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^{15}N Tracer studies of the primary nitrite maximum[1]

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ABSTRACT

^{15}N tracers were used in sub-tropical and Antarctic waters to study the production of nitrite from nitrate and ammonia, and the uptake of nitrate and ammonia. It was found that ammonia was the predominant source of nitrite in the primary nitrite maximum in sub-tropical waters and in the Ross Sea in summer, while nitrate and ammonia were both important in the Scotia Sea in early spring. Mean turnover times for nitrite ranged from 13 days off Southern California to 50 days in the Scotia Sea.

Light had a stimulating effect on phytoplankton nitrite production from nitrate and an inhibitory effect on ammonia oxidation and nitrite oxidation by nitrifying bacteria. Nitrate reduction by phytoplankton may be important as a source of nitrite in situations where nitrate is present in near-surface waters; in most situations, however, nitrite uptake by phytoplankton outweighs its production by this source.

A half-saturation constant value of 0.07 μM was determined for a natural population of nitrite oxidizers, and the results of several experiments indicate that the K_s for natural populations of ammonia oxidizers is similarly low or lower. The data suggest that variations in rates of nitrification with depth largely account for the observed nitrite profiles.

1. Introduction

A subsurface maximum in nitrite concentration occurs over wide areas of the tropical and temperate oceans. At higher latitudes nitrite is often found through-

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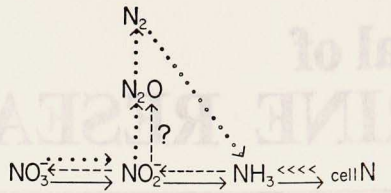

Figure 1. Marine nitrogen cycle processes, showing the intermediate position of nitrite. Direct evidence for nitrous oxide (N_2O) production during marine nitrification is still lacking. Key: • • • • • = denitrification; ——— = assimilation; o o o o o = nitrogen fixation; <<<<< = ammonification; – – – – – = nitrification.

out the upper water column and sometimes forms seasonal subsurface maxima, depending on the stability of the water column. Rakestraw (1936) suggested that study of nitrite distributions is of importance in understanding the marine nitrogen cycle because of the intermediate position of nitrite in the processes of nitrogen assimilation and regeneration and in denitrification (Fig. 1). Brandhorst (1959) suggested that nitrite near the bottom of the euphotic zone in well-oxygenated waters (the primary nitrite maximum) was the result of ammonia oxidation by nitrifying bacteria, while Vaccaro and Ryther (1960) postulated that excretion of nitrite by phytoplankton during nitrate reduction could be the source of the nitrite. It has also been suggested that nitrate reduction by bacteria could be important, either aerobically (Hattori and Wada, 1971) or in microzones of low oxygen concentration (Gundersen, 1977).

Field studies in which changes in nitrite concentration in incubated samples were measured by a sensitive chemical method led to the conclusion that ammonia was the major source of nitrite at the depth of the nitrite maximum in the central North Pacific Ocean (Wada and Hattori, 1971). Miyazaki *et al.* (1973, 1975), using an ^{15}N-tracer method, concluded that both nitrate and ammonia were sources for nitrite in the euphotic zone in Sagami Bay and in the western North Pacific.

In the work reported here the rates of nitrite production in the nitrite-containing layer from the central North Pacific gyre, Southern California coastal waters, and Antarctic waters have been estimated using ^{15}N tracers. In addition, estimates of nitrite-utilizing processes (nitrite uptake and nitrite oxidation to nitrate) have been made. The results have been obtained from *in situ* (IS) or simulated *in situ* (SIS) incubations, in contrast to previous work.

2. Methods and materials

a. Station locations. The results discussed below were obtained during several different cruises. The central North Pacific gyre work was done aboard cruise

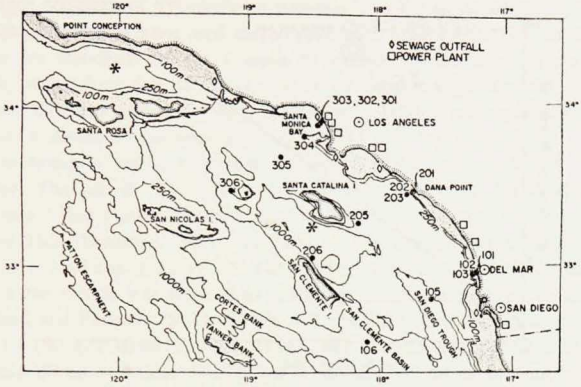

Figure 2. Location of Southern California coastal stations (SCBS = •; *Thompson* = *; small boat station ≅ SCBS 103).

IndoPac-15 (June 1977) at 30N, 155W. The Antarctic work was done on the U.S.C.G.C. *Glacier* cruise to the Ross Sea (December 1977-January 1978) and on A.R.A. *Islas Orcadas* cruise 17 to the Scotia Sea (September-October 1978); these stations were given in a previous paper (Olson, 1980). The Southern California coastal work was done on several Southern California Bight Studies (SCBS) cruises, a cruise of the R.V. *T. G. Thompson,* and small boat sampling trips to a station three miles off Scripps Institution of Oceanography (SIO) in 500 m of water (See Fig. 2). Some experiments were also done using water samples from the SIO pier.

b. ^{15}N tracer methods. Nitrite and nitrate production and N uptake were estimated by determining the ^{15}N content of the component of interest after incubation of the sample in the presence of an ^{15}N-labelled precursor. Samples were collected using a 30-liter or 5-liter Niskin bottle. After incubation, the particulate matter was collected on a precombusted (500°C for 4 hours) microfine glass fiber filter (Reeve-Angel 984-H). The filter was dried and the particulate N was converted to N_2 by the micro-Dumas combustion procedure of Wada *et al.* (1977) for $^{15}N/^{14}N$ determination in a mass spectrometer.

The filtrate was analyzed for nitrite or nitrate production by a variation on the method first described by Wada and Hattori (1972). The method is based on the formation of an azo dye compound whose N=N moiety consists of one N atom derived from an added reagent and one N atom derived from ambient nitrite. This compound is extracted from the seawater sample and converted to N_2 gas, which is labelled wtih ^{15}N according to the proportion of the nitrite which was

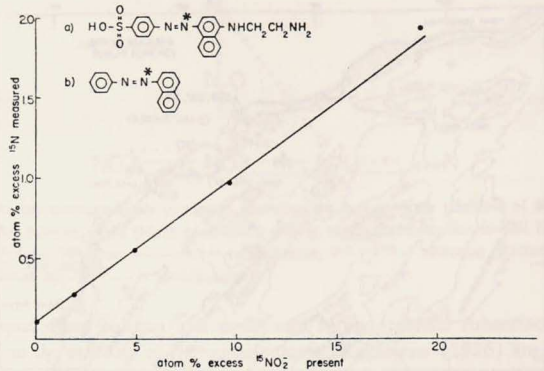

Figure 3. Measured ^{15}N enrichment of nitrite samples with varying amounts of ^{15}N-nitrite added, using the sulfanilic acid/NEDA system. The structures of the labelled compound and the modified compound formed using the aniline/β-napthol system are shown, with the nitrite-N identified by (*).

labelled. Several variations on the original method were used during this study, with varying sensitivity. In the original method sulfanilic acid and N-(1-napthyl)-ethylenediamine (NEDA) were used to form the dye, and it was extracted from the seawater samples by using an ion-exchange column (Dowex 1×8). Although standard curves with $^{15}NO_2^-$-spiked samples gave linear results (Fig. 3), the sensitivity was low due to dilution of the label by excess N in the NEDA moiety of the dye and in excess reagent carried over with the extraction procedure. Substitution of aniline and beta-naphthol for the original reagents reduced the dilution of label by excess N in the dye molecules to the minimum value possible (reagent-N:nitrite-N = 1:1), and washing the extracted dye to remove excess aniline resulted in a total dilution factor of 2.0. The present method as outlined below is essentially similar to that described by Schell (1978).

To four-liter seawater samples, 24 ml of aniline (5 ml/liter of 2N HCl) are added and mixed. After 10 minutes, 24 ml of beta-naphthol (5 g/liter of 3N NaOH) are added and mixed. After another 15 minutes, 5 ml of concentrated HCl are added to re-acidify the sample; the dye is then extracted by shaking with 80 ml of chloroform. The extract is washed with 100 ml volumes of 1N HCl (twice) to remove excess aniline, 2N NaOH (twice) to remove excess beta-naphthol, and distilled water. The washed extract is placed in a 100 ml beaker with a pre-combusted glass fiber filter (GF/C) and the chloroform evaporated. The ^{15}N-containing dye remains on the filter, which is treated as a particulate ^{15}N sample.

Standard additions of ^{15}N-nitrite to seawater samples extracted in parallel with the incubated samples were used to calibrate each experiment. The coefficient of variation for standards (at the 2 atom % excess ^{15}N level), using the various methods, ranged from 6% for the *Glacier* cruise samples to 1% for the *Thompson* cruise and laboratory samples (using the present method); corresponding standard deviations in absolute rates were about 0.5 and 0.1 nM/day, respectively.

The oxidation of nitrite to nitrate was measured by an extension of the same technique. The added ^{15}N-nitrite tracer was destroyed after the incubation by adding to a 1-liter seawater sample 1 ml of 20% sulfamic acid and 2 ml of concentrated HCl (Bremner, 1965). The pH of the sample was then adjusted to above 10 (by adding 4 ml of 8N NaOH) to inactivate the sulfamic acid. The nitrate in the sample was reduced to nitrite by passage through a Cd-Cu column (Strickland and Parsons, 1972) (10 cm × 1 cm I.D.) after the addition of 30 ml of NH_4Cl (50 g/200 ml distilled water). The sample was collected in a flask containing 20 ml of aniline/HCl. After 3 minutes, 40 ml of beta-naphthol were added. After another 15 minutes, the sample was acidified by adding 10 ml of concentrated HCl and the dye extracted with 80 ml chloroform. The extract was washed as described above, except that 3 NaOH washes were employed.

Since the mass spectrometer requires at least 1 μmole N for an analysis, it was sometimes necessary to add carrier nitrite or nitrate to samples from depths other than the nitrite maximum. This was done prior to the incubation rather than afterward, so that uptake of newly formed nitrite or nitrate by phytoplankton (or oxidation of nitrite) would be minimized by competition with the carrier.

Ammonia regeneration rates were measured by a modification of the ^{15}N isotope dilution method of Harrison (1978), in which 20 μM $^{15}NH_3$ was added to a seawater sample, which was then split. Half the sample was filtered and steam-distilled immediately to extract the ammonia; the other half was incubated under SIS conditions for 24 hours before filtration and steam-distillation. No carrier $^{14}NH_3$ was added.

c. Incubation conditions. For most of the experiments, 4-liter pyrex bottles with silicone rubber stoppers were used; for the *Thompson* cruise *in situ* experiments 4-liter polyethylene bottles were used, while 4-liter polycarbonate bottles were used for the *Islas Orcadas in situ* work. Light penetration into the polyethylene bottles was equivalent to that into the Pyrex bottles between 400-700 nm wavelengths as determined by a quantum scalar irradiance meter (Booth, 1976), and there was no significant difference between ^{15}N-nitrate uptake values obtained using the two types of bottles in parallel laboratory incubations.

In the laboratory experiments, light was supplied by Cool-White fluorescent bulbs. *In situ* conditions of light intensity and spectral distribution were approximated by attenuating the light with blue Plexiglas (No. 2424) and neutral density

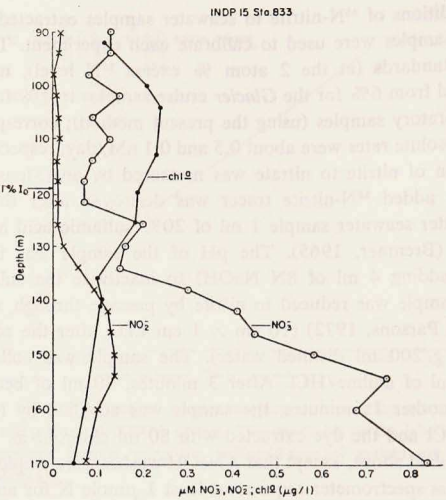

Figure 4. Nitrate, nitrite and chlorophyll profile from INDP-15 station 833, 24 July 1977 in the central North Pacific gyre. Ammonia concentrations were undetectable (<0.05 μM) throughout the profile; nitrite and nitrate were undetectable above 90 m and are not shown. The 1% light level is indicated by an arrow.

screening, as necessary. Samples were incubated in a Plexiglas aquarium whose temperature was maintained at the *in situ* value (±1°C) by a circulating water bath.

On deck incubations were carried out in Plexiglas aquaria with temperature regulation by flowing surface seawater; light intensity and quality were adjusted by blue Plexiglas and neutral density screening.

In situ incubations were carried out for 24 hours or less, with the sampling and addition of tracers carried out in the dark to avoid light shock to the organisms.

d. Other measurements. Nutrients (nitrite, nitrate, and ammonia) were measured after filtration of the sample through a GF/C glass fiber filter; the filter was used to determine chlorophyll *a* fluorometrically (all according to Strickland and Parsons, 1972). Particulate nitrogen (PN) was determined by filtration of the sample through a precombusted 984-H Reeve-Angel glass fiber filter and subsequent analysis of the filter on a Hewlett-Packard 185-B CHN Analyzer (Gordon, 1971). Calculations of ^{15}N uptake rates followed the procedures of Dugdale and Goering (1967) and calculations of nitrite and nitrate production rates were done in an

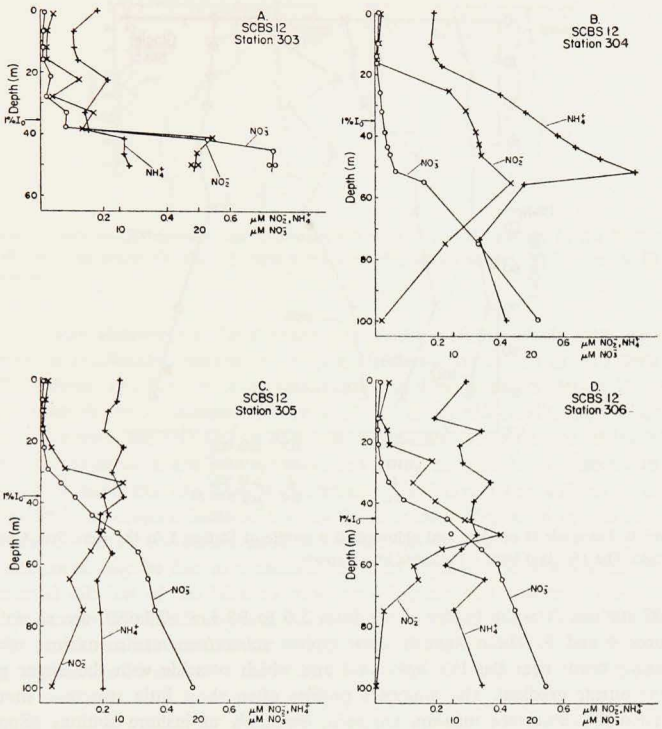

Figure 5. Inorganic-N nutrient profiles at SCBS-12 stations 303-306, with the 1% light level indicated by arrows.

analogous fashion. *In situ* and laboratory light measurements were done with a quantum scalar irradiance meter (Booth, 1976), except for the samples obtained with a small boat for laboratory incubation, when Secchi-disk readings were used to estimate light penetration depths (using 3 × Secchi depth = 1% I_0).

^{15}N tracers were obtained from Prochem, Ltd. ($K^{15}NO_3$, $Na^{15}NO_2$; 99 atom %) and from Biorad ($^{15}NH_4Cl$; 99 atom %). All chemicals used were of reagent grade.

3. Results and discussion

a. Nutrient profiles. Nutrient profiles from the Central North Pacific gyre and

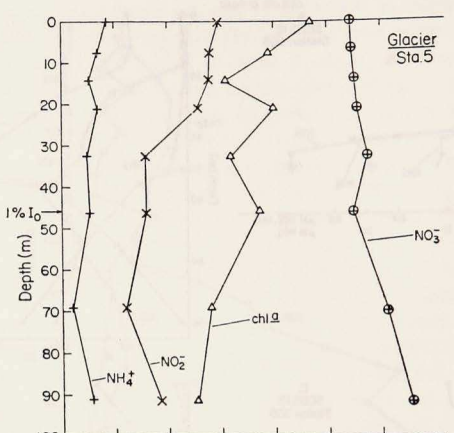

Figure 6. Inorganic-N nutrient and chlorophyll *a* profile at Station 5 in the Ross Sea; *Glacier* cruise. The 1% light level is indicated by an arrow.

SCBS stations 303-306 (which range from 5.6 to 98 km offshore), are shown in Figures 4 and 5. These stations show typical subsurface nitrite maxima which generally occur near the 1% light level and which coincide with the upper part of the nitrate gradient. The ammonia profiles often show little structure, though occasionally subsurface maxima are seen, especially at inshore stations (Eppley *et al.*, 1979). Profiles of nutrients and chlorophyll from the Ross Sea in summer and the Scotia Sea in early spring are shown in Figures 6 and 7; these show near-uniform nutrient distributions in the upper water column, probably associated with very low static stability values (unpublished data, this laboratory).

Figure 8 shows two nutrient profiles taken 12 hours apart at a station 5 miles off San Onofre, Southern California (Fig. 2), in which the disappearance of a large ammonia maximum apparently did not affect the nitrite maximum. Figure 9 shows nitrite profiles taken at SCBS station 305 at four different times of year; the nitrite maximum is present in all of them, though its position and intensity change somewhat. It is difficult, however, to infer a mechanism for nitrite formation from such static measurements; tracer experiments such as those described below are necessary to determine what kinds of transformations are actually occurring.

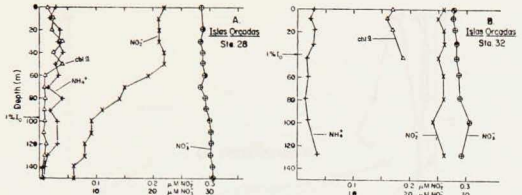

Figure 7. Inorganic-N nutrient and chlorophyll *a* profiles at two stations in the Scotia sea; *Islas Orcadas* cruise 17. The 1% light levels are indicated by arrows. A) Station 28; B) Station 32.

b. ^{15}N-tracer experiments. Time course experiments. Bottle effects must be considered in incubation experiments. Figure 10 shows the results of two tests for such artifacts. The first set of measurements was done at SCBS station 106 with water from the nitrite maximum layer incubated under SIS conditions, the second with water from the SIO pier in darkness. ^{15}N-nitrate reduction was undetectable in the SIS experiment and was not measured in the dark-bottle experiment. In the first set of ammonia oxidation measurements Gentamycin (an antibiotic) added to inhibit heterotrophic bacterial activity (it decreased ^{3}H-glucose uptake in these samples by 80%) apparently enhanced nitrite production at longer times. This enhancement may be due to decreased bacterial regeneration of ammonia, hence decreased dilution of the labelled substrate (Dugdale and Goering, 1967). The activity under SIS conditions was lower during daylight hours, while the dark-bottle experiment showed constant activity. Overall, however, these results indicate that incubations of up to 24 hours are reasonable for measuring nitrification, in

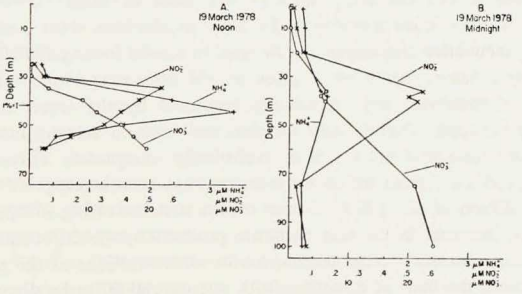

Figure 8. Inorganic-N nutrient profiles at a station 5 miles off San Onofre, Southern California at A) noon, and B) midnight of 19 March 1978.

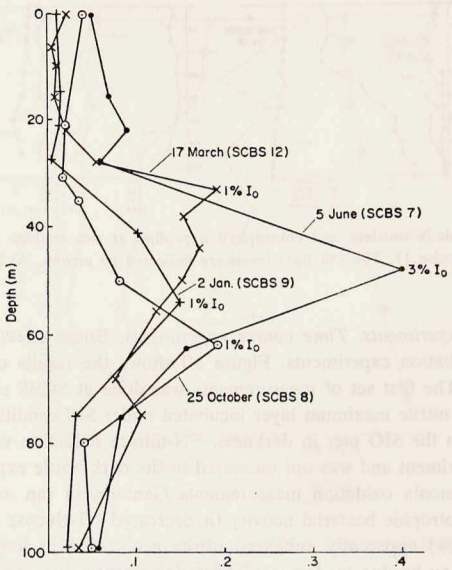

Figure 9. Nitrite profiles at SCBS station 305 during four different times of year. Light levels of the bottles containing maximal nitrite concentrations are indicated.

contrast to the situation with most heterotrophic bacterial activities. This is probably due to the extremely slow growth rates of these chemolithotrophic organisms. To maximize sensitivity, 24 hour incubations were routinely used; this full day incubation also eliminates the need to correct for any diel effects.

Effects of substrate enrichment. Since in ^{15}N tracer experiments the ambient substrate concentrations are significantly increased by the tracer addition, the relationship between substrate concentration and reaction velocity must be determined before measured rates can be realistically interpreted. This relationship, for nitrite production from nitrate by a diatom, has been investigated in a laboratory study (Olson *et al.*, 1980). It was shown that increasing nitrate concentrations caused increases in the rate of nitrite production, which saturated at about 20 μM nitrate. In sub-tropical waters, nitrate concentrations at the surface were usually close to the limit of detection (with one exception, to be discussed below) so the values reported for these samples must be regarded as potential rates only. Nitrate was present in appreciable quantities in the samples from the nitrite maxi-

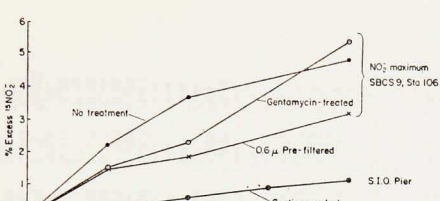

Figure 10. Two time course experiments of ^{15}N-nitrite production from ammonia. Nitrite production from nitrate was undetectable in the nitrite-maximum experiment and was not measured in the dark-bottle experiment.

mum (0.5-10 μM nitrate) and in all the Antarctic samples (20-30 μM), so the values for nitrite production from nitrate from these samples reflect naturally occurring rates. Rate enhancements due to tracer additions then varied according to the individual experimental conditions; the range of estimated enhancements is from 0% (Antarctic work) to 50% (North Pacific gyre work). The uptake of nitrate by phytoplankton is also enhanced by increased nitrate concentrations (MacIsaac and Dugdale, 1969), so the reported uptake rates are subject to constraints similar to those for the corresponding nitrite production measurements.

The effects of varying ammonia concentrations on ammonia oxidation and uptake are shown in Figure 11. No consistent increase in the rate of ammonia oxidation is apparent over the concentration range 0.1-20 μM, indicating that the organisms responsible have a very high affinity for ammonia (half-saturation constant (K_s) = 0.1 μM or less). This result is contrary to that reported for cultures of nitrifying bacteria, which have K_s values in the millimolar range (Carlucci and Strickland, 1968). However, pure cultures are usually isolated using highly enriched medium which could select strains atypical of those active in the sea. Wada and Hattori (1971) reported a half-saturation constant of 5 μM for the net production of nitrite from ammonia in natural samples.

It is possible that the lack of response of the ammonia oxidizers to increases in ammonia concentration is not due to their possessing a K_s value below detection limits. Rather the inability to respond may result from limitation by other factors (Dugdale, 1967) or prolonged substrate depletion (R. Dugdale, pers. comm.). It is not possible to evaluate here "other" limitation, but the latter situation would imply that even the organisms sampled from waters containing more than 1 μM ammonia (a near-maximum value for open ocean conditions) were substrate-starved. This seems unlikely in view of the abilities of other organisms such as phytoplankton and heterotrophic bacteria to utilize substrates at similarly low concentrations.

Table 1. Nitrite production from ammonia ($NH_3 \rightarrow NO_2$) and from nitrate ($NO_3 \rightarrow NO_2$), ammonia uptake (ρNH_3) and nitrate uptake (ρNO_3) rates, and estimated nitrite uptake ("ρNO_2") rates (all in units of nM/day) for central North Pacific gyre, Southern California coastal, and Antarctic waters. The values in parentheses under "ρNO_2" are measured values obtained from incubations with ^{15}N-nitrite.

Date	Cruise/Station		Depth (m)	NO_2 (μM)	(A) $NH_3 \rightarrow NO_2$	(B) $NO_3 \rightarrow NO_2$	(C) $2NO_2$ prod.	(D) ρNO_3	(E) "ρNO_2"	(F) ρNH_3	(G) A/C	(H) E/C	(I) A/F
					(nM/day)								
Central North Pacific gyre													
6 Jun '77	INDP-15	SIS	130	0.06	2.24	1.60	3.84	3.1	0.41	1.6	0.58	0.017	0.9
8 Jun '77	INDP-15	IS	133	0.10	7.30	0.04	7.34	0.2	0.03	0.3	0.99	0.005	24
13 Jun '77	INDP-15	SIS	109	0.18	4.60	0.40	5.0	1.1	0.33	2.1	0.92	0.067	2.2
14 Jun '77	INDP-15	IS	148	0.14	4.40	0.11	4.51	0.1	0.03	0.5	0.98	0.007	8.8
20 Jun '77	INDP-15	IS	134	0.11	3.0	0.34	3.34	0.2	0.05	2.1	0.90	0.012	1.7
Southern California coastal													
8 Jun '76	SCBS 7/206	IS	56	0.61	32.1	1.0	33.1	---	---	---	0.97	---	---
24 Oct '76	SCBS 8/306	SIS	35	0.21	7.6	0.0	7.6	16.5	0.60	13.0	1.00	0.077	0.58
31 Jan '77	SCBS 9/106	SIS	70	0.32	17.9	0.5	18.4	0.75	0.10	3.9	0.97	0.006	4.6
3 Mar '77	Scripps/103	(dark)	58	0.17	23.0	6.0	29.0	---	---	---	0.79	---	---
6 Aug '77	SCBS 11/203	IS	10	0.12	0.84	---	---	---	---	---	---	---	---
	"	IS	50	0.33	5.73	---	---	---	---	---	---	---	---
	"	IS	90	0.09	25.8	---	---	---	---	---	---	---	---
8 Aug '77	SCBS 11/105	IS	64	0.32	19.7	5.0	24.7	2.1	0.05	19.6	0.80	0.002	1.01
16 Mar '78	SCBS 12/204	SIS	50	0.40	17.6	---	>17.6	1.25	0.15	27.3	---	<0.008	0.64
20 Mar '78	SCBS 12/203	IS	34	0.59	60.2	3.0	63.2	3.8	0.38	92.3	0.95	0.006	0.65
21 Mar '78	SCBS 12/203	IS	31	0.54	39.9	1.88	41.8	5.2	0.63	80.7	0.96	0.015	0.49
	"	SIS	31	0.54	43.0	1.08	44.1	7.5	0.92	74.2	0.98	0.007	0.58
7 Nov '79	Thompson/"103"	IS	0	0.06	0.02	3.36	3.38	19.1	10.4	110.7	0.01	3.12	0.00
	"	IS	30	0.32	14.2	1.88	16.1	12.0	0.60	63.6	0.88	0.037	0.22
	"	IS	60	0.28	11.7	0.0	11.7	2.5	0.07	---	1.0	0.006	---
9 Nov '79	Thompson/	SIS	0	0.08	0.70	7.31	8.01	189.4	66.8	334	0.09	8.33	0.002
	34°15'N, 120°3'W	SIS	32	0.24	13.1	2.82	15.9	31.5	1.50	32.0	0.82	0.094	0.41
		SIS	100	0.02	30.7	1.76	32.5	1.9	0.002	1.83	0.94	0.000	16.8
11 Nov '79	Thompson/	SIS	0	0.02	0.0	5.43	5.43	31.1	25.2	91.1	0.0	4.55	0.0
	33°15'N, 118°35'W	SIS	45	0.36	26.1	2.44	28.5	43.1	2.06	34.0	0.92	0.072	0.77
12 Nov '79	Thompson/"103"	SIS	0	0.02	0.13	5.65	5.78	56.1	14.2	33.8	0.02	2.44	0.004
	"	SIS	45	0.29	140.1	1.23	141.3	4.55	0.08	45.7	0.99	0.001	3.1
	"	SIS	100	0.08	73.4	3.34	76.7	1.65	0.01	7.45	0.96	0.000	9.9
13 Nov '79	Thompson/"103"	IS	0	0.03	0.19	10.0	10.2	42.1	5.58	139.2	0.02	0.55	0.00
	"	IS	20	0.06	2.74	2.36	5.10	35.6	3.80	146.5	0.54	0.75	0.02
	"	IS	35	0.12	26.4	1.42	27.8	5.99	0.08	10.9	0.95	0.003	2.4
	"	IS	50	0.07	37.0	1.56	38.6	3.26	0.02	6.63	0.96	0.001	5.6
	"	IS	75	0.03	25.2	0.92	26.2	2.59	0.01	2.28	0.96	0.000	11.1
	"	IS	100	0.04	21.4	1.53	22.9	1.64	0.00	1.25	0.93	0.000	17.1

Laboratory SIS: samples from 3 miles off SIO													
13 May '79			2	0.20	0.76	13.02	13.2	441	11.8		696	0.06	0.90	0.001
"			11	0.28	1.56	4.36	5.92	303	6.43		1112	0.26	1.47	0.001
12 July '79			26	0.23	17.7	4.93	22.63	30.2	0.44	(0.66)	35.8	0.78	0.02	0.49
"			42	0.01	11.38	2.14	13.52	11.5	0.02	(0.01)	15.9	0.84	0.000	0.85
"			84	0.02	14.29	0.51	14.80	---	---	(0.001)	1.6	0.97	0.000	9.4
31 July '79			20	0.09	0.62	6.93	7.53	194	5.10	(4.59)	317	0.08	0.07	0.002
"			40	0.38	44.4	0.0	44.4	5.1	0.13	(0.18)	153	1.0	0.003	0.29
"			100	0.06	25.3	0.16	25.4	1.25	0.003	(0.003)	2.6	0.99	0.000	9.7
11 Feb '80			20	0.21	6.73	7.81	14.5	171	35.8		106.8	0.46	2.5	0.06
"			100	0.01	4.76	---	---	---	---		---	---	---	---
"			280	0.03	2.06	---	---	---	---		---	---	---	---
Ross Sea														
31 Dec '77	75°14'S, 170°42'E		30	0.22	8.9	<2.5	<11.4	79.8	0.54		163	>0.78	0.059	0.06
5 Jan '78	78°05'S, 179°42'E		300	0.13	6.0	<2.5	<8.5	---	---		---	>0.71	---	---
Scotia Sea														
6 Sept '78	2	IS	5	0.14	3.5	---	>3.5	---	---		124	---	---	0.03
	"	IS	24	0.12	2.3	2.1	4.3	8.28	0.052		94.4	0.53	0.012	0.02
	"	IS	50	0.14	4.6	0.66	5.3	9.07	0.049		40.5	0.87	0.011	0.11
	"	IS	77	0.12	6.7	0.39	7.1	1.68	0.008		7.12	0.94	0.001	0.94
10 Sept '78	5	IS	0	0.09	5.16	9.36	14.4	48.2	0.25		42.0	0.36	0.17	0.12
	"	IS	24	0.09	6.35	---	>6.4	58.6	0.21		54.8	---	---	0.12
	"	IS	40	0.09	---	3.95	>4.0	24.4	0.09		---	---	---	---
	"	IS	88	0.04	3.31	1.32	4.6	1.89	0.004		5.11	0.72	0.001	0.65
	"	SIS	0	0.09	0.82	4.84	5.7	35.2	0.18		31.8	0.15	0.03	0.03
	"	SIS	40	0.09	3.19	1.37	4.6	8.89	0.044		15.6	0.70	0.01	0.20
11 Sept '78	7	SIS	0	0.24	0.35	3.15	3.5	5.96	0.051		5.85	0.10	0.014	0.06
	"	SIS	30	0.23	0.37	0.17	0.5	3.13	0.025		2.37	0.69	0.05	0.16
15 Sept '78	15	IS	0	0.10	5.30	2.66	8.0	20.0	0.07		17.8	0.67	0.009	0.30
	"	IS	24	0.10	5.83	3.26	9.1	13.1	0.045		19.2	0.64	0.005	0.30
	"	IS	58	0.08	4.45	4.28	8.7	2.74	0.007		4.42	0.51	0.001	1.0
	"	IS	90	0.03	12.90	2.34	15.2	1.84	0.002		2.12	0.85	0.000	6.1
21 Sept '78	19	SIS	0	0.30	0.76	4.91	5.7	13.1	0.13		15.0	0.13	0.023	0.05
	"	SIS	38	0.30	1.87	3.62	5.5	10.7	0.10		14.7	0.34	0.019	0.13
25 Sept '78	23	SIS	0	0.16	0.54	6.54	7.1	86.8	0.50		36.7	0.08	0.067	0.02
	"	SIS	20	0.16	0.82	1.21	2.0	71.1	0.40		54.4	0.40	0.20	0.02
28 Sept '78	28	SIS	0	0.23	0.0	2.18	2.2	8.60	0.074		12.5	0.0	0.034	0.0
3 Oct '78	29	SIS	0	0.29	0.49	2.27	2.8	101.0	0.98		66.9	0.18	0.36	0.01
4 Oct '78	32	SIS	0	0.26	1.54	13.35	14.9	122.6	1.14		118	0.10	0.077	0.01
	"	SIS	20	0.26	3.74	3.57	7.3	73.7	0.68		83.9	0.51	0.093	0.05

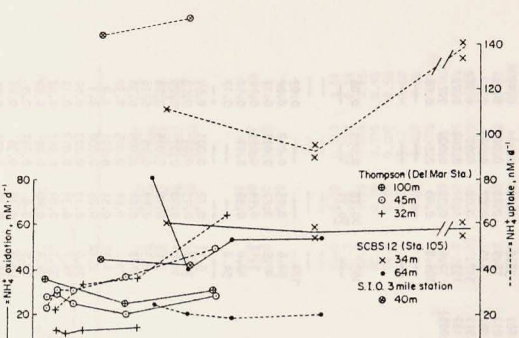

Figure 11. Effects of increasing ammonia concentration on the rate of ammonia oxidation (————) and the rate of ammonia uptake (– – – – –). The arrows at the abscissa indicate the ambient ammonia concentrations for each experiment before the tracer was added.

Most of the data presented here were obtained with 1 μM $^{15}NH_3$ additions; this minimizes uncertainties due to: 1) error in the measurement of ambient ammonia concentration ($\pm$0.05 μM), which affects the absolute rate calculation; 2) dilution of the label by regenerative processes occurring during the incubation; and 3) possible depletion of the labelled substrate by phytoplankton uptake. In no experiment was the ^{15}N tracer depleted by more than 50% by the end of the incubation, as determined by ^{15}N calculations and changes in total substrate. That phytoplankton respond to increased ammonia concentrations by increasing their uptake rates is well documented (MacIsaac and Dugdale, 1969; also see Fig. 11); thus ammonia uptake is overestimated by varying degrees, depending on the characteristics of the phytoplankton populations and the ambient ammonia levels.

No attempt has been made to correct any of the rates for enhancement due to nutrient enrichment. This means that nitrite production from nitrate and uptake of nitrate and ammonia may all be overestimated, while nitrite production from ammonia is accurate as reported.

Southern California coastal and central North Pacific gyre results. 1) Source of nitrite. The results of ^{15}N-tracer experiments in Southern California coastal waters and the central North Pacific gyre are presented in Table 1. It is clear that in these regions, ammonia is the main source of the nitrite in the nitrite maximum. The fraction of the total measured nitrite production that is derived from ammonia (column G) ranges from 0.78 to 1.0 (mean = 0.93, std. dev. = 0.07, n = 13) in samples from the nitrite maximum. Above the depth of the maximum, the

potential for nitrite production from nitrate increases and ammonia oxidation decreases, so that the ammonia-derived fraction decreases to very low values near the surface. The mean turnover time for nitrite in the nitrite maximum layer was 12.5 days in Southern California coastal waters (std. dev. = 6 days, $n = 15$) and 25 days in the central North Pacific gyre (std. dev. = 10 days, $n = 5$).

Indications that the mediators of the observed ammonia-oxidizing activity are nitrifying bacteria include size fractionation and inhibitor experiments. The results of three experiments in which water samples were pre-filtered through various pore-size Nuclepore filters (142 mm diameter) are shown in Table 2. In each case a large proportion of the ammonia-oxidizing activity passed a 0.6 μ filter but was removed by a 0.2 μ filter, suggesting that bacteria are responsible. In tests with enrichment cultures of marine ammonia-oxidizers, both $^{14}CO_2$ fixation and ammonia oxidation activity were largely inhibited by nitrapyrin (6-chloro(trichloromethyl)pyridine), an inhibitor of nitrifying bacteria (Goring, 1962) (unpublished data, this laboratory). In the experiment of 20 June 1977 during IndoPac-15, 1 ppm nitrapyrin inhibited $^{15}NH_3$ oxidation by 60%; this is consistent with the findings of Miyazaki *et al.* (1973).

The finding that ammonia is the primary source of nitrite in the nitrite maximum agrees with the conclusion of Wada and Hattori (1971) for work in the central North Pacific; however, it contradicts the conclusions of Miyazaki *et al.* (1973, 1975), who found nitrate and ammonia to contribute about equally to nitrite production in the western North Pacific and in Sagami Bay. It should be pointed out that the authors mentioned above incubated samples from depth under full natural sunlight or in darkness rather than under IS or SIS conditions. Increased

Table 2. Size fractionation of ammonia oxidation activity. Seawater samples were prefiltered through 142-mm diameter Nuclepore filters of the indicated pore-sizes.

Sample	Pore-size	$^{15}NO_2^-$, % excess	% of control
SIO pier, 20 Oct '79	not filtered	0.078	—
4 m	0.6 μ	0.030	38%
	0.2 μ	0.006	8%
SCBS 9, Sta. 106	12 hr not filtered	2.20	—
72 m	12 hr 0.6 μ	1.52	69%
	24 hr not filtered	3.70	—
	24 hr 0.6 μ	1.90	51%
	48 hr not filtered	4.90	—
	48 hr 0.6 μ	3.25	66%
SCBS 11, Sta. 105	not filtered	4.04	—
64 m	1.0 μ	3.70	92%
	0.6 μ	3.51	87%
	0.2 μ	0.30	7%

light intensity would be expected to increase phytoplankton nitrite production (Olson *et al.*, 1980; Wada and Hattori, 1971) and can inhibit nitrifying bacteria, as discussed below.

2) *Competition for ammonia.* In the nitrite maximum layer in these sub-tropical regions, rates of ammonia oxidation are similar to the rates of ammonia uptake (presumably by phytoplankton). Column I in Table 1 presents the ratio of ammonia oxidation: ammonia uptake; it can be seen that at the depth of the nitrite maximum, ammonia uptake has decreased (due to light limitation of growth rate) so that the nitrifying bacteria are utilizing ammonia at rates comparable to those of the phytoplankton. In view of the low K_s for ammonia oxidation discussed above, it seems that nitrifying bacteria should be able to compete with phytoplankton for ammonia in the upper waters of the euphotic zone as well, where ammonia regeneration is much more rapid (Table 3; Harrison, 1978). However, the profiles of ammonia oxidation activity indicate that little or no activity is seen above the depth of the nitrite maximum.

3) *Light effects.* Light has opposing effects on nitrification and phytoplankton nitrite production (Columns A and B, Table 1). Experiments with laboratory cultures (Schön and Engel, 1962; Hooper and Terry, 1974) indicate that photoinhibition of ammonia oxidation could occur in the surface waters, and comparison of light and dark bottles in field incubations (Table 4) indicates that light can inhibit the activity to some extent even at the depth of the nitrite maximum. The results of a laboratory incubation of seawater samples from the SIO pier (from 4 m depth in a 7-m water column, and from the surface) are shown in Table 5. Each sample was split and incubated under three light intensities, ranging from darkness to 4.3×10^{16} quanta/cm^2/s (about 20% of full sunlight). The results show the same pattern as the field experiments in that nitrate reduction increases with light intensity while ammonia oxidation decreases. The threshold for inhibition of ammonia oxidation is apparently between 0.2% and 2% of sunlight.

Table 3. Ammonia regeneration rates in the euphotic zone at a station three miles off Del Mar, California. Each measurement is the mean of triplicate determinations of ^{15}N enrichment; the standard deviation for the rate measurement was calculated on the basis of a coefficient of variation of 1.5% for triplicate standards.

Sample	Depth (m)	% $^{14}NH_3$ (begin)	% $^{14}NH_3$ (end)	NH_3 regeneration (nM/day)
31 July '79, SIO	20	2.803	4.317	303 ± 10.7
	40	4.062	4.355	59 ± 12.6
	100	2.034	2.018	−3.2 ± 6.0
12 Nov '79, *Thompson*	0	1.540	2.611	214 ± 6.0
	45	2.378	2.656	56 ± 7.5
	100	1.301	1.421	22 ± 4.1

Both the rate of nitrite production from nitrate and the ratio of this nitrite production to nitrate uptake increased with light intensity. This relationship was found in the depth profiles from the *Thompson* cruise as well and agrees with the behavior found by Olson *et al.* (1980) in a laboratory study of diatom nitrite metabolism.

4) *Upwelling experiment.* The experiment of 13 May 1979 (Table 1) was a special case, in that it was carried out during a period of upwelling off the Southern California coast. The surface concentrations of nitrate, nitrite and particulate nitrogen were relatively high (7.3, 0.20, and 4.01 μM, respectively). Under these conditions of high irradiance, high nitrate and high phytoplankton standing stock, the production of nitrite from nitrate reached its highest measured value, 13.02 nM/day. However, the production of nitrite from ammonia was nearly nil and the calculated uptake of nitrite (see below) was 11.8 nM/day, so that little or no accumulation of nitrite was occurring at this time.

These findings indicate that the production of nitrite by phytoplankton is potentially important when the surface nitrate concentration is high and the phytoplankton crop is large. The nitrite present at the surface during periods of upwelling, however, could also be due to "upwelled nitrite maximum" water, considering the relatively long turnover time for nitrite (about two weeks) and the short duration of most upwelling episodes.

5) *Nitrite uptake and oxidation.* An analysis of the accumulation of nitrite requires, in addition to a source, a consideration of sinks for nitrite. On the basis of changes in nitrite concentration in incubated bottles, Wada and Hattori (1971) concluded that in the upper waters of the euphotic zone in the central North Pacific the potential for uptake of nitrite was greater than the potential for its production. In an attempt to address this question for the studies reported here, parallel $^{15}NO_2^-$ and $^{15}NO_3^-$ uptake experiments were carried out in two of the depth profiles (12 July and 31 July 1979; Table 1). The results indicated that nitrite and nitrate uptake can be considered as being competitive processes, as has been shown for laboratory cultures of phytoplankton (Eppley and Coatsworth,

Table 4. Nitrite production from ammonia in light *vs* dark bottles.

Sample	Incubation Type	Depth (m)	$NH_3 \rightarrow NO_2$ Light (nM/day)	Dark (nM/day)	Light/Dark
14 June '77 INDP-15	IS	148	4.4	4.6	0.96
20 June '77 INDP-15	IS	134	3.0	4.3	0.70
3 Mar '77 *Scripps*	Laboratory	58	5.5	23.0	0.24
31 Dec '77 *Glacier*	SIS	30	8.9	15.6	0.57
8 Aug '77 SCBS 11/105	IS	63	3.23	4.05	0.80

1968; Olson *et al.*, 1980). This allows an estimate of nitrite uptake to be obtained by referring to the nitrate uptake data (Column D, Table 1) and the measured nitrate: nitrite ratio for each sample. Because the nitrate uptake estimates were maximum estimates, the nitrite uptake estimates (Column E, Table 1) are also maximum estimates. The results of this calculation indicate that the potential for uptake of nitrite in the upper waters is usually greater than the total nitrite produc-

Table 5. Effects of light on nitrite production from nitrate and ammonia, and on uptake of nitrate and ammonia.

Sample	Light intensity (quanta/cm²/s)	$NH_3 \rightarrow NO_2$	$NO_3 \rightarrow NO_2$	ρNH_3	ρNO_3	$\frac{NO_3 \rightarrow NO_2}{\rho NO_3}$
		(nM/day)			)	
27 Sept '79,	0.0	1.48	0.04	123.9	4.33	0.009
SIO pier,	4.3×10^{14}	1.69	0.13	151.5	9.60	0.014
4 m depth	4.3×10^{15}	0.14	2.32	165.9	99.2	0.023
19 Oct '79,	0.0	—	0.20	—	12.65	0.016
SIO pier,	4.3×10^{15}	—	1.16	—	57.6	0.020
surface	4.3×10^{16}	—	18.9	—	279	0.068

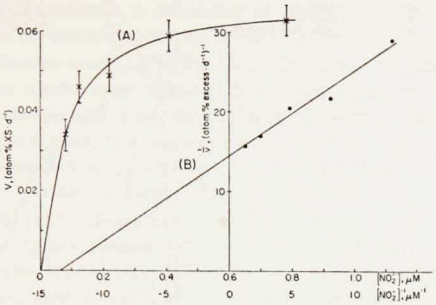

Figure 12. Effect of increasing nitrite concentration on the rate of nitrite oxidation. The sample was taken from three miles off SIO at a depth of 60 m (below the nitrite maximum). Ambient nitrite concentration was 0.02 μM. A) Rate *vs* substrate concentration (error bars indicate ± 1 std. dev. in ^{15}N determination); B) Lineweaver-Burk plot of the data.

tion, while by the depth of the nitrite maximum, uptake is less than 5% of the total production of nitrite (Column H, Table 1). Thus, nitrite accumulation at this depth is unlikely to be much affected by phytoplankton uptake.

Wada and Hattori (1971) also considered the loss of nitrite into waters below the maximum layer and concluded that eddy diffusion could balance the production of nitrite, given a turnover time of 30 days. However, they pointed out that the choice of a coefficient of eddy diffusion is critical to this calculation and is difficult to obtain with any degree of accuracy. In any case, the nitrite mixed away into deeper water must obviously still be further oxidized to nitrate, since nitrite has not accumulated in the deep water.

To investigate this potential sink for nitrite, the ^{15}N-tracer method for ammonia oxidation measurement was adapted for measuring nitrite oxidation, as detailed in the Methods section. This second step of nitrification showed a marked dependence on the substrate concentration (Fig. 12), in contrast to ammonia oxidation. A Lineweaver-Burk plot of the data (fitted by the weighted linear regression method of Wilkinson (1961)) resulted in an apparent half-saturation constant value of 0.07 μM nitrite. This value is much lower than estimates of K_s for laboratory cultures of nitrite-oxidizers (Carlucci and Strickland, 1968), but is similar to nitrite concentrations found in many parts of the marine habitat. Since the levels of $^{15}NO_2^-$ added in subsequent experiments (0.3 to 0.5 μM) were high compared to ambient levels (except within the nitrite maximum), the estimates of nitrite oxidation rates are actually estimates of potential activity.

The results of two field experiments in which both ammonia oxidation and nitrite oxidation were measured are shown in Figure 13; while obviously not a

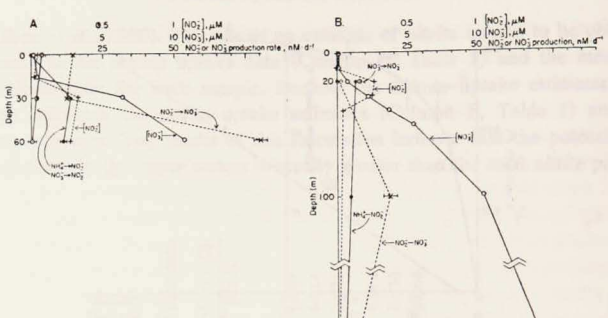

Figure 13. Profiles of inorganic-N nutrients, ammonia oxidation, nitrite oxidation, and nitrate reduction at two stations off Southern California. A) *Thompson, in situ*, 7 Nov 1979; B) small boat sampling, 3 miles off SIO, 11 Feb 1980.

comprehensive data set, these experiments show an interesting pattern. Although the precision of the nitrite oxidation measurement worsens as depth and nitrate concentration increase (because of the resulting lower ^{15}N enrichments), it is clear that the highest nitrite oxidation potential is found *below* the nitrite maximum layer, while ammonia oxidation activity is highest within the maximum. Ammonia oxidation activity is still appreciable at 280 m depth. The relatively low rate of nitrite oxidizing activity within the nitrite maximum is probably due to a photinhibition effect similar to that demonstrated for ammonia oxidation. The relatively *high* rate of nitrite oxidation at the surface in the first experiment is probably due to chemical photolysis of nitrite as suggested by Zafiriou and True (1979) and confirmed by $HgCl_2$-killed control ^{15}N tracer experiments in this laboratory (unpublished data).

In both of these experiments, nitrite oxidation exceeds total nitrite production at all depths except for the depth of the nitrite maximum. This is consistent with the concept that differential nitrification rates determine the structure of the nitrite profile (although phytoplankton uptake of nitrite above the maximum makes this a moot point there), even without considering such important modifiers as turbulent diffusion.

Antarctic waters. Two experiments are reported from the Ross Sea in the middle of the austral summer (Table 1). In both of these ammonia was predominant over nitrate as the source of nitrite (a problem with high blanks precluded the determination of low rates of activity in these samples). The turnover time for nitrite was about 13.5 days, a value similar to that obtained for sub-tropical waters.

In the near-surface experiment, a stimulation of activity was seen in the darkened bottle compared to the bottle at simulated *in situ* irradiance.

A.R.A. *Islas Orcadas cruise* 17, to the Scotia Sea and along the pack ice in the early spring, gave results quite different from those described above. On this cruise, nitrate reduction and ammonia oxidation rates were roughly equivalent, though the total nitrite production was much lower than that found in Southern California coastal waters (6.9 *vs* 36 nM/day); the turnover time for nitrite was also very long (mean = 50 days; std. dev. = 43 days, $n = 10$). However, the patterns of activity followed the trends outlined above for sub-tropical waters. The relationship of nitrite production from nitrate to nitrate uptake for these samples has been discussed elsewhere (Olson *et al.*, 1980); it appears that here as well as off Southern California, phytoplankton produce nitrite more rapidly in surface samples than at depth. The opposite relationship is found for ammonia oxidation (See Table 6): Rates generally increased with depth. The upper water column at most of the *Islas Orcadas* stations was well mixed, with chlorophyll and nutrient profiles showing very little structure (Fig. 7). If the distribution of ammonia-oxidizing organisms was similarly uniform with depth, then the increase in ammonia oxidation rate with depth would be further evidence for photoinhibition of nitrification.

Possible reasons for the observed differences between the Ross Sea and Scotia Sea results include seasonal considerations and species differences. The onset of nitrite accumulation at high latitude stations such as Station P in the northeastern Pacific typically occurs in mid- to late summer (Robertson *et al.*, 1965), although its mechanism is not known. Both increased water column stability and increased grazing (and so ammonia input) would be expected as the summer progresses. Another factor, the species composition of the phytoplankton crop, differed between the sets of Antarctic data; the Scotia Sea was dominated by diatoms during the spring cruise, while the Ross Sea experiments were done in a region dominated

Table 6. Effect of depth on ammonia oxidation in the Scotia Sea. The data presented are from stations at which both surface and 10% light level samples were tested.

Station	$NH_3 \rightarrow NO_2$ (surface) (nM/day)	$NH_3 \rightarrow NO_2$ (10% I_0) (nM/day)	surface rate / 10% I_0 rate
5	0.82	3.19	0.26
7	0.35	0.37	0.95
15	5.30	5.83	0.91
19	0.76	1.87	0.41
23	0.54	0.82	0.66
32	1.54	3.74	0.41
			$\bar{x} = 0.60$
			std. dev. = 0.29

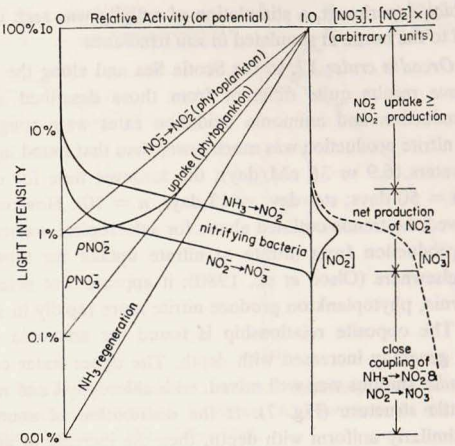

Figure 14. Conceptual model of biological processes contributing to the formation of a primary nitrite maximum. The relationship between ρNO_3 and $NO_3 \rightarrow NO_2$ is from Olson *et al.* (1980); that between $NH_3 \rightarrow NO_2$ and $NO_2 \rightarrow NO_3$ is discussed in Olson (1981). The divergence of ρNO_2 from ρNO_3 at the top of the nitrate gradient is due to competition with nitrate for uptake. Abiotic photochemical reactions are not considered here, but would roughly resemble the $NO_3 \rightarrow NO_2$ and ρNO_2 curves (unpublished data, this laboratory).

by *Phaeocystis,* a haptophyte. The phenomenon of nitrite production is widespread among diatoms but is much less well known in other taxonomic groups of phytoplankton.

4. Conclusions

On the basis of these results, a model can be visualized to account for the accumulation of nitrite at depth (Fig. 14). It seems that despite the circumstantial evidence linking nitrite and nitrate (Voituriez and Herbland, 1977; Kiefer *et al.,* 1976), the main source for nitrite in oxygenated waters is ammonia oxidation. The observed co-occurrence of nitrate and nitrite is probably the result of protection of nitrite from consumption by phytoplankton by the presence of a competing substrate. There seems to be a continuum of nitrite production potential with depth, gradually switching from nitrate reduction to ammonia oxidation as light intensity decreases. An accompanying decrease in nitrite uptake potential by phytoplankton, caused partly by decreasing light intensity but largely by increasing nitrate concentration, allows the accumulation of nitrite at some depth. At a greater depth the potential for oxidation of nitrite increases and a close coupling of ammonia oxidation and nitrite oxidation prevents accumulation of nitrite.

The biological processes which contribute to the formation of a primary nitrite maximum are thus extremely complex, and the balance between them would be expected to be sensitive to many environmental influences such as insolation, water clarity, nitrate input into the euphotic zone, and ammonia regeneration; all of these require further investigation. The difference in depth between the onset of ammonia oxidation and the onset of nitrite oxidation, which is critical to this model, is addressed in another paper Olson, (1981).

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