric, electrochemical or spectrophotometric techniques. KOLASA and WARDENKY (1974) gave an extensive review of these methods.

Nitrification and Nitrate Reduction - A Brief Review

The importance of the biochemically mediated processes involving

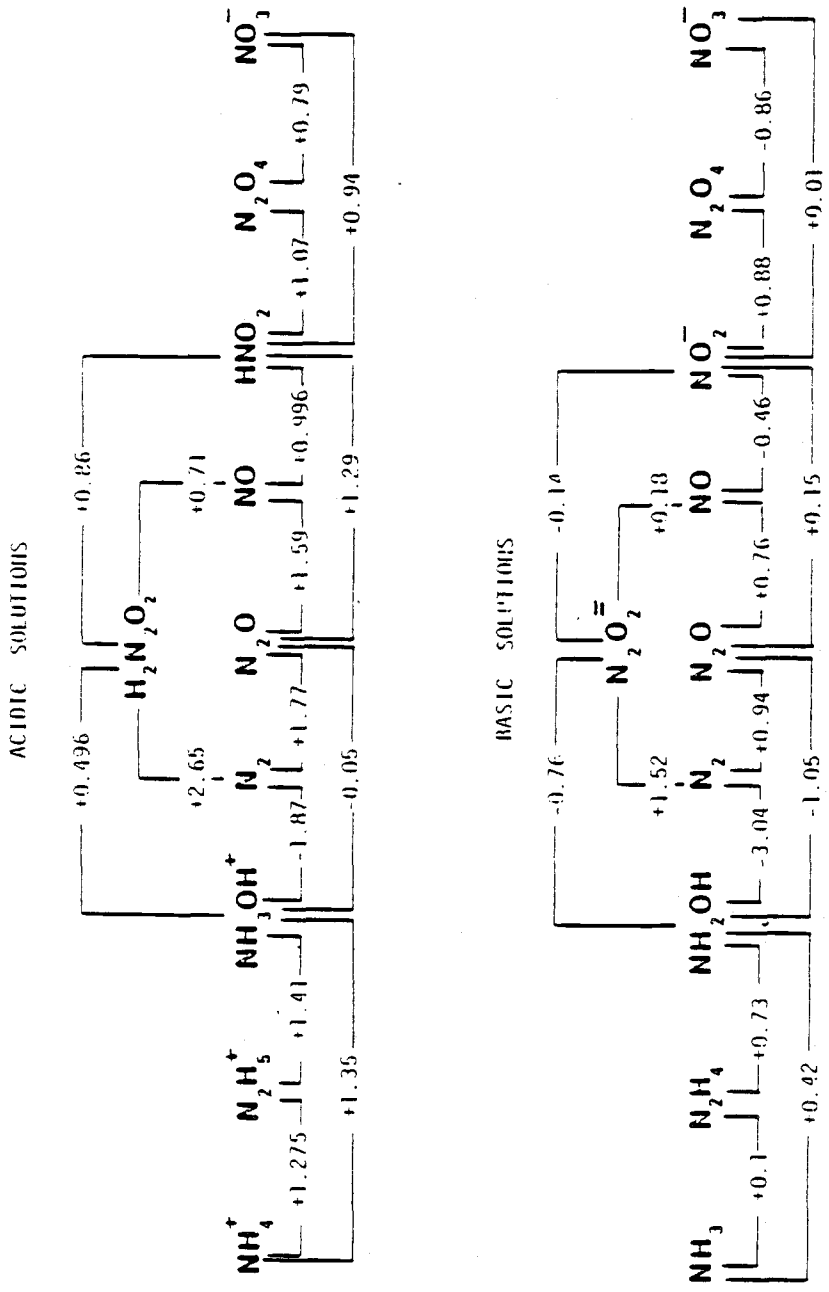

Figure 1. Redox potentials for nitrogen species in aqueous solutions

Redrawn from Latimer (1952)

nitrogen species was readily recognized by 19th century microbiologists. The biological nitrogen cycle is summarized in Figure 2. The formation of nitrate in soils was attributed, over two centuries ago, to the decay of organic matter as noted by LIPMANN, (1908). Nitrification, the microbial oxidation of ammonia to nitrate, has also been studied in the aqueous environment. BRANDHORST (1959) suggested that nitrification occurred near the bottom of the euphotic zone in well oxygenated waters. Nitrification can also take place under low partial pressures of oxygen (GUNDERSEN, 1966; GUNDERSEN et al. 1966). CARLUCCI and MCNALLY (1969) showed nitrification to occur in media containing less than 0.1 ml O_2/ℓ The rate of oxidation of ammonia to nitrite has been measured in both the oceanic euphotic zone (WADA and HATTORI, 1971) and in Sagama Bay (MIYAZAKI et al., 1973) using ^{15}N techniques. Qualitative measurements have been reported on a coral reef (WEBB and WIEBE, 1975), and in rivers (CURTIS et al, 1975; TUFFEY et al., 1974).

Nitrification can be carried out by both chemoautotrophic and heterotrophic microorganisms. Autotrophic nitrifiers depend entirely on the oxidation of nitrogen for their energy supply. The discovery and isolation of the chemoautotrophic bacteria Nitrosomonas and Nitrobacter was achieved by WINOGRADSKY (1890). The oxidation of ammonium to nitrite involves the participation of molecular oxygen which serves in two ways: by direct incorporation into the substrate, and as terminal electron acceptor. The biochemical pathway is still not well known, but it is now generally accepted that it occurs via two-electron changes, with hydroxylamine as an intermediate (Figure 2).

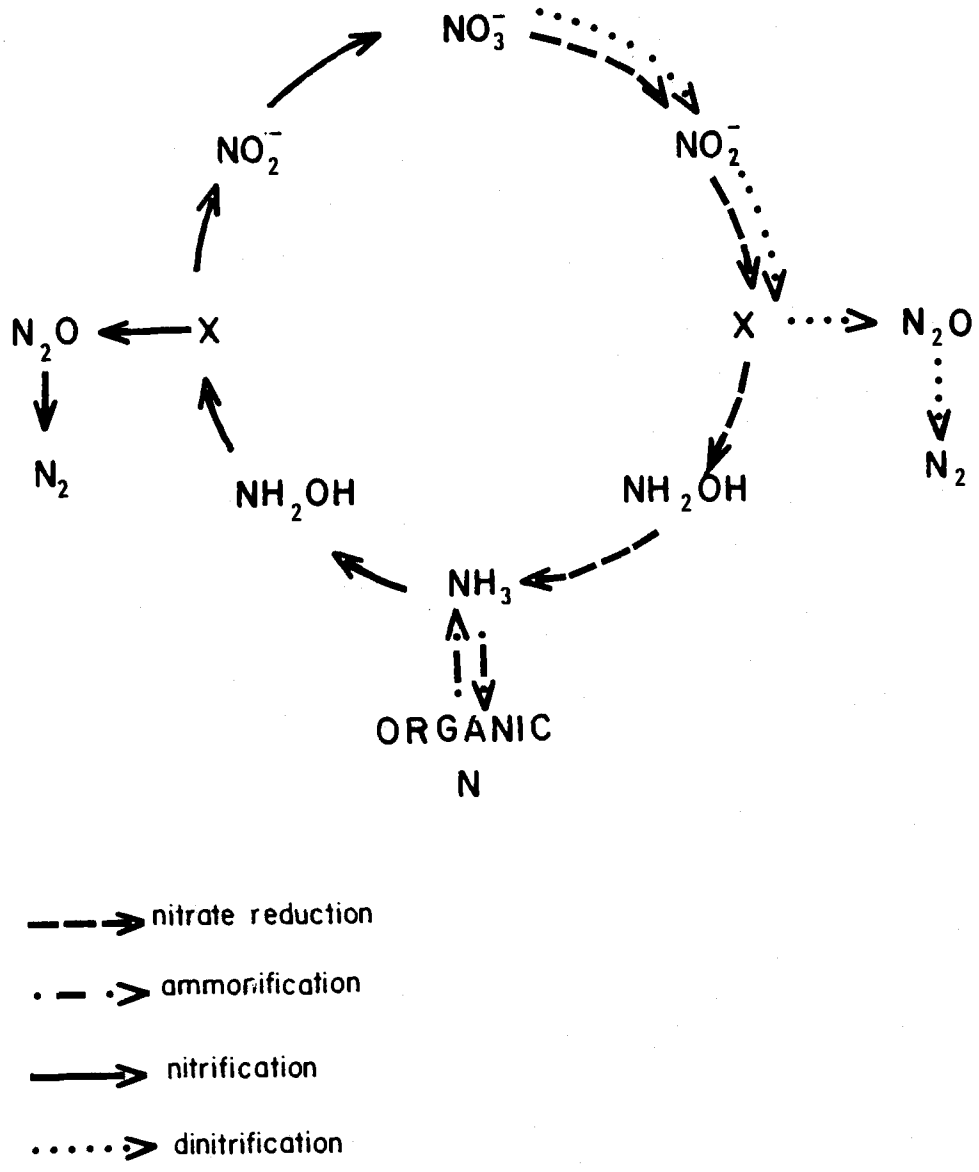

Figure 2. The marine nitrogen cycle

The species with nitrogen in the formal oxidation state of +1 has not yet been satisfactorily defined.

Heterotrophic nitrification may account for some of the nitrogen oxidized in nature, though probably its contribution is small. Studies on axenic cultures of a nitrifying strain of Arthrobacter revealed that this heterotroph excreted hydroxylamine, a hydroxamic acid, nitrite and nitrate (VERSTRAETE and ALEXANDER, 1973).

Hydroxylamine has thus been positively identified as an intermediate in both autotrophic and heterotrophic nitrification in in vitro studies. However, no attempt to measure hydroxylamine as related to nitrification processes in aquatic systems has been reported.

A number of pathways for biological nitrate reduction are known. Assimilatory reduction is the process by which certain microorganisms reduce nitrate via nitrite to ammonia, which ends up as cell nitrogen. In this case, nitrite, whether supplied exogenously or as a consequence of nitrate reduction, is reduced in a sequence of two-electron transfers to ammonia, with hydroxylamine as the intermediate with the -1 formal oxidation state (COLE et al.; 1974, GARRET, 1972; HUGES, 1975).

Dissimilatory reduction of nitrate to nitrite is carried out by a variety of facultative and obligately anaerobic bacteria genera. In this process nitrate acts as an electron acceptor. Further reduction varies among species with molecular nitrogen, nitrous oxide and ammonia all having been identified as products (YORDY and RUOFF, 1981). The reduction of nitrate to molecular nitrogen and nitrous oxide is known as denitrification. PAYNE (1981) has reviewed the extensive literature on denitrifying bacteria. However, few studies have dealt

with the reduction of nitrite to ammonia by either assimilatory or dissimilatory reducers. Very few ammonia producing dissimilatory nitrate-reducing bacteria have been identified; these include Escherichia coli (ZAROWNY and SANWAL, 1963), Veillonela alcalescens (YORDY and DELWICHE, 1979) and Achromobacter fisheri (PRAKASH and SADANA, 1972).

In the marine environment, investigations into the reduction of nitrate have also been carried out. A genus of nitrate reducing bacteria in oxygen depleted waters off Peru was reported by CARLUCCI and SCHUBERT (1969). Denitrification has been quantitatively measured using ^{15}N techniques in oxygen deficient waters of Darwin Bay, in the Galapagos Islands (GOERING and DUGDALE, 1966) and in the deep waters of the Eastern Tropical Pacific (GOERING, 1968). Denitrification has also been reported in an intermittently anoxic fjord (COHEN, 1978), and in sediments of rivers and estuaries (TERRY and NELSON, 1975; CHEN et al., 1972).

While denitrification has been clearly observed in natural aquatic systems, nitrate reduction to ammonia has not been so definitely reported. One reason may be the fact that this process has been considered to have little environmental significance compared with denitrification, and nitrification processes. However, studies on deep Central Pacific waters and Sagama Bay, using ^{15}N techniques, seemed to indicate that "assimilatory" nitrate reduction could be an important process in deep sea waters (WADA and HATTORI, 1972). During this study, denitrification was insignificant, if present at all, and an increase in nitrite and ammonia concentrations led to the conclusion that nitrate

reduction by heterotrophic bacteria was taking place. Ammonia formation from nitrate has also been shown to occur in anaerobic coastal sediments (KOIKE and HATTORI, 1978).

The measurement of hydroxylamine in the marine environment could be a very useful parameter in the elucidation of the kind of nitrate reduction taking place in a given system, since it does not appear to be produced during the denitrification sequence.

CHAPTER I

GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION FOR DETERMINATION OF NH_2OH IN SEAWATER

Analytical Chemistry, 1982, 54, 1209-1210

ABSTRACT

A new method for the determination of nanomolar concentrations of hydroxylamine in seawater has been developed. It involves its immediate oxidation to nitrous oxide using Fe (III) ions, and subsequent determination using electron capture gas chromatography. The method is linear in the range tested, from 1.2 to 560 nM hydroxylamine, with a relative standard deviation of 4% at the 12.5 nM level. The limit of detection, as calculated from the precision of the blanks was 0.6 nM at a 95% confidence level. Samples can be stored for at least 16 days before analysis. Ammonium and sulfide ions do not interfere.

The method described has been applied to samples from various natural marine environments over a wide range of salinities and oxygen levels. The hydroxylamine levels observed ranged from undetectable to 7.8 nM.

INTRODUCTION

Hydroxylamine has been identified as an intermediate in the nitrogen cycles in nature (HUGHES, 1975; YOSHIDA and ALEXANDER, 1964). The oxidation of ammonia to nitrate by several chemoautotrophic microorganisms has been proven to be a source of hydroxylamine in several in-vitro studies (YOSHIDA and ALEXANDER, 1971; RAJENDRAN and VENUGOPALAN, 1976; PATEMAN, et al., 1967). The study of its distribution in relation to other nitrogen species can be useful in understanding the nitrogen cycle, one of the basic areas of research in our laboratory. However, few measurements of hydroxylamine in natural environments have been made; TANAKA (1953), KOYAMA and TOMINO (1967) and PITWELL (1975) have reported the accumulation of hydroxylamine in relatively deep lakes.

Numerous methods for the determination of hydroxylamine have been described (KOLASA and WARDENKY, 1974) including a method for the spectrophotometric determination in seawater (FIADEIRO et al., 1967) and a gas chromatographic method in human colonic fluid (DARKE and ROEDIGER, 1980). The latter method involved conversion of hydroxylamine to an oxime which was subsequently measured by flame ionization gas chromatography. The limit of detection for this method was reported as 3 μM. RAO and RAO (1942) used a titrimetric method to determine the quantitative oxidation of hydroxylamine using ferric ammonium sulfate with a copper sulfate catalyst. This method is applicable in the millimolar range. In general, the previous methods employed were applicable only in media containing relative high concentrations of hydroxylamine or in instances where the analysis could be carried out

immediately after sampling.

Direct determination of hydroxylamine is difficult in natural waters because of its low concentration and extreme lability. Aqueous solutions will be oxidized quite readily by atmospheric oxygen. It has been reported that millimolar solutions of hydroxylamine are stable for several hours at pH 4.0 (FIADEIRO et al., 1967) but for only 60 minutes at pH 7.8 in the presence of air (ANDERSON, 1964). Furthermore, trace amounts of transition metal ions catalyze its autoxidation (JAMES, 1942; MOEWS and AUDRIETH, 1959; ANDERSON, 1964). Nevertheless, the relative kinetics of the two competing reactions-microbial production and autoxidation-may allow the accumulation of hydroxylamine in natural oxygenated waters.

In this paper, we present a new method for the determination of nanomolar concentrations of hydroxylamine in seawater by its immediate oxidation to nitrous oxide using Fe (III) ions, adapted from the method of RAO and RAO (1942). The nitrous oxide produced in this manner is subsequently measured by using electron capture gas chromatography. The method is linear in the range tested from 12 to 560 nM hydroxylamine with a relative standard deviation of 4% at the 12.4 nM level.

EXPERIMENTAL

Water samples were taken in either 125 or 25 cc Pyrex^R gas sampling flasks with Teflon^R stopcocks at either end, or 125 cc Pyrex^R reagent bottles with greased, ground glass stoppers. Care was taken in sampling to avoid contamination with atmospheric oxygen. All sample containers were previously washed with dilute (1:1) HCl and rinsed thoroughly with double distilled water. Hydroxylamine in the sample was immediately converted to nitrous oxide gas using a 40 mM reagent grade ferric ammonium sulfate solution to a final concentration of 200 μM Fe (III). Samples taken in 125 cc gas flasks or reagent bottles were transferred to 25 cc gas flasks immediately before analysis. Replicate samples taken in all three types of sample containers showed no appreciable difference in either nitrous oxide or hydroxylamine concentrations. No detectable nitrous oxide or hydroxylamine was lost during the transfer processes.

The nitrous oxide generated was measured by electron capture gas chromatography using the method of COHEN (1977) with minor modifications. Helium was used as the stripping gas instead of nitrogen and a Molecular Sieve-5A trap at liquid nitrogen temperatures was used to trap the nitrous oxide. A nitrous oxide blank for each sample was run consisting of a duplicate water sample without the addition of Fe (III) ions. This gave the original amount of dissolved nitrous oxide in the sample. Hydroxylamine was then calculated as ($\Delta N_2O \times 2$) to take into account the stoichiometry of the reaction, where ΔN_2O is the difference between the nitrous oxide present in the treated and untreated samples. Both treated and untreated samples were

poisoned with a 5% solution of reagent grade mercuric chloride to a concentration of 75 ppm Hg^{+2} in the 125 cc samples and 375 ppm Hg^{2+} in the 25 cc samples, immediately after sampling. Previous experiments performed in this laboratory have shown that $HgCl_2$ preserves nitrous oxide in seawater for at least 16 days. Additional storage experiments have shown that samples treated with the hydroxylamine-oxidizing reagents and $HgCl_2$ were also stable for this period of time. All samples were run within this time frame.

Hydroxylamine standards were run by the addition of a standard solution of reagent grade NH_2OH-HCl to the gas flask prior to the introduction of the sample into the stripping system. A yield of 50% was obtained for the conversion of hydroxylamine to nitrous oxide; after taking into consideration the stoichiometry of the reaction as generally accepted (RAO & RAO, 1942). The actual concentration of the primary standard (25 nM) was obtained using a modification of the titrimetric method described by Rao and Rao (1942). In our method, Fe (III) was reduced by the hydroxylamine and the resultant Fe (II) was titrated with reagent grade potassium dichromate using sodium diphenylaminesulfonate as the indicator.

Nitrous oxide standards for the gas chromatographic method were run by injecting a 25 ppm N_2O (in nitrogen) standard from a 0.5 cc gas loop into a stripper that had been purged of all dissolved nitrous oxide.

RESULTS AND DISCUSSION

A series of hydroxylamine standards ranging from 1.2 to 560 nM NH_2OH was run to obtain a calibration curve and to determine the efficiency of the conversion of hydroxylamine to nitrous oxide. Matrix and reagent blanks were run to determine the nitrous oxide and hydroxylamine levels of the water used to prepare the standards. Hydroxylamine values were usually undetectable and nitrous oxide concentrations were close to saturation values with respect to the atmosphere (6-9 nM) in these blanks.

Calibration curves were run on four different occasions using five different matrix water types. Standards were made up in double distilled water, artificial seawater, open ocean surface seawater from 14 miles off the Oregon coast and both filtered and unfiltered estuarine water from Yaquina Bay, Oregon. On all four occasions and with all water types used, the standard curve was linear in the concentration range measured and did not vary significantly from one water type to the next, as shown in Figure 3. Linear regression of the data points in these figures resulted in an intercept of 0.2 nM and a slope of 0.4988, i.e., a conversion factor of 50% from hydroxylamine to nitrous oxide. The limit of detection, as calculated from the precision of the blanks, was 0.6 nM at a 95% confidence level.

A number of experimental conditions were then varied in order to observe their effect on the conversion factor and to discover interferences with the method. Time of reaction does not appear to be a critical factor. Replicate standards samples were prepared and those run ten minutes after the addition of the oxidizing reagents were found

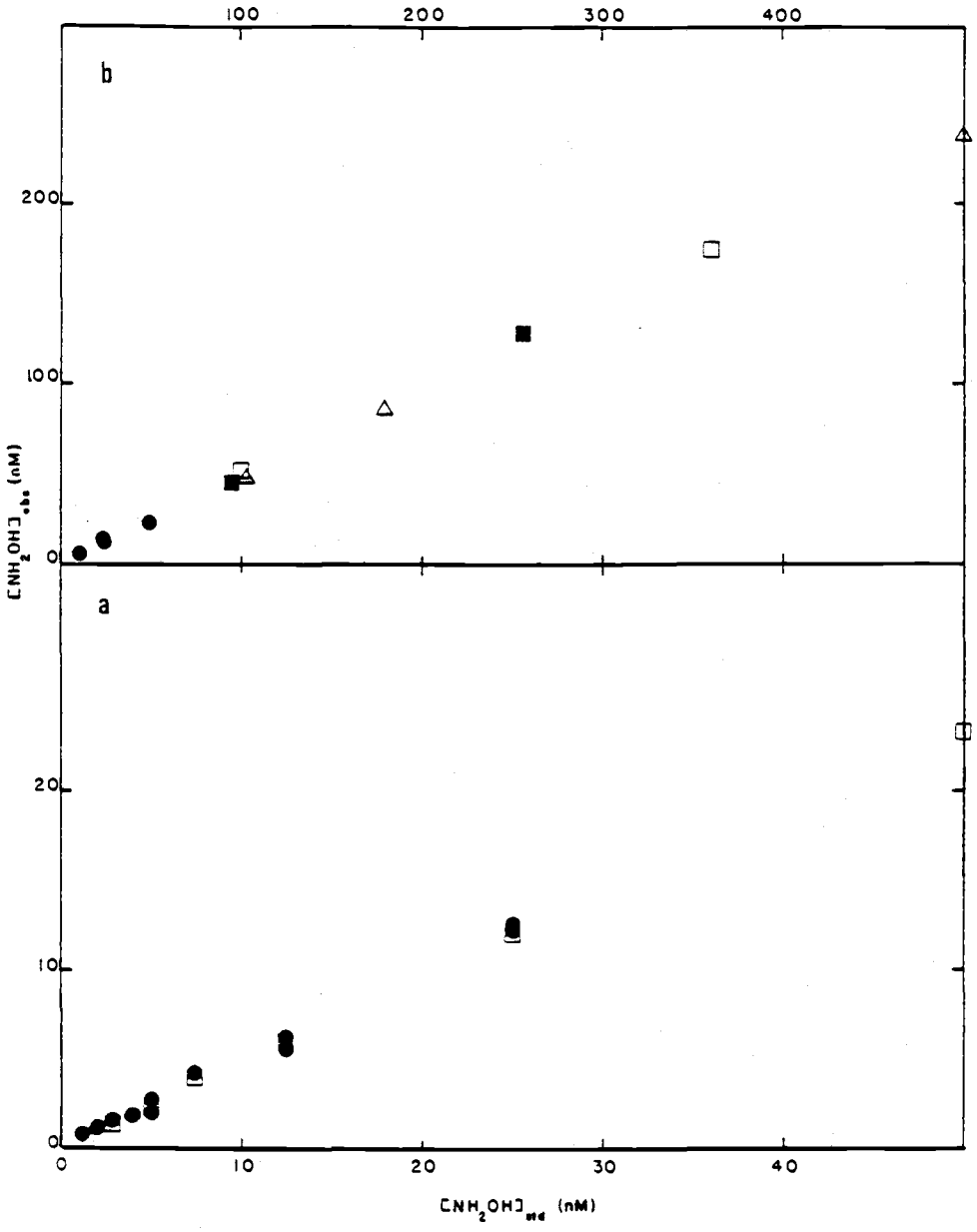

Figure 3. Calibration curves for NH_2OH standards (a) from 1.2 to 50 nM and (b) from 12.5 to 560 nM. Open triangles (△) represent standards run in double distilled water; closed circles (●) indicate standards run in open ocean seawater; open squares (□) indicate standards run in estuarine water; closed squares (■) represent standards run in artificial seawater.

to be identical, within experimental error, with those run two weeks later. Samples allowed to react at room temperature (22-25°C) yielded the same conversion factor as those allowed to react at approximately 40°C for the same period of time. Therefore, it appears that as long as the samples are poisoned with mercuric chloride and are treated with the hydroxylamine-oxidizing reagent immediately after sampling, they may be stored for a least two weeks at room temperature without adverse effects. Salinity also does not appear to affect the reaction. Results obtained with the addition of hydroxylamine standard to distilled water were identical to those obtained with estuarine water (salinity = 29°/oo) and seawater (salinity = 33°/oo). Hence, the method is applicable to aquatic systems other than seawater.

Since hydroxylamine is a reduced form of nitrogen, we investigated the possibility that ammonium ion in seawater might interfere with the method by also being oxidized by the Fe (III) ion. This was tested by analyzing duplicate standard hydroxylamine samples with and without the addition of ammonium ion to a final concentration of 8 μM, which is an upper limit to the range of concentrations observed from ammonia in the ocean. No significant difference in the production of nitrous oxide was observed with or without ammonium ion. In order to test the applicability of this method in anoxic environments, a sulfide solution was added to a standard water sample to a final concentration of 5 mM immediately before the addition of the oxidizing reagents. Again, no change in hydroxylamine concentrations was seen with or without the presence of sulfide.

Since it was reported (RAO and RAO, 1942; MOEWS and AUDRIETH,

1959) that Cu (II) ions catalyze the oxidation of hydroxylamine to nitrous oxide, a series of samples with and without the addition of Cu (II) ion to the final concentration of 2.5 mM was analyzed. Samples were measured from 5 minutes to 5 hours after the addition of the reagents. No difference was observed in the amount of nitrous oxide produced between the samples with and without Cu (II).

Varying the concentration of Fe (III) ion from 40 μM to 200 μM had no effect on the conversion factor. Cu (II) alone, without the addition of Fe (III) in natural seawater standard solutions, resulted in reduced conversions. The results indicate that Fe (III) must be added for 50% conversion of hydroxylamine to nitrous oxide to take place; 40 μM was the lowest Fe (III) concentration tested.

This method was tested on seawater samples in July, 1981, on the continental shelf fourteen miles off the Oregon coast. A profile of NH_2OH, N_2O and O_2 was taken from the surface to 160 meters (Figure 4). Duplicate samples of N_2O and NH_2OH were run for all samples and a relative standard deviation of 4% for hydroxylamine was obtained at concentrations higher than 5.0 nM.

The profile shows a maximum for hydroxylamine of 7.8 nM at 5 meters, corresponding to a dissolved oxygen concentration of 303 μM. Hydroxylamine then decreases to undetectable values from 50 to 160 meters. The vertical distribution of hydroxylamine is similar to that of oxygen and inversely correlated with that of nitrous oxide. The method has been applied to other regimes with similar success, and proven to be a practical method for the study of hydroxylamine in natural marine environments.

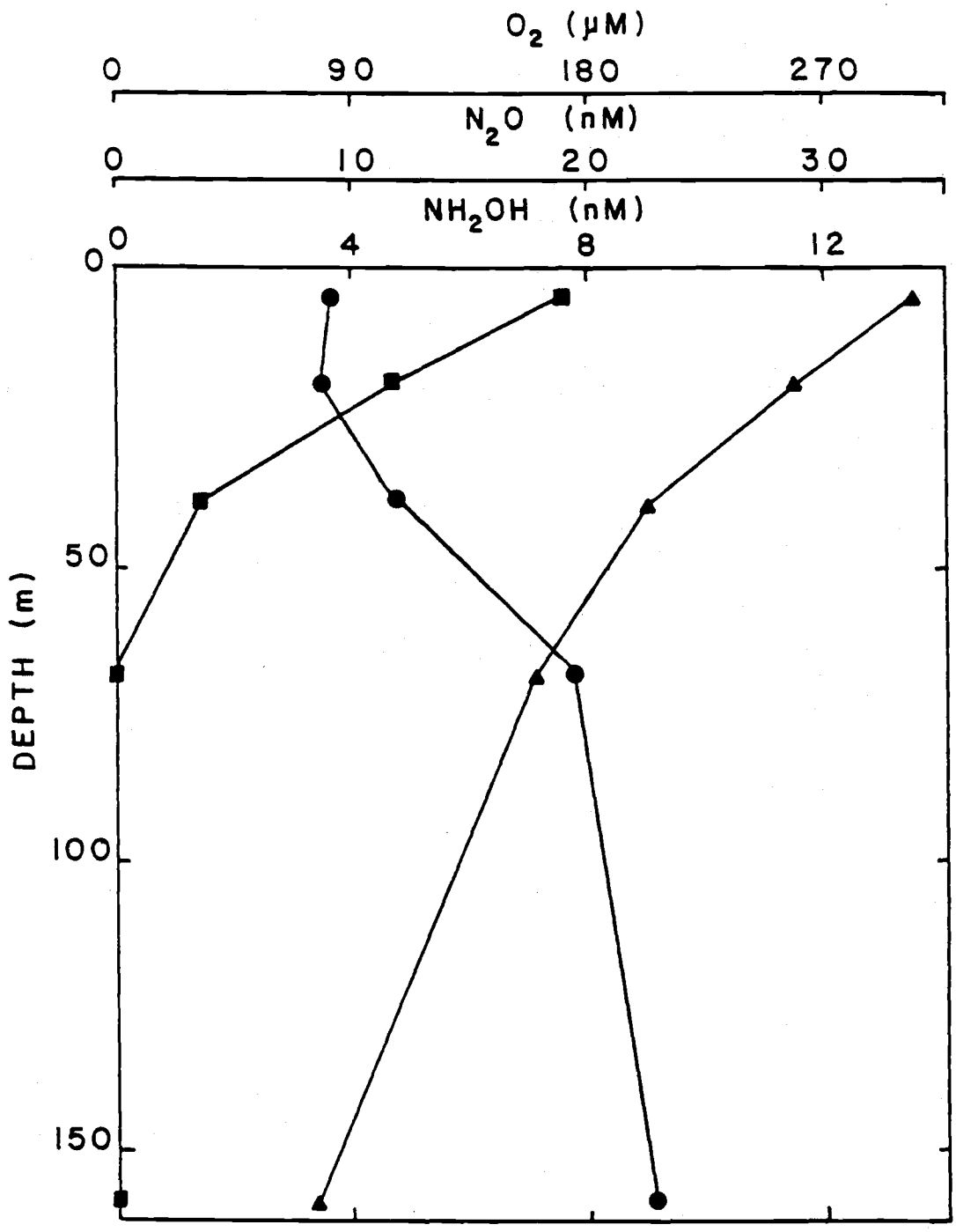

Figure 4. Vertical distributions of hydroxylamine (■), nitrous oxide (●), and oxygen (▲) at a station 14 miles off the Oregon coast.

CHAPTER II

MEASUREMENTS OF NH_2OH IN COASTAL AND ESTUARINE ENVIRONMENTS

ABSTRACT

The distributions of hydroxylamine, oxygen and inorganic nitrogen species in Saanich Inlet, an intermittently anoxic fjord, and at a station 14 miles off the Oregon coast were investigated. The results indicated that hydroxylamine is produced in the well oxygenated subsurface waters of these sampling sites, where nitrification takes place. In the oxygen depleted deep waters, hydroxylamine was observed to be correlated with an increase in the ammonia concentration and with a decrease in the nitrate and nitrite levels; this set of conditions suggest that build up of hydroxylamine in low oxygen systems is related to microbial nitrate reduction.

Production of hydroxylamine in Yaquina Bay, a coastal plain estuary, is also reported. It varies systematically with the other nitrogen species studied near tidal flats and commercial oyster beds. The complexity of the system, and the scarcity of data make it difficult to determine the specific mechanisms which led to the accumulation of hydroxylamine in this environment.

The concentrations of hydroxylamine observed on all of the environments studied ranged from undetectable to 9.6 nM.

MEASUREMENTS OF HYDROXYLAMINE IN COASTAL AND ESTUARINE ENVIRONMENTS

INTRODUCTION

Hydroxylamine has been known for a long time to be an intermediate in the biological nitrogen cycle. As early as 1926, KLUYVER and DONKER postulated hydroxylamine as a likely intermediate in the oxidation of ammonia to nitrite. Since then a number of studies have been carried out with chemoautotrophs to show the formation of hydroxylamine during nitrification (YOSHIDA and ALEXANDER, 1971; RAJENDRAN and VENUGOPALAN, 1976). Although the biochemical mechanism of nitrification is still poorly defined, it is now generally accepted (CORBET, 1935, COOPER, 1937, HUGHES, 1975) that the oxidation of ammonia to nitrite proceeds via two electron changes with hydroxylamine as an intermediate in the following sequence:

$$\underset{-3}{NH_3} \longrightarrow \underset{-1}{NH_2OH} \longrightarrow \underset{+1}{X} \longrightarrow \underset{+3}{NO_2^-}$$

The oxidation of ammonia to hydroxylamine has been demonstrated with the use of intact cells (ENGEL and ALEXANDER, 1958). Further oxidation of hydroxylamine to nitrite will occur only in well aerated environments. In less oxygenated waters, however, nitrous oxide will be evolved (FALCONE et al., 1963).

The accumulation of hydroxylamine in well oxygenated waters would generally not be expected. The biological rate of oxidation of hydroxylamine is comparable to that of ammonia (HOFMAN and LEES, 1953). However, there is evidence that specific inhibitors affect the rate of

oxidation of ammonia to hydroxylamine, and retard the further oxidation of hydroxylamine, allowing for the accumulation of the latter. Autoxidation of hydroxylamine can also remove it from oxygenated waters. However, the relative kinetics of all the processes taking place may be such that accumulation of hydroxylamine in natural oxygenated waters could occur.

Microbially mediated hydroxylamine production occurs not only during nitrification, but also during the formation of ammonia from nitrate (JENSEN, 1951; TANAKA, 1953). The pathway between nitrite and ammonia is not yet well elucidated, however, a sequence of two-electron transfers has been suggested (PATEMAN, et al., 1967).

$$\underset{+5}{NO_3^-} \longrightarrow \underset{+3}{NO_2^-} \longrightarrow \underset{+1}{X} \longrightarrow \underset{-1}{NH_2OH} \longrightarrow \underset{-3}{NH_3}$$

The study of the distribution of hydroxylamine, and its relationship with other nitrogen species, could be useful in understanding the nitrogen cycle in the marine environment. Few measurements of hydroxylamine in natural environments have been reported, most probably due to the problems associated with the previous methods of analysis. TANAKA (1953) and KOYAMA and TOMINO (1967) have reported accumulation of hydroxylamine in a relatively deep lake during a period of marked stratification. Hydroxylamine was only detected at low oxygen levels and this finding was attributed to bacterial reduction of nitrate. PITWELL (1975) reported measurable concentrations of hydroxylamine in relatively deep lakes with reedy or swampy shorelines, and in rivers with quiet flows and relatively narrow and deep beds. He also found the hydroxylamine to be destroyed by rapids, presumably by autoxidation.

I used a new analytical procedure for the measurement of hydroxylamine in seawater by electron capture gas chromotography (VON BREYMANN, DE ANGELIS and GORDON, 1982) to carry out preliminary studies of the distributions of hydroxylamine in coastal and estuarine environments. The objective was to elucidate the relationship of hydroxylamine distributions with those of other nitrogen species and pertinent oceanographic variables. The areas of study were Saanich Inlet (Figure 5), an intermittently anoxic fjord on Vancouver Island, British Columbia, a station 14 miles off the Oregon coast, and Yaquina Bay, a coastal plain estuary on the Oregon coast (Figure 6) during the summer and fall of 1981.

This is the first set of profiles taken for hydroxlamine in the marine environment. These and the concurrent data on oxygen, and other nitrogen species lead to some hypotheses on the mechanisms controlling the hydroxylamine distributions.

METHODS

Experimental Sites

The objective of this study was to observe the distributions of hydroxylamine in environments covering a wide range of salinity and oxygen levels. To achieve this, two kinds of sampling patterns were employed: deep casts in areas of varying dissolved oxygen concentrations, and surface sampling in a coastal plain estuary.

Environments of varying dissolved oxygen concentrations would provide clues to the processes controlling hydroxylamine levels in natural systems. For this reason I selected areas where both an upper oxic layer, as well as low oxygen zones were known to occur. Since it was expected that hydroxylamine would be found in areas of high bacterial activity coastal and estuarine environments were selected and sampling was done during the summer and fall months.

The Oregon coastal waters were sampled during a period when the expected upwelling and resulting biomass increase would favor bacterial growth. Our observations were made during three separate trips to a station 14 miles off the Oregon coast, at 124°25' W and 44°58' N, on July 23, September 10 and October 1, 1981. The station is 180 m deep, located just off the continental shelf break. HUYER (1977) described the physical oceanography of the general area, and the average chemical characteristics were studied by ATLAS, GORDON and TOMLINSON (1977).

In Saanich Inlet, a shallow sill at its northern end restricts replenishment of the deep water, allowing for the development of anoxic conditions during the summer months. Flushing by dense oxygenated

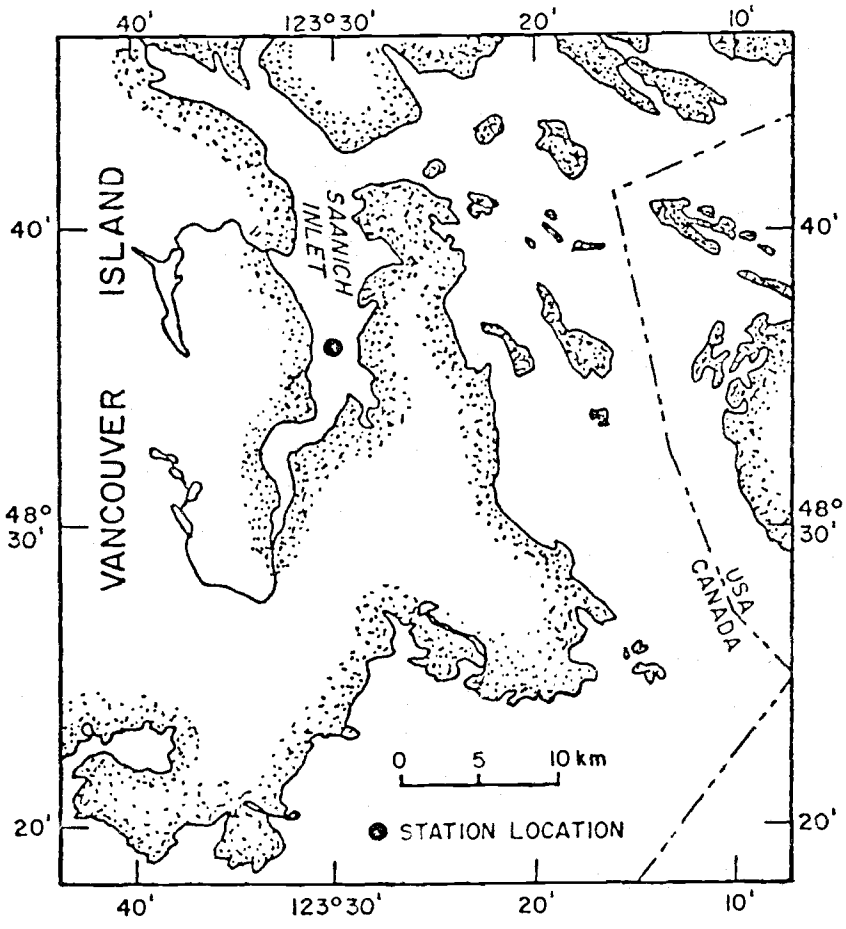

Figure 5. Location map, Saanich Inlet, British Columbia

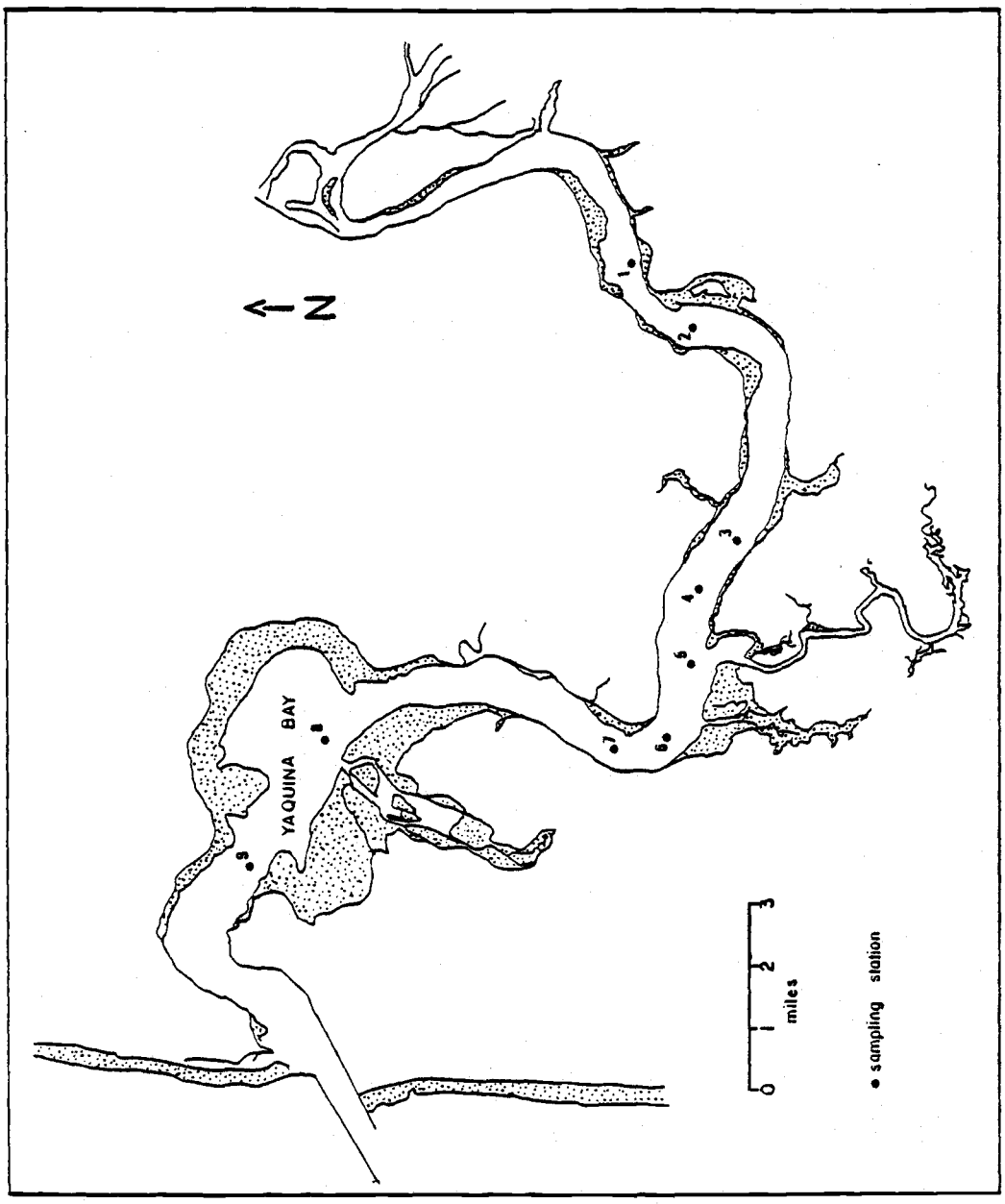

Figure 6. Location map, Yaquina Bay, Oregon.

waters from the Strait of Georgia usually occurs in late summer or fall (ANDERSON and DEVOL, 1973). HERLIVEAUX (1962) described the general hydrography of this fjord. Saanich Inlet was sampled at a station located at 48°36' N and 123°30' W, as shown in Figure 5, on June 25, 1981.

Yaquina Bay on the Oregon coast was sampled on July 27, 1981 to obtain an estuarine distribution of hydroxylamine (Figure 6). It is a drowned river valley, or a coastal plain estuary. Based on observed circulation and salinity patterns, BURT and MCALISTER (1959) classified it as being well to partially well mixed. The relatively large tidal range and low river runoff prevailing for most of the year prevent stratification. The tidal regime is semi-diurnal.

Sampling

During the coastal trips samples were collected in 10 ℓ PVC samplers, in Saanich Inlet 10 ℓ Niskin bottles were used, and 5 ℓ Niskin bottles were used for the Yaquina Bay study. Samples for analysis of gases and hydroxylamine were transferred immediately after collection to 125 cc or 25 cc Pryex^{R} gas sampling flasks with Teflon^{R} stopcocks. Hydroxylamine samples were also taken in 125 cc Pyrex reagent bottles with greased ground glass stoppers. All these samples were poisoned with a 5% solution of reagent grade $HgC\ell_2$ to a concentration of 75 ppm Hg^{+2} in the 125 cc samples and to 375 ppm Hg^{+2} in the 25 cc samples. A 40 mM reagent grade ferric ammonium sulfate solution was added (to the hydroxylamine samples) to a final concentration of 200 μM Fe^{+3}. After addition of the reagents all samples were shaken to insure

thorough mixing. Storage experiments have shown that samples treated in this way result in stable concentrations of both nitrous oxide and hydroxylamine for a period of at least 16 days (VON BREYMANN, DE ANGELIS and GORDON, 1982). All samples were run within these time periods. Nutrient samples were frozen immediately after collection in dry ice, and were kept in a freezer until thawed for analysis.

Analytical Methods

The method used for the determination of hydroxylamine (VON BREYMANN, DE ANGELIS and GORDON, 1982) involves the oxidation of hydroxylamine with ferric ions, immediately after sampling, and the subsequent measurement of the resulting nitrous oxide by electron capture gas chromatography using the method of COHEN (1977) with minor modifications. A duplicate sample to which no Fe (III) was added was used to correct for the the background nitrous oxide present in the sample. The limit of detection for hydroxylamine as calculated from the precision of the banks was 0.6 nM at the 95% confidence level. The standard deviation was 4% at 12.5 nM.

Nutrients were measured using an Autoanalyzer system (GORDON et al., 1975). The average standard deviations found for nitrate, nitrite and ammonia were 0.02, 0.01, and 0.1 μM respectively. Dissolved oxygen was measured using the CARPENTER (1965) modification of the Winkler method, with an average standard deviation of 0.8 μM. The standard deviations were calculated from sets of duplicate determinations of samples taken from the same bottle sampler. Salinities were obtained using a Guildine Autosal^{R} model 8400 with an instrumental precision

of ± 0.001°/oo. Temperatures were obtained with reversing thermometers for the Saanich Inlet study, general use laboratory thermometers with one division per degree Celcius were used for the Yaquina Bay survey, and a thermistor bridge for the work off the Oregon coast.

RESULTS AND DISCUSSION

Vertical Distributions

The concentrations of hydroxylamine observed in vertical profiles off the Oregon coast and in Saanich Inlet ranged from non-detectable to 9.6 nM. The profiles are shown in Figure 7. The data for hydroxylamine and other variables are given in the Appendix.

A. Surface and subsurface oxygenated waters

The surface values ranged from undetectable to 5.6 nM. During September off the Oregon coast, there was no detectable hydroxylamine in the surface sample. However, by a depth of 5 m a concentration of 2.4 nM had developed. This indicates that a marked concentration gradient can occur in the upper surface layers. Note there is no surface data for the July cruise, however, the above observations indicate that a subsurface value does not necessarily reflect the surface concentration and therefore the July data are not usable in consideration of surface effects.

The undetectable hydroxylamine concentration reported for the September trip off the coast coincided with very choppy surface water. Experiments in our laboratory have shown that hydroxylamine in standard solutions are almost completely destroyed when the solution is stirred rapidly in an open container for as little as three minutes. This is in agreement with the chemical behavior reported for this compound (ANDERSON, 1964; FIADIERO et al., 1967), and PITWELL'S (1975) finding that hydroxylamine was destroyed presumably by oxidation in the river rapids. On the other hand, during the October cruise off the Oregon

Figure 7. Vertical profiles of hydroxylamine in Saanich Inlet on June 25, 1981; and off the Oregon coast on July 23, September 10 and October 1, 1981.

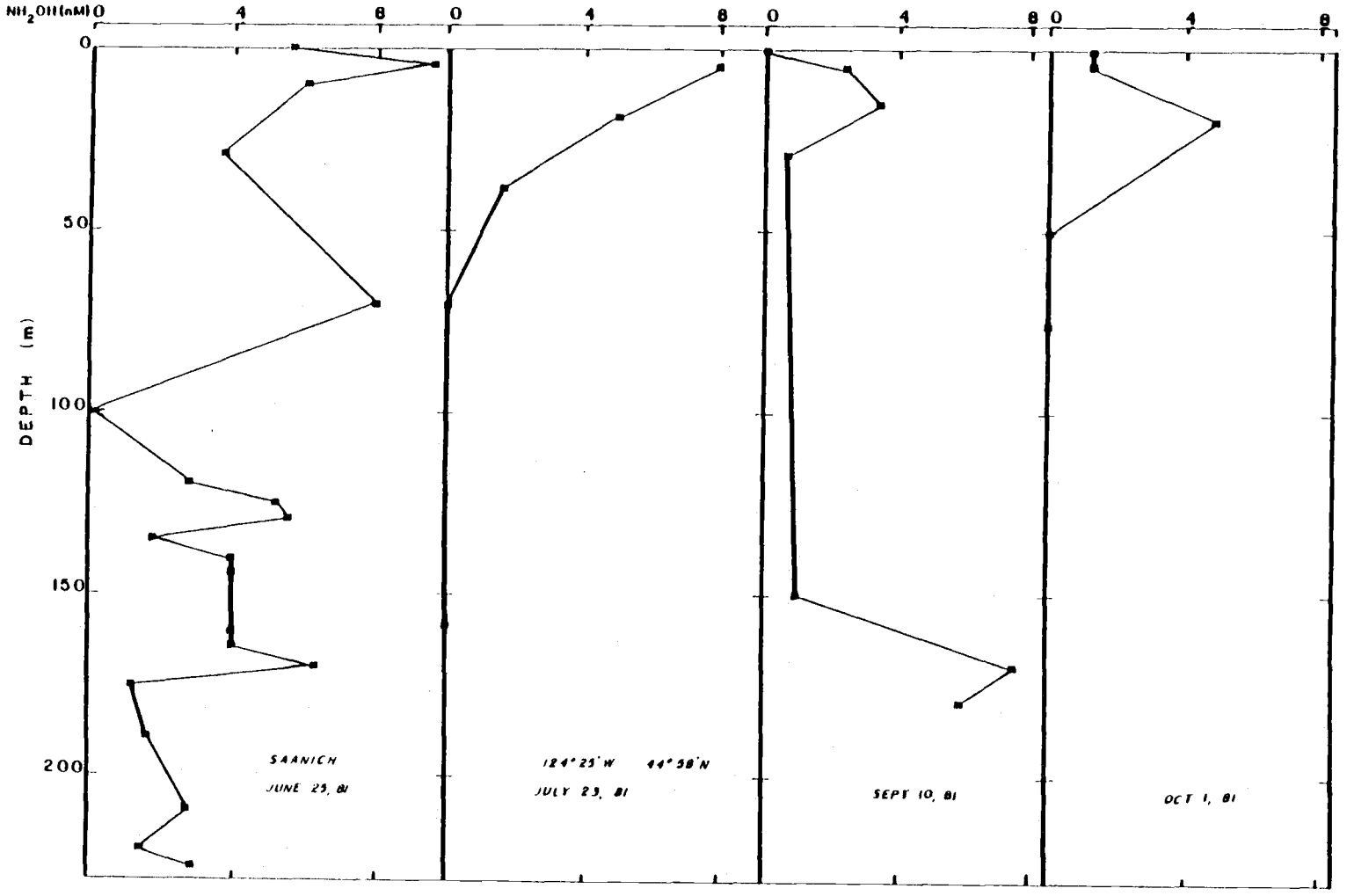
NH2OH(nM)
0
4
8
DEPTH (m)
0
50
100
150
200
SAANICH
JUNE 25, 81
124°25'W 48°58'N
JULY 23, 81
SEPT 10, 81
OCT 1, 81

Figure 7

coast and the Saanich Inlet work, the water surface was calm. In these instances measurable hydroxylamine concentrations were observed.

These observations lead to the hypothesis that the surface concentrations are determined by weather and water surface conditions - a choppy surface may result in the autoxidation of surface hydroxylamine concentrations causing a marked gradient in the upper surface layers.

Characteristic of all of the profiles is the presence of a subsurface maximum between 5 and 20 m depth which usually coincides with the oxygen maximum. Note the relationship of this maximum with the behavior of the other nitrogen species. In the Saanich Inlet profile (Figure 8) the hydroxylamine maximum occurred at 5 m, followed by maxima in the nitrite concentration at 15 m, and in the nitrate concentration at 30 m. The nitrite and nitrate maxima in Saanich Inlet have been discussed previously (COHEN, 1978).

In this study off the Oregon coast, there was also a subsurface maximum in the hydroxylamine concentration, followed by a nitrite maximum (Figures 9-11). In the profiles obtained for the October trip, the sequence of maxima of ammonia and hydroxylamine, then nitrite and nitrate was present. BRANDHORST (1959) postulated ammonia oxidation by nitrifying bacteria as the source of the primary nitrite maximum near the bottom of the euphotic zone in well oxygenated waters. Based on ^{15}N tracer studies OLSON (1981a) concluded that the main source of nitrite in oxygenated waters is the oxidation of ammonia by nitrification. It is therefore likely that the presence of a subsurface maximum in the hydroxylamine profiles is the result of nitrification. Futhermore, concurrent measurements of the nitrification potential activities (oxidation

Figure 8. Vertical profiles of hydroxylamine (■), oxygen (▲), nitrous oxide (●), ammonia (●), nitrite (▲) and nitrate (■) in Saanich Inlet, June 25, 1981. The bottom depth was 227 m.

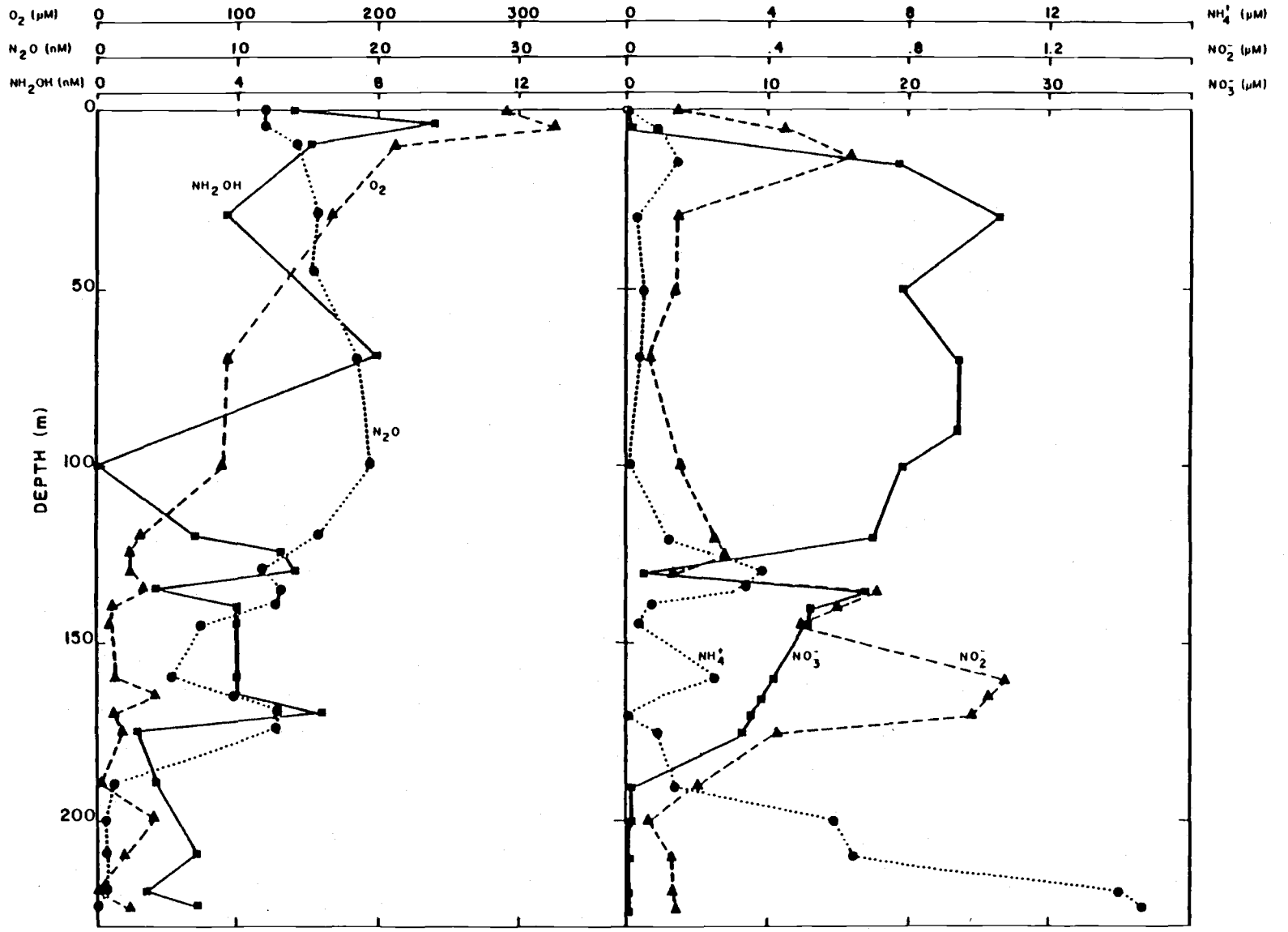
O_2 (μM)
N_2O (nM)
NH_2OH (nM)
NH_4^+ (μM)
NO_2^- (μM)
NO_3^- (μM)
DEPTH (m)
NH_2OH
O_2
N_2O
NH_4^+
NO_3^-
NO_2^-

Figure 8.

Figure 9. Vertical profiles of hydroxylamine (■), oxygen (▲), nitrous oxide (●), ammonia (●), nitrite (▲) and nitrate (■) off the Oregon coast on July 23, 1981. The bottom depth was 180 m.

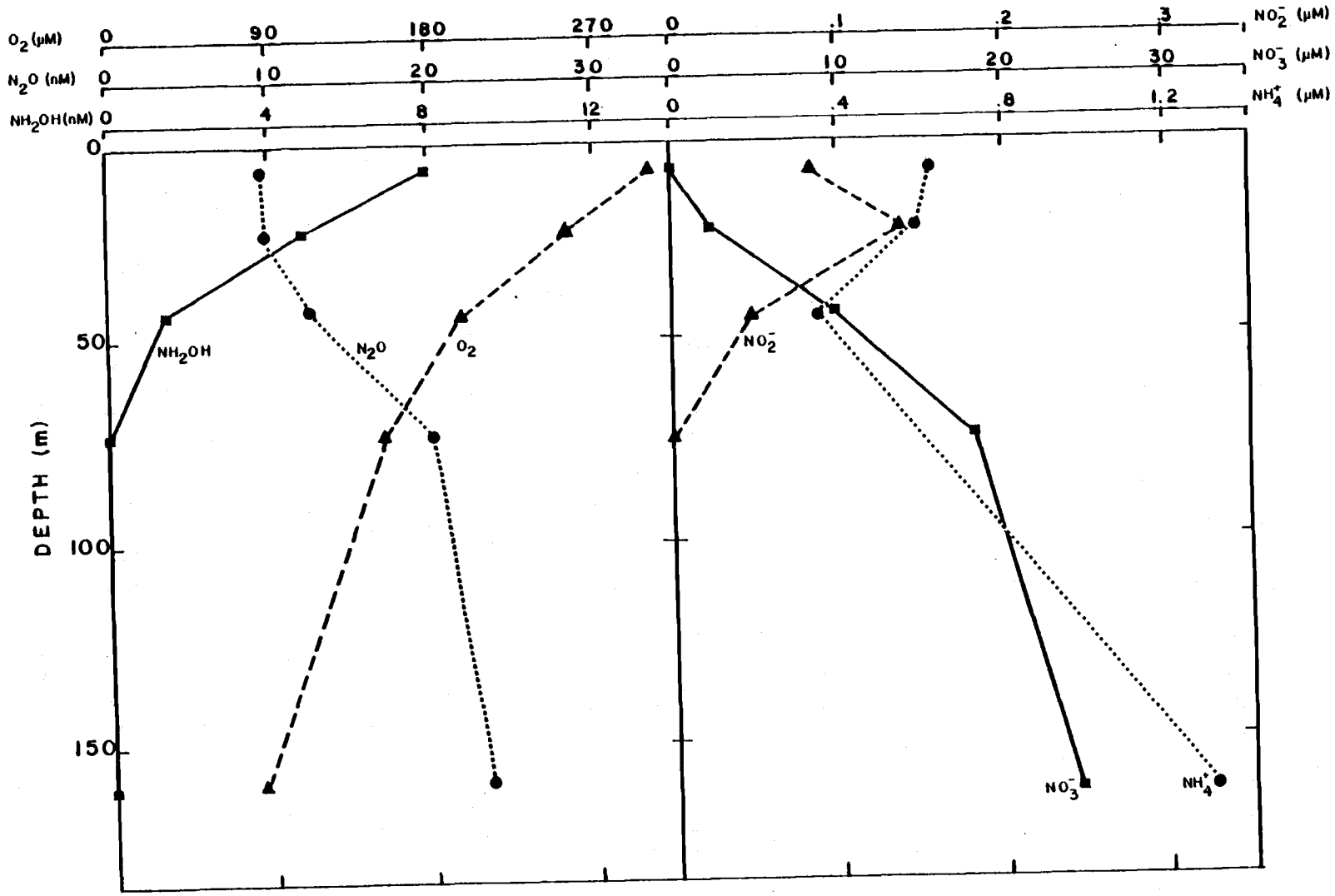
O_2 (μM) 0 90 180 270
N_2O (nM) 0 10 20 30
NH_2OH(nM) 0 4 8 12
0 1 2 3 NO_2^- (μM)
0 10 20 30 NO_3^- (μM)
0 .4 .8 1.2 NH_4^+ (μM)
DEPTH (m)
0
50
100
150
NH_2OH
N_2O
O_2
NO_2^-
NO_3^-
NH_4^+

Figure 9.

Figure 10. Vertical profiles of hydroxylamine (■), oxygen (▲), nitrous oxide (●), nitrite (▲) and nitrate (■) off the Oregon coast on September 10, 1981. Bottom depth was 180 m.

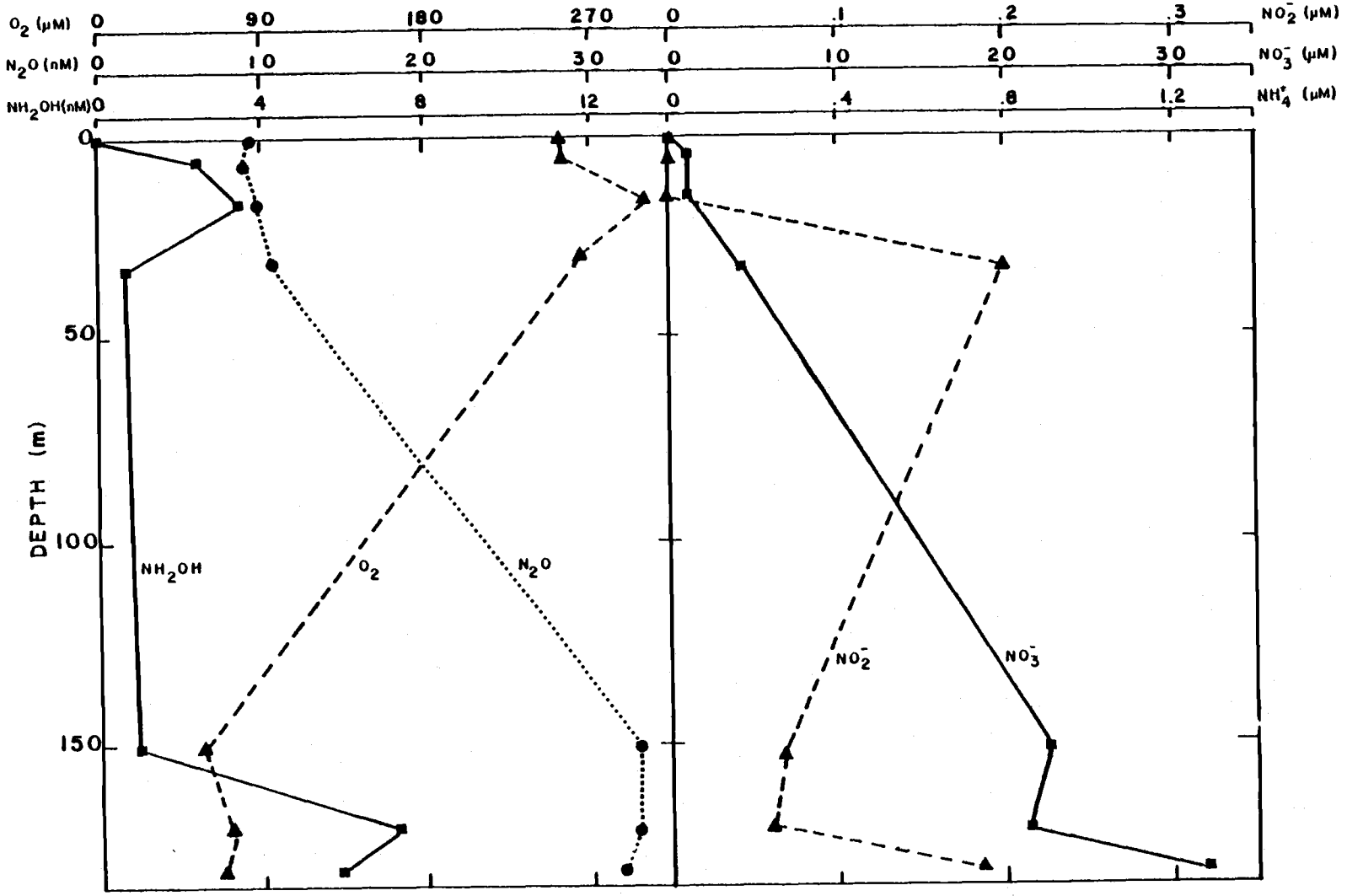
O_2 (μM) 0 90 180 270
N_2O (nM) 0 10 20 30
NH_2OH(nM) 0 4 8 12
NO_2^- (μM) 0 .1 .2 .3
NO_3^- (μM) 0 10 20 30
NH_4^+ (μM) 0 .4 .8 1.2
DEPTH (m)
0
50
100
150
NH_2OH
O_2
N_2O
NO_2^-
NO_3^-

Figure 10.

Figure 11. Vertical profiles of hydroxylamine (■), oxygen (●), nitrous oxide (▲), ammonia (●), nitrite (▲) and nitrate (■) off the Oregon coast on October 1, 1981. Bottom depth was 180 m.

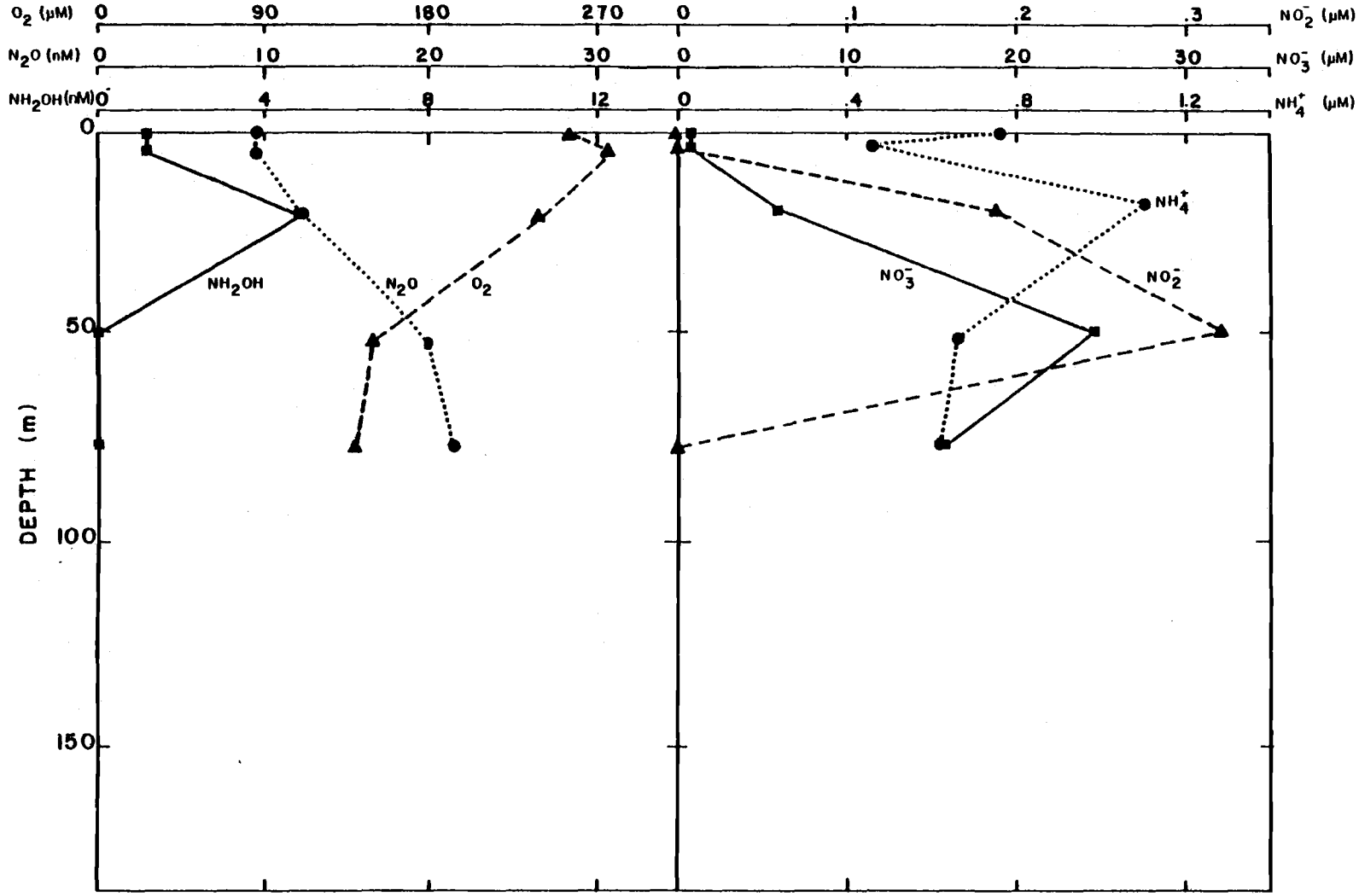
O_2 (μM) 0 90 180 270
N_2O (nM) 0 10 20 30
NH_2OH (nM) 0 4 8 12
0 .1 .2 .3 NO_2^- (μM)
0 10 20 30 NO_3^- (μM)
0 .4 .8 1.2 NH_4^+ (μM)
0
50
100
150
DEPTH (m)
NH_2OH
N_2O
O_2
NO_3^-
NH_4^+
NO_2^-

Figure 11.

of NH_4^+ to NO_2^-) for the Saanich Inlet samples (DE ANGELIS, personal communication) showed a maximum in this depth zone.

In the series of samples taken off the coast, the depth of the hydroxylamine subsurface maximum varies with time, being at 5 m in July, at 15 m in September and at 20 m in October. OLSON (1981b) found evidence for photoinhibition of the autotrophic oxidation of ammonia in coastal waters off the California coast. He reported (OLSON, 1981a) that the rate of ammonia oxidation decreased with increased exposure to light. Consequently the nitrite maximum moved to deeper zones from June to October. A similar mechanism could account for the observed variations in the depth of the hydroxylamine maximim in the samples off the coast.

Below the subsurface maximum, the levels of hydroxylamine decreased to undetectable levels. This is expected since in this area of the water column the nitrification rates have decreased and the lability of the compound is such that it will not exist for a prolonged period of time in oxygenated waters. However, there is a maximum in the Saanich Inlet profile at 70 m accompanied by variations in the concentrations of other species at this depth. A slight increase in the nitrite level, and a decrease in the nitrate concentration at 50 m, were observed. The missing point for hydroxylamine at 50 m, (the sample was lost during transportation) and lack of a duplicate for the hydroxylamine sample at 70 m makes it harder to reject or explain this point.

B. Low oxygen deep waters

In the low oxygen deep waters, variations in the hydroxylamine

concentrations were also observed. In the Saanich Inlet profile there were maxima at 130 m and at 170 m, while another maximum was observed at 170 m in the September cruise off the Oregon coast.

Two of these maxima, off the coast at 170 m, and in Saanich Inlet at 170 m coincided with singularities in the concentrations of the other nitrogen species in a way that seems to indicate the involvement of hydroxylamine in microbial nitrate reduction. In low oxygen waters, two types of nitrate reduction are known to occur: assimilatory, or the process of nitrate reduction via nitrite to ammonia, which ends up as cell nitrogen, and dissimilatory nitrate reduction, which is a respiratory process. Denitrification is a kind of dissimilatory nitrate reduction in which nitrate is reduced to nitrous oxide and molecular nitrogen. Denitrification has been identified as taking place in this area at low oxygen levels (COHEN, 1978). However, some nitrate reduction may be taking place concurrently, leading to formation of ammonia. It has been shown that ammonia formation via nitrate reduction is an important process in coastal anaerobic sediments in Japanese bays (KOIKE and HATTORI, 1978). Determination of ammonia, molecular nitrogen and particulate organic nitrogen using ^{15}N tracers demonstrated the simultaneous occurrence of denitrification and bacterial reduction of nitrate in anaerobic marind coastal environments.

During the September station off the Oregon coast, the hydroxylamine maximum at 130 m occurred simultaneously with decreases in nitrite and nitrate concentrations. The hydroxylamine maximum in the Saanich Inlet profile at 130 m also coincided with minima in the

nitrite and nitrate profiles. At this depth, there was also a maximum in the ammonia concentration. The above observations suggest that these maxima are the result of microbiological nitrate reduction, which is in agreement with the findings reported by KOYAMA and TOMINO (1967) in some stratified lakes.

The situation observed at 170 m in the Saanich Inlet profiles, is different, however. Here the hydroxylamine maximum coincided with increases in the nitrous oxide and nitrite concentrations, and a decrease in the ammonia levels. There is a slight increase in the oxygen concentration at 170 m, which could be the result of partial flusing of the Inlet. Concurrent measurements of the nitrification potential activities showed a maximum at this depth zone (DE ANGELIS, personal communication). Therefore, it seems likely that this hydroxylamine maximum is the result of nitrification. Nitrification at low oxygen levels can lead to nitrous oxide production (FALCONE et al., 1962; HUGHES, 1975) which accounts for the nitrous oxide maximum observed at this depth.

Surface Distributions

The levels of NH_2OH observed during the surface sampling cruise in Yaquina Bay, Oregon ranged from undetectable to 4.8 nM (Figure 12). The NH_2OH concentrations and other variables measured are given in the Appendix.

Concentrations of NH_2OH of approximately 1 nM were observed in the two extreme locations sampled: station 9, the station located closest to the mouth and station 1, located ten miles upstream from the mouth.

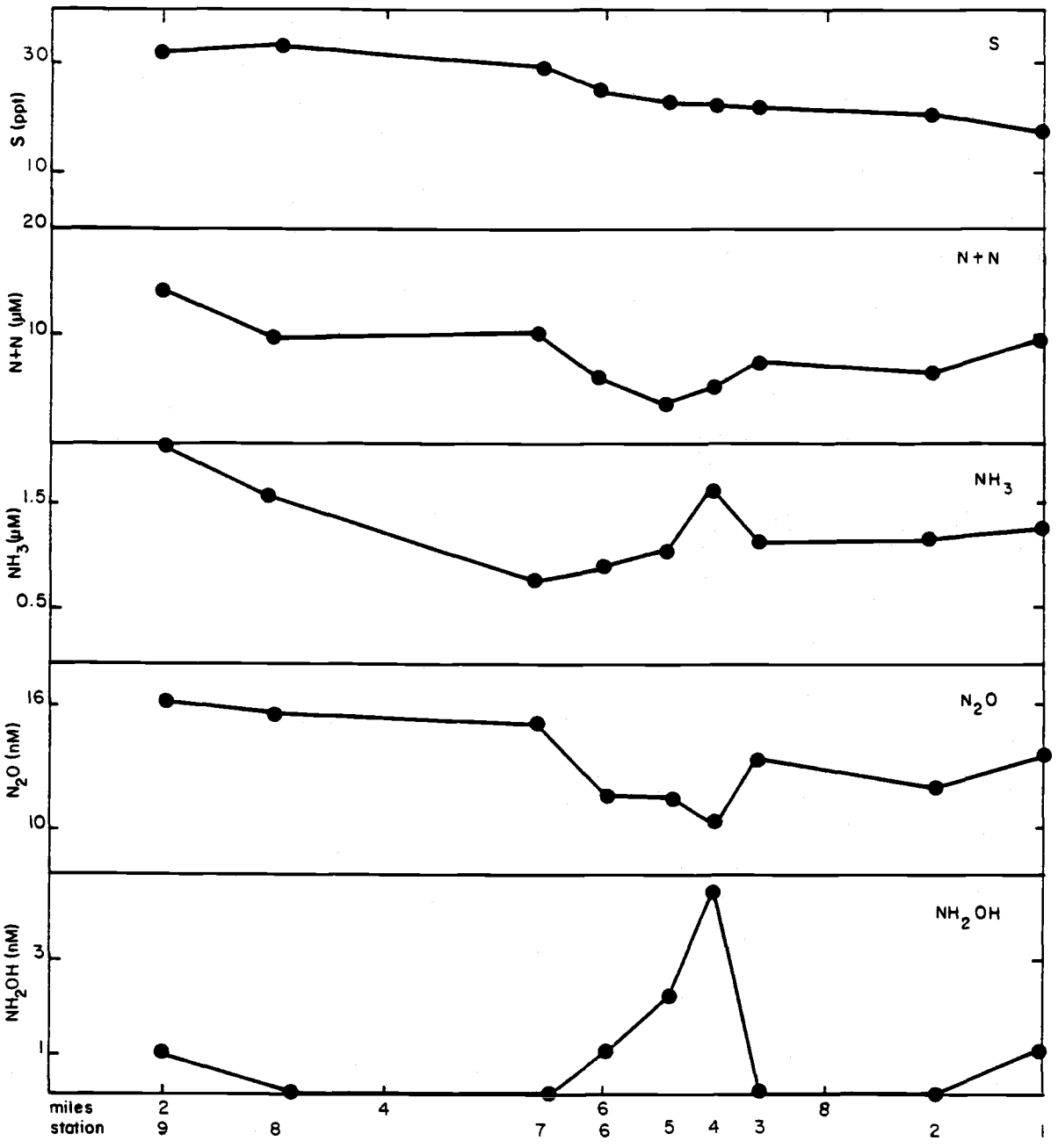

Figure 12. Surface distributions of salinity, (nitrate + nitrite), ammonia, nitrous oxide, and hydroxylamine in Yaquina Bay, Oregon, on July 27, 1981.

The hydroxylamine concentrations observed at station 9 could be due to the influence of incoming seawater. The subsurface maxima discussed in the previous section related to the samples off the Oregon coast may also have been present in the water column outside the Bay at the time of this survey.

Station 1 is located in a area of very slow flow, and it is the shallowest station sampled (approximately 0.5 m depth). Bacterial activity occurring in the sediments could be the source of the hydroxylamine present in the water sample. The greater abundance of organic matter and consequently higher bacterial population in the sediments has been held responsible for the greater denitrification (TERRY and NELSON, 1975) and nitrification (CURTIS et al., 1975) activity in the sediments of fresh water and estuarine systems. Studies on diffusion of NH_2OH from sediments and mixing processes in a particular area of study would be necessary to draw any definite conclusions on this matter.

From Figure 12, a marked increase in the hydroxlymine concentration between river miles six and eight was observed. A three point transect across the estuary showed an increase in hydroxylamine concentrations from south to north. Tidal flats and commercial oyster beds are located on the north shore of this area. Previous nutrient data (U.S. GEOLOGICAL SURVEY, 1974) have shown drastic variations of nitrate and ammonia concentrations within short time period in this area (Figure 13). The maximum in the hydroxylamine concentration could be the result of net production in this area. However, the complexity of the system and the scarcity of data make it difficult to determine the specific

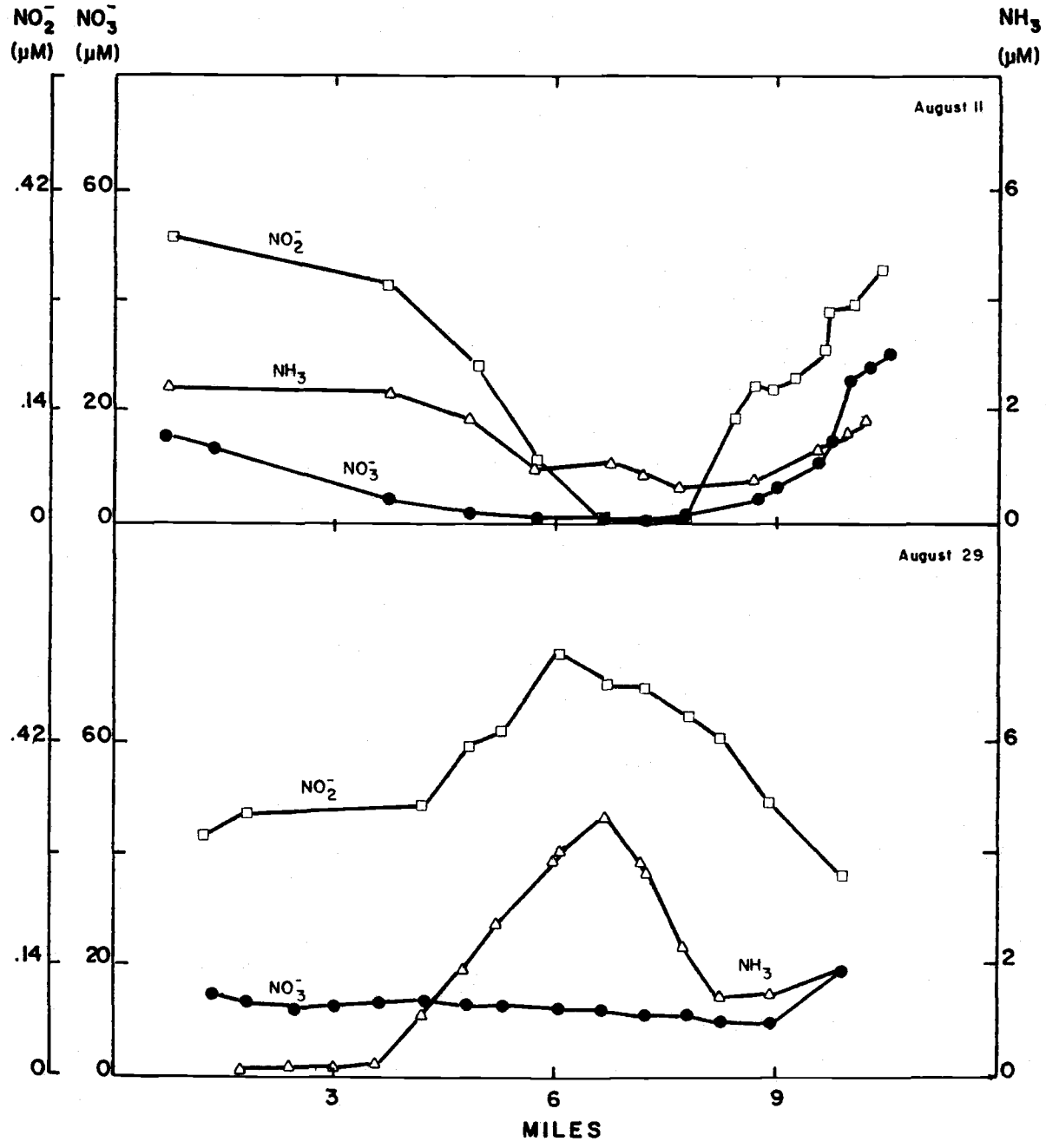

Figure 13. Surface distributions of nitrogen nutrients on August 11, and August 29, 1974 in Yaquina Bay, Oregon. (U.S.G.S. unpublished data)

mechanisms taking place.

During the study, the maximum in hydroxylamine concentration in this area coincided with a maximum in ammonia, and a slight decrease in the nitrate + nitrite concentrations. No separate nitrite data were available. The presence of this identifiable zone of activity could be explained by the presence of the oyster beds, which constitute an appropriate environment for intensive bacterial growth. Variations in the retention time of the water due to the characteristic circulation of the estuary, average depth (TUFFEY et al., 1974), and sediment texture (WAGNER and SMITH, 1960; FOCHT and VERSTRAETE, 1977) are other factors that could lead to the observed zonation.

CONCLUSIONS

It has been shown that NH_2OH concentration levels of 0.6 to 560 nM can be measured when gastight seawater samples are treated with ferric ammonium sulfate immediately after collection. The nitrous oxide formed, with an efficiency of 50% can then be measured using electron capture gas chromatography with an overall precision of 4% at the 12.5 nM level. Samples can be stored for at least a week before analysis.

Concentrations of hydroxylamine at nanomolar levels have been measured in the water columns of three different environments: coastal waters, a coastal plain estuary and an intermittently anoxic fjord. Variations at the station off the coast were recorded from July to October 1981. Hydroxylamine concentrations ranging from undetectable levels to 9.6 nM were observed.

Hydroxylamine was found to vary in a systematic way with other variables, reflecting its involvement in the nitrogen cycle. The presence of subsurface maxima was observed in all the profiles between 5 and 20 m depth. It usually occurred in the area of the oxygen maxima, followed by the primary nitrite maxima, thus suggesting that the presence of the subsurface maxima in the hydroxylamine profiles is the result of nitrification.

In the low oxygen deep waters, an increase in the hydroxylamine concentrations occurred simultaneously with a marked decrease in the nitrite and nitrate levels and an increase in the ammonia concentration, which indicates the involvement of hydroxylamine in the microbial reduction of nitrate to ammonia.

A different situation is observed however in the low oxygen waters of Saanich Inlet at 170 m depth. Here, the hydroxylamine maximum occurred simultaneously with increases in the nitrous oxide, nitrite and oxygen levels. A partial flushing of the inlet could account for this increase in the oxygen concentration. Nitrification in these circumstances can lead to nitrous oxide production with hydroxylamine as an intermediate.

Additional research suggested by this study include:

- the effect of water surface conditions on hydroxylamine concentrations.
- the effect of light levels on hydroxylamine subsurface maxima.
- the effects of the presence of oyster beds and tidal flats on hydroxylamine distributions and on the nitrogen cycle activity in Yaquina Bay.
- the effect of nitrification and nitrate reduction taking place in the sediments on hydroxylamine concentrations in the overlying water.

BIBLIOGRAPHY

BIBLIOGRAPHY

Anderson, J. H. (1964) Copper-catalysed oxidation of hydroxylamine. Analyst 89, 357-362.

Anderson, J. J. and A. H. Devol (1973) Deep water renewal in Saanich Inlet, an intermittently anoxic basin. Estuar. Coastal Mar. Sci. 1, 1-10.

Atlas, E. L., L. I. Gordon and R. D. Tomlinson (1977) Chemical characteristics of Pacific Northwestern coastal waters - nutrients, salinities, seasonal fluctuations. In R. Krauss (ed.) The Marine Plant Biomass of the Pacific Northwest Coast. Oregon State Univ. Press. pp. 57-79.

Brandhorst, W. (1959) Nitrification and denitrification in the eastern tropical North Pacific. J. Cons. Perm, Int. Explor. Mer. 25, 2-20.

Burt, W. V. and W. B. McAlister (1959) Recent studies in the hydrography of Oregon estuaries. Oregon Fish Commission Research Briefs 7, 14-27.

Carlucci, A. F. and P. M. McNally (1969) Denitrification by marine bacteria in low concentrations of substrate and oxygen. Limnol. Oceanog. 14, 736-739.

Carlucci, A. F. and H. R. Schubert (1969) Nitrite reduction in sea water of the deep nitrite maximum off Peru. Limnol. Oceanog. 14, 187-193.

Carpenter, J. H. (1965) The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnol. Oceanog. 10, 141-143.

Chen, R. L., D. R. Keeney, D. A. Graetz and A. J. Holding (1972) Denitrification and nitrate reduction in Wisconsin lake sediments. J. Environ. Qual. 1, 158.

Cohen, Y. (1977) Shipboard measurement of dissolved nitrous oxide in seawater by electron capture gas chromatography. Anal. Chem. 49, 1238-1240.

Cohen, Y. (1978) Consumption of dissolved nitrous oxide in an anoxic basin, Saanich Inlet, British Columbia. Nature 272, 235-237.

Cole, J. A., K. L. Coleman, B. E. Compton, B. M. Kavanagh and C. W. Keevil (1974) Nitrite and ammonia assimilation by anaerobic continuous cultures of Escherichia Coli. J. Gen. Microbiol. 85, 11-22.

Cooper, L. H. N. (1937) The nitrogen cycle in the sea. J. Mar. Biol. Assoc. U. K. 22, 183-204.

Corbet, A. (1935) The formation of hyponitrous acid as an intermediate compound in the biological and photochemical oxidation of ammonia to nitrous acid. I. Chemical Reactions. Biochem. J. 28, 1575-1582.

Curtis, E. J. C., K. Jurrant and M. M. Harman (1975) Nitrification in rivers in the Trent Basin. Water Res. 9, 255.

Darke, D. J. and W. E. W. Roediger (1980) Method for the measurement of hydroxylamine in colonic fluid using derivatisation and gas chromatography. J. Chromatog. 181, 449-452.

Engel, M. S. and M. Alexander (1958) Growth and autotrophic metabolism of Nitrosomonas europaea. J. Bacteriol. 76, 217.

Falcone, A. B., A. L. Shug and D. J. D. Nicholas (1963) Some properties of a hydroxylamine oxidase from Nitrosomonas europaea. Biochim. Biophys. Acta 77, 199-208.

Fiadeiro, M., L. Solórzano and J. D. H. Strickland (1967) Hydroxylamine in seawater. Limnol. Oceanog. 12, 555.

Focht, D. D. and W. Verstraete (1977) Biochemical Ecology of Nitrification and Denitrification. In M. Alexander (ed.) Advances in Microbial Ecology. Vol. 1, pp. 135-214.

Garret, R. H. (1972) The induction of nitrite reductase in Neurospora crassa. Biochim. Biophys. Acta 264, 481-489.

Goering, J. J. (1968) Denitrification in the oxygen minimum layer of the eastern tropical Pacific Ocean. Deep-Sea Res. 15, 157-164.

Goering, J. J. and R. C. Dugdale (1966) Denitrification rates in an island bay in the equatorial Pacific Ocean. Science 154, 505-506.

Gordon, L. I., C. N. Dahm, W. H. Dickinson. P. K. Park and D. R. Standley (1975) A precision oceanographic nutrient data system. In Proceedings of the UNOLS Working Conference on Oceanographic Data Systems, Woods Hole Oceanographic Institution, Nov. 12-14, pp. 38-56.

Gundersen, K. (1966) The growth and respiration of Nitrosocystis oceanus at different partial pressures of oxygen. J. Gen. Microbiol. 42, 387-396.

Gundersen, K., A.F. Carlucci and K. Bostrom (1966) Growth of some chemoautotrophic bacteria at different oxygen tensions. Experientia 22, 229-230.

Herliveaux, R. H. (1962) Oceanography of Saanich Inlet in Vancouver Island, British Columbia. J. Fish. Res. Board Canada 19, 1-37.

Hoffman, T. and H. Lees (1953) The biochemistry of nitrifying organisms. 4. The respiration and intermediary metabolism of Nitrosomonas. Biochem. J. 54, 579-583.

Hughes, M. N. (1975) Nitrogen Fixation and the nitrogen cycle. In The Inorganic Chemistry of Biological Processes, J. Wiley and Sons, London. pp. 200-229.

Hughes, M. N. and H. G. Nicklin (1970a) The chemistry of peroxynitrites Part I. Copper (II) catalysed reaction between hydroxylamine and peroxynitrite in alkali. J. Chem. Soc. (A), 925.

Hughes, M. N. and H. G. Nicklin (1970b) A possible role for the species peroxynitrite in nitrification. Biochim. Biophys. Acta 222, 660-661.

Hughes, M. N. and H. G. Nicklin (1971) Autoxidation of hydroxylamine in alkaline solutions. J. Chem. Soc. (A). 164-168.

Huyer, A. (1977) Seasonal variations in temperature, salinity and density over the continental shelf off Oregon. Limnol. Oceanog. 22, 442-453.

James, T. H. (1942) Effect of catalysis upon the oxidation products of hydroxylamine. J. Amer. Chem. Soc. 64, 731-735.

Jensen, H. L. (1951) Nitrification of oxime compounds by heterotrophic bacteria. J. Gen. Microbiol. 5, 360-368.

Kluyver, A. J. and H. J. L. Donker (1926) Die Einheit inder Biochemic. Chem. Zelle Gwebe. 13, 134-190.

Koike, I and A. Hattori (1978) Denitrification and ammonia formation in anaerobic coastal sediments. Appl. Environ. Microbiol. 35, 278-282.

Kolasa, T. and W. Wardenky (1974) Quantitative determination of hydroxylamine. Talanta 21, 854-857.

Koyama, T. and T. Tomino (1967) Decomposition process of organic carbon and nitrogen in lake water. Geochem. J. 1, 109.

Latimer, W. M. (1952) Oxidation Potentials. Second Edition. Prentice Hall, Englewood Cliffs, New Jersey.

Lipman, J. G. (1908) Bacteria in relation to country life. Macmillan, New York.

Moews, P. C. and L. F. Audrieth (1959) The autoxidation of hydroxylamine. J. Inorg. Nucl. Chem. 11, 242-246.

Miyazaki, T., E. Wada and A. Hattori (1973) Capacities of shallow waters of Sagami Bay for oxidation and reduction of inorganic nitrogen. Deep Sea Res. 20, 571-577.

Olson, R. (1981a) ^{15}N Tracer studies of the primary nitrite maximum. J. Mar. Res. 39, 204-226.

Olson, R. (1981b) Differential photoinhibition of marine nitrifying bacteria, A possible mechanism for the formation of the primary nitrite maximum. J. Mar. Res. 39, 227-238.

Pateman, J. A., B. M. Rever and D. J. Cove (1967) Genetic and biochemical studies of nitrate reduction in Aspergilius nidulans. Biochem. J. 104, 103.

Payne, W. J. (1981) Denitrification. John Wiley and Sons, Inc., New York.

Pitwell, L. R. (1975) The determination of hydroxylamine in Ethiopian rivers and lakes. Mikrochimica Acta 425, 429.

Prakash, O. and J. C. Sadana (1972) Purification, characterization and properties of nitrate reductase of Achromobacter fischeri. Arch. Biochem. Biophys. 148, 614-632.

Rajendran, A. and V. K. Venugopalan (1976) Hydroxylamine formation in laboratory experiments on marine nitrification. Mar. Chem. 4, 93-98.

Rao, K. B. and G. G. Rao (1942) Determination of hydroxylamine-use of copper sulfate as a catalyst. Z. Anal Chem. 157, 100.

Tanaka, M. (1953) Occurrence of hydroxylamine in lake waters as an intermediate in bacterial reduction of nitrate. Nature 171, 1160.

Terry, R. E. and D. W. Nelson (1975) Factors influencing nitrate transformations in sediments. J. Environ. Qual. 4, 549.

Tuffey, T. J., J. V. Hunter and V. A. Matulewich (1974) Zones of nitrification. Water Res. Bull. 10, 555.

U.S. Geological Survey (1974). Unpublished data.

Verstraete, W. and M. Alexander (1972) Heterotrophic nitrification by Arthrobacter species. J. Bacteriol. 110, 955-961.

Verstraete, W. and M. Alexander (1973) Heterotrophic nitrification of samples of natural ecosystems. Environ. Sci. Technol. 7, 39-42.

von Breymann, M. T., M. A. de Angelis and L. I. Gordon (1982) Gas chromatography with electron capture detection for determination of hydroxylamine in seawater. Anal. Chem. 54, 1209-1210.

Wada, E. and A. Hattori (1971) Nitrite metabolism in the euphotic layer of the central North Pacific Ocean. Limnol. Oceanog. 16, 766-772.

Wada, E. and A. Hattori (1972) Nitrite distribution and nitrate reduction in deep sea waters. Deep Sea Res. 19, 123-132.

Wagner, G.H. and G. E. Smith (1960) Recovery of fertilizer nitrogen from soils. Missouri Agric. Exp. Sta. Res. Bull. 738, 1.

Webb, K. L. and W. J. Wiebe (1975) Nitrification on a coral reef. Can. J. Microbiol. 21, 1427.

Winogradsky, S. (1890) Sur les organismes de la nitrification. C. R. Acad. Sci., Paris 110, 1013.

Yordy, D.M. and E. A. Delwiche (1979) Nitrite reduction in Veiollonella alcalescens. J. Bacteriol. 137, 905.911.

Yordy, D. M. and K. L. Ruoff (1981) Dissimilatory nitrate reduction to ammonia. In C. C. Delwiche (ed.) Denitrification, nitrification and atomspheric nitrous oxide. John Wiley and Sons, New York.

Yoshida, T. and M. Alexander (1964) Hydroxylamine formation by Nitrosomonas europaea. Can. J. Microbiol. 10,923-926.

Yoshida, T. and M. Alexander (1971) Hydroxylamine oxidation by Nitrosomonas europaea. Soil Sci. 111, 307.

Zarowny, D. P. and B. D. Sanwal (1963) Characterization of a nicotinamide adenine dinucleotide-specific nitrite reductase from Escherichia coli strain K-12. Can. J. Microbiol. 9, 531-539.

APPENDIX

DATA LISTINGS

The following notations are used in the data listing:

Z	Depth in meters
Stn	Station number (for the Yaquina Bay samples only). See figure 6 for station locations.
T	Temperature in degrees Celcius.
S	Salinity in parts per thousand.
O_2	Dissolved oxygen in micromoles per liter.
NO_3^-	Nitrate in micromoles per liter.
NO_2^-	Nitrite in micromoles per liter.
NH_3	Ammonia in micromoles per liter.
NH_2OH	Hydroxylamine in micromoles per liter.
N_2O	Nitrous oxide in nanomoles per liter.
N+N	Nitrate plus nitrite in micromoles per liter.
$[NH_2OH]_{std}$	Hydroxylamine standard concentration in nanomoles per liter.
$[NH_2OH]_{obs}$	Hydroxylamine concentration observed in nanomoles per liter.
nd	non-detectable concentrations.

For replicate measurements the mean value is given.

References to the analytical procedures used are made in chapters one and two.

CALIBRATION CURVE FOR THE ANALYSIS OF HYDROXYLAMINE BY ELECTRON CAPTURE GAS CHROMATOGRAPHY, AFTER OXIDATION TO NITROUS OXIDE.

$[NH_2OH]_{std}$ (nM)	$[NH_2OH]_{obs}$ (nM)	Observations
105	47	
180	88	double distilled water
500	241	
550	279	
94	47	artificial sea-water
254	128	
100	51	
250	122	Yaquina Bay water
360	187	
560	287	
1.2	0.6	
2.0	0.8	
2.5	1.3	
4.0	2.0	Seawater from 124°25'W 44°58'N
5.0	2.2	
7.5	4.6	
12.5	5.9	
25.0	12.9	
50.0	22.7	
2.5	1.3	
5.0	3.0	Yaquina Bay water
7.5	4.0	
12.5	6.1	
25.0	11.8	
50.0	21.9	
1.2	0.8	
2.5	1.2	
5.0	3.4	Seawater from 124°25'W 44°58'N
7.5	4.3	
12.5	6.2	
25.0	12.6	

OREGON COASTAL SAMPLES

124°25'W 44°58'N JULY 23, 1981

Z (m)	T (°C)	S (ppt)	O_2 (μM)	NO_3^- (μM)	NO_2^- (μM)	NH_3 (μM)	N_2O (nM)	NH_2OH (nM)
5	13.5	30.72	301	0.13	0.08	0.59	9.3	7.6
20	10.0	--	256	3.10	0.14	0.67	8.9	4.9
40	9.1	32.59	202	10.10	0.06	0.38	11.9	1.5
70	7.8	33.46	170	17.90	nd	--	18.8	nd
160	6.9	33.98	93	24.00	nd	2.27	25.3	nd

OREGON COASTAL SAMPLES

124°25'W 44°58'N SEPTEMBER 10, 1981

Z	T	S	O_2	NO_3^-	NO_2^-	NH_3	N_2O	NH_2OH
(m)	(°C)	(ppt)	(μM)	(μM)	(μM)	(μM)	(nM)	(nM)
0	--	31.488	258	nd	nd		8.2	nd
5	15.4	31.484	259	0.87	nd		7.9	2.4
15	10.5	32.302	311	nd	nd		9.5	3.6
30	9.4	32.440	265	4.46	0.20		10.8	1.6
150	7.3	33.941	54	22.50	nd		29.0	1.2
170	7.1	33.968	63	20.11	0.06		28.8	7.0
180	7.1	33.963	63	31.72	0.15		28.6	5.6

OREGON COASTAL SAMPLES

124°25'W 44°48'N

OCTOBER 1, 1981

Z	T	S	O_2	NO_3^-	NO_2^-	NH_3	N_2O	NH_2OH
(m)	(°C)	(ppt)	(μM)	(μM)	(μM)	(μM)	(nM)	(nM)
0	--	31.600	261	0.51	nd	0.61	9.3	1.2
5	14.4	31.600	281	0.25	nd	0.44	9.5	1.2
20	12.0	32.142	246	7.72	0.18	1.04	12.0	4.8
50	8.1	33.2164	157	24.30	0.32	0.65	18.2	nd
75	8.0	33.714	146	14.57	nd	0.79	20.8	nd

YAQUINA BAY SAMPLES JULY 27,1981

Stn #	T (°C)	S (ppt)	O_2 (μM)	N+N (μM)	NO_2^- (μM)	NH_3 (μM)	N_2O (nM)	NH_2OH (nM)
1	21.0	18.02	233	9.61	--	1.18	12.4	1.2
2	20.0	21.53	249	6.10	--	1.12	11.9	nd
3	18.0	23.83	---	6.78	--	1.11	13.3	nd
4	20.5	23.30	231	5.16	--	1.53	10.2	4.8
5	20.5	23.68	253	3.84	--	1.05	11.7	2.4
6	19.5	25.03	256	5.97	--	0.83	11.7	0.8
7	15.5	30.31	235	10.30	--	0.76	15.3	nd
8	12.0	32.83	272	9.70	--	1.48	15.7	nd
9	14.0	31.91	251	13.90	--	2.0	16.3	0.8