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Ammonium distribution in southern California coastal waters and its role in the growth of phytoplankton[1]

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Abstract

The average concentration of ammonium in the euphotic zone of the Southern California Bight was about 0.35 μg-atom·liter^{-1} in 1974–1977. Concentrations up to 5–20 μg-atoms·liter^{-1} were found in Santa Monica Bay surface and bottom waters. Elsewhere maximum concentrations were 2–3 μg-atoms·liter^{-1} at the bottom of the euphotic zone of inshore stations.

Ammonium provided an average of 35% of the nitrogen assimilated by the phytoplankton and a much higher proportion in Santa Monica Bay. The rate of ammonium assimilation was modified primarily by the phytoplankton standing stock, and also by ambient concentration of ammonium, temperature, irradiance, and the carbon:chlorophyll *a* ratio of the phytoplankton. These variables accounted for 52% of the explained variance in three "winter" cruises and 83% in a summer cruise. Remineralization processes account for most of the ammonium in the bight, while human inputs account for about 10%.

The purpose of this study was to determine the distribution of ammonium in southern California coastal waters (the Southern California Bight), to evaluate the importance of ammonium in the nitrogen economy of the phytoplankton, and to assess those factors that modify the rate of ammonium assimilation by the phytoplankton in natural seawater.

The principal forms of nitrogen for phytoplankton growth in the sea are regarded as nitrate, ammonium, urea, and molecular nitrogen in oligotrophic tropical and subtropical areas. Near shore, certain littoral and neritic forms may also assimilate dissolved free amino acids (North 1975). The ability to assimilate urea-N and amino acid-N is somewhat limited in its distribution among phytoplankton species and clones (Wheeler et al. 1974; Carpenter et al. 1972; McCarthy 1971).

The sources of these nitrogenous nutrients differ. Typically a large reservoir of nitrate exists below the euphotic zone even though the surface layers may be depleted by previous plant growth (Dugdale and Goering 1967). Other than the deep ammonia pool in anoxic basins such as the Black Sea (Sen Gupta 1971), no reservoirs of ammonium or urea usually exist, and concentrations of free amino acids are vanishingly low in the open sea. What dissolved organic nitrogen (other than urea) there is in the open ocean does not appear to be much used by ocean phytoplankton but may be present as a refractory residuum (Thomas et al. 1971).

The sources of ammonium in southern California coastal waters are both local, such as sewage and refinery outfalls, and dispersed, such as rain, aerial fallout, and advection. They also include in situ regeneration processes such as animal excretion and microbial decomposition within the euphotic zone and transport from the sediment, an active site of mineralizing processes. The principal sink is the phytoplankton, either by its direct assimilation of ammonium or its assimilation of transformed products as nitrite and nitrate. In the littoral zone, macroalgae constitute a large sink for inorganic nitrogen (Jackson 1977).

Ammonium is an important source of N for phytoplankton growth in the oceans generally and the Southern California Bight in particular. McCarthy (1972)

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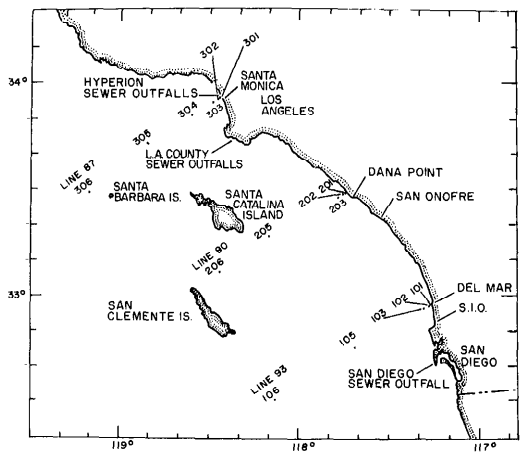

Fig. 1. Station locations.

found in several stations in July 1970 and June 1971 in this area, using ^{15}N-labeled substances, that ammonium assimilation accounted for an average of 42% of the sum of ammonium, nitrate, and urea-N assimilation by the phytoplankton. In the cruises here discussed, Harrison (1978) measured rates of ammonium regeneration in the euphotic zone and found that small (<35 μm) particles carried out most of the activity, while micro- and macrozooplankton were less important, and that the rate of ammonium regeneration was of comparable magnitude and was proportional to its assimilation rate by the phytoplankton.

Modifiers of the rate of ammonium assimilation by phytoplankton are, first, the standing stock and its specific growth rate. Irradiance modifies the rate either directly, or more probably indirectly by regulating the specific growth rate, as some assimilation of ammonium takes place in darkness in laboratory cultures. Some dark uptake of ammonium is usually observed with natural samples and irradiance about 3% of full sunlight is rate saturating (MacIsaac et al. 1974). In laboratory experiments the ambient ammonium concentration can also be rate determining, as seen in short term ammonium uptake experiments (Eppley et al. 1969) and in continuous cultures with ammonium as a source of nitrogen for algal growth (Caperon and Meyer 1972).

Several studies of ammonium concentration in local waters have been carried out (McCarthy and Kamykowski 1972), some with respect to evaluating effects of sewage discharges on phytoplankton standing stocks (i.e. Stevenson and Grady 1956; Thomas 1972; Eppley et al. 1972). In bioassay experiments some adverse effects of sewage were observed on phytoplankton growth. However, these were not attributed to the ammonium in the sewage effluent (Thomas et al. 1974).

Methods

Eleven cruises were made approximately quarterly between September 1974 and August 1977; ammonium was determined on all but the first. Stations were occupied on each cruise at the locations shown in Fig. 1 where stations were omitted in order to free the ship for special studies. The stations were grouped on three CalCOFI (California Cooperative Oceanic Fisheries Investigations) lines perpendicular to the shoreline and were spaced approximately in a logarithmic progression of distance offshore. This was done since gradients in biological properties were expected to be steepest near shore. Two or three stations were made each day, so the time of arrival was irregular, but all sampling was done during daylight hours.

The work at each station included measurement of a depth profile of quantum scalar irradiance with the instrument described by Booth (1976). When the quanta-meter readings were not available, the euphotic depth (i.e. the 1% light depth) was taken as three times the Secchi depth. It also included a cast of 5-liter PVC Niskin bottles: six in the euphotic zone and two to greater depth when depth permitted. Samples for nutrient analysis, phytoplankton rate measurements, particulate carbon and nitrogen, extracted chlorophyll *a*, and ATP were taken from the bottle casts.

Samples for nutrient analysis were filtered (washed Whatman GF/C filters) di-

rectly into acid-cleaned polyethylene, screwcapped 250-ml bottles, put into a freezer in the ship's laboratory, and kept frozen until analysis ashore 1–3 weeks later.

Ammonium was determined in duplicate by the indophenol blue method of Solórzano (1969). The method was scaled down for the present study and 10-ml sample volumes were used. Analyses were carried out in 50-ml screwcapped test tubes.

Photosynthetic carbon assimilation and rates of nitrate and ammonium assimilation by phytoplankton were measured with ^{14}C and ^{15}N tracers. We added 4 μCi of [^{14}C]carbonate to about 200 ml of seawater in 250-ml bottles and 0.1 μg-atom of [^{15}N]nitrate or ammonium (99% ^{15}N) to 1,000-ml samples in 1-liter screwcapped bottles. Within an hour of collection, the bottles were placed into shipboard incubators fitted with neutral density light filters, cooled with flowing surface seawater, and exposed to sunlight. They were incubated 24 h and surface irradiance was recorded with a pyranometer or quantum-scalar irradiance meter. The pyranometer values were multiplied by 0.5 to estimate photosynthetically active solar irradiance. The 24-h incubation period was selected because of the irregular sampling times and to encompass one full light-dark cycle, thus minimizing effects related to circadian periodicity in phytoplankton metabolism. After incubation the samples were filtered to collect the particulate matter. Reeve-Angel 984H glass-fiber filters were used for ^{15}N samples and either these or Whatman GF/C filters for ^{14}C samples. The ^{15}N filters were placed in glassine envelopes and put in a vacuum desiccator for storage before mass spectrometer analysis (Dugdale and Goering 1967). The ^{14}C filters were carefully rinsed with filtered seawater and placed directly into cocktail, such as Aquasol, for scintillation counting with a Beckman model LC 100 instrument. Standardization was carried out both with the external standard device provided with the instrument and by adding standard [^{14}C] toluene to the samples. Photosynthetic rates were measured on all cruises. Rate measurements with ^{15}N were limited to cruises 2, 3, and 5 and line 87 of cruise 6.

Particulate material was collected by filtration onto combusted glass-fiber filters (GF/C or 984H) and stored in a vacuum desiccator until analysis with a Hewlett-Packard model 185B CHN analyzer. Weighed quantities of EDTA were analyzed with each set of samples for standardization. Phytoplankton carbon was calculated from POC according to the equation given by Eppley et al. (1977*a*). Samples for chlorophyll *a* and pheopigment analysis were filtered (GF/C) and the filters were placed in 90% acetone and ground in a Teflon-glass homogenizer. The extracts were then made up to volume and placed in darkness in a refrigerator (3°–4°C) for longer than 1 h. They were then centrifuged, the fluorescence of the clear supernatant was recorded, a drop of 10% HCl was added to the cuvette and a second, lower fluorescence value was recorded. Calculations and filter combinations for chlorophyll and pheopigment analysis with a Turner model 111 fluorometer were taken from Holm-Hansen et al. (1965).

The data were treated by multiple regression analysis. The *F*-values reported for the independent variables are based on the decomposition by the standard regression method of the explained variance into components attributable to each independent variable (Kim and Kohout 1975).

Results

Precision of ammonium analysis of field samples—The precision of the present ammonium method at the 95% confidence level is $\pm 0.15/n^{1/2}$ and $0.10/n^{1/2}$ μg-atom·liter^{-1} at the 3 and 1 μg-atom·liter^{-1} levels (Strickland and Parsons 1972) where n is the number of replicate samples measured. The smallest concentration that can be reliably detected is about 0.1 μg-atom·liter^{-1}.

We did duplicate analyses for each

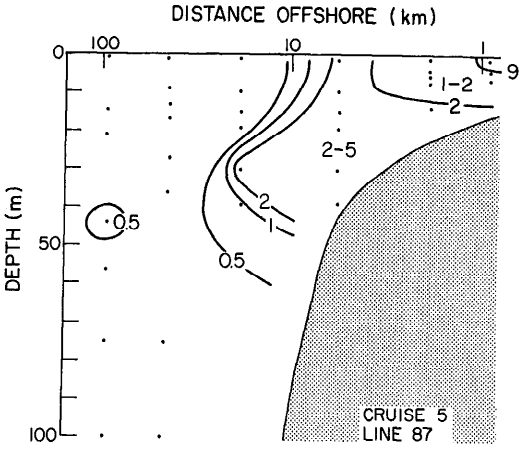

Fig. 2. Ammonium concentration, μg-atom·liter^{-1}, in a section through Santa Monica Bay, December 1975. Note log scale for distance offshore.

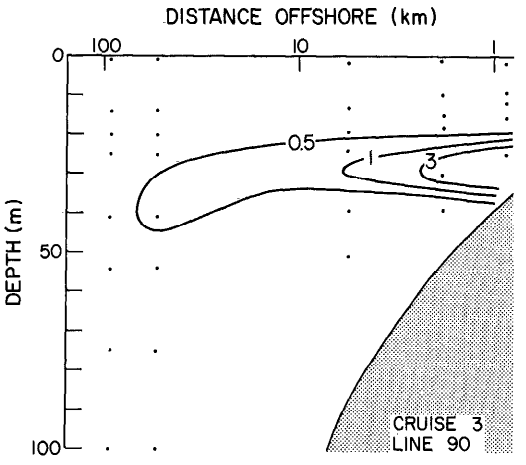

Fig. 3. As Fig. 2, but in a section showing a maximum at 30-m depth. June 1975.

sampling depth. The 82 samples of cruise 10 were selected arbitrarily as a data set for examining the precision of the method. Let X_1 and X_2 be the absorbance of the two replicates and $\omega = 100|X_1 - X_2|$. Let $\bar{X}$ be the average of X_1 and X_2. The ratio $\omega:\bar{X}$ was then calculated and its frequency distribution examined. The distribution was highly skewed to low values. Logarithmic transformation of $(\omega:\bar{X} + 1)$ resulted in a distribution more nearly normal and this distribution was used to estimate a 95% confidence limit, i.e. an upper cut-off limit. That value in absorbance units for $|X_1 - X_2|$ was 0.0209 with 10-cm absorption cells or about 0.16 μg-atom·liter^{-1} as NH_4^+-N. Thus, when replicate samples are analyzed 95% confidence limits about the mean ammonium concentration would be about this value divided by $2^{1/2}$ or 0.113 μg-atom·liter^{-1}, in rough agreement with the precision indicated by Strickland and Parsons, ±0.106 for $n = 2$ at 1 μg-atom·liter^{-1}.

This estimate of precision suggests that measurements of ammonium concentrations <1.0 μg-atom·liter^{-1} are not very precise and that the precision at the 0.22 μg-atom·liter^{-1} level would be ±50% (0.11–0.33 μg-atom·liter^{-1}) for 95% confidence. The relative precision, as percent of the mean, is of course improved as concentrations increase, being ±11% at 1.0 μg-atom·liter^{-1} and ±5.5% at 2.0.

Distribution of ammonium off southern California—Figure 2 shows a transect of ammonium concentration vs. depth and distance offshore and demonstrates several recurring features common to the data. A high surface concentration was found inshore in Santa Monica Bay; this feature was noted on eight of the ten cruises on which ammonium was measured. The highest concentration observed was 23 μg-atoms·liter^{-1} on cruise 9. Elevated ammonium concentrations were noted near the bottom at the inshore stations on line 87; this feature was present on all ten cruises. Maximum observed concentrations ranged from >1 to 9 μg-atoms·liter^{-1}. Elevated concentrations were often found above the bottom at nearshore stations on lines 90 and 93 (Fig. 3). Maximum concentrations were 0.4–3 μg-atoms·liter^{-1}. Six of the nine cruises with data for lines 90 and 93 showed the feature. Concentrations were low, <0.3 μg-atom·liter^{-1} in inshore surface waters on lines 90 and 93 and at all offshore stations except for occasional middepth maxima with concentrations of the order 0.5 μg-atom·liter^{-1} (*see Fig. 2*: sta. 306, line 87).

The surface inshore values, shown as 9 (Fig. 2) and 5 μg-atoms·liter^{-1} (Fig. 4),

resulted from the effluent of a petroleum refinery outfall inshore of station 301 at a depth of about 8 m. The concentrations at the 30-m depth range, 2–5 (Fig. 2) and 3 μg-atoms·liter^{-1} (Fig. 4), represent sewage.

The elevated ammonium concentrations near the bottom at inshore stations on CalCOFI lines 90 (Fig. 3) and 93 are of uncertain origin. They could result from decomposition of the large phytoplankton crops often found at these stations, from fish schools, or from the sediments, as well as perhaps from the longshore transport of sewage by coastal currents.

On two of the cruises (cruise 3, Fig. 3; cruise 11), plumes with middepth ammonium maxima within the euphotic zone were noted on line 90 with maximum concentrations >2 μg-atoms·liter^{-1}. The ammonium concentration at the inshore stations on lines 90 and 93 often increased abruptly at the 1% light level.

We did over 800 ammonium analyses, in duplicate. The frequency distribution of the ammonium concentration was obviously different in Santa Monica Bay, where 52% of the values >1 μg-atom·liter^{-1}. Elsewhere, only 4% were as high and 89% were <0.5 μg-atom·liter^{-1} (Table 1).

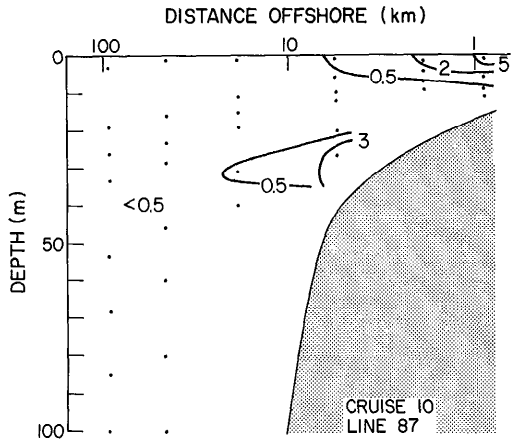

Fig. 4. As Fig. 2, but in a section through Santa Monica Bay, April 1977.

Ammonium assimilation by phytoplankton—The contribution of ammonium to the total N assimilation of the phytoplankton was assessed in two ways. First, the ratio of ammonium uptake to the sum of nitrate and ammonium uptake was considered. Second, total N assimilated was calculated as the photosynthetic carbon assimilation rate multiplied by the ratio of particulate nitrogen to particulate carbon and the measured ammonium uptake compared with that value. The first method gave an average of 52 ± 18%. The second method gave an average value of 34%. These values are rec-

Table 1. Frequency distribution of ammonium concentration (μg-atoms·liter^{-1}).

Santa Monica Bay (sta. 301–303)			All other stations		
$[NH_4^+]$	No. Samples	% of total	$[NH_4^+]$	No. Samples	% of total
0–0.99	70	48	0–0.49	624	89.1
1.0–1.99	27	18	0.5–0.99	48	6.9
2.0–2.99	18	12	1–1.49	13	1.9
3.0–3.99	12	8.2	1.5–1.99	6	0.86
4.0–4.99	3	2.0	2.0–2.49	4	0.57
5.0–5.99	2	1.4	2.5–2.99	2	0.29
6.0–6.99	1	0.7	>3.0	3	0.43
7.0–7.99	2	1.4		Σ = 700	100
8.0–8.99	3	2.0			
9.0–9.99	1	0.7			
10.1–19.9	5	3.4			
>20.0	3	2.0			
	Σ = 147	100			
Total samples	847				

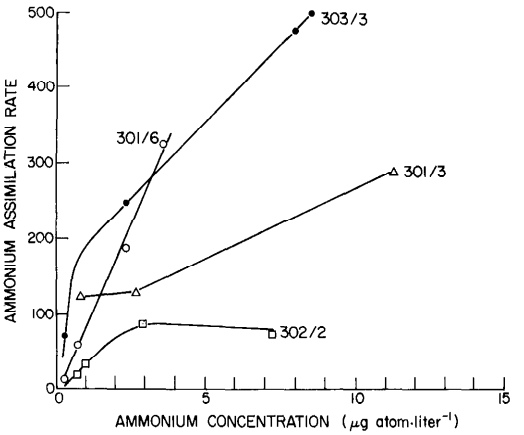

Fig. 5. Ammonium assimilation rate (ng-atom N·μg Chl a^{-1}·d^{-1}) vs. ammonium concentration in the seawater. Lines labeled by station/cruise.

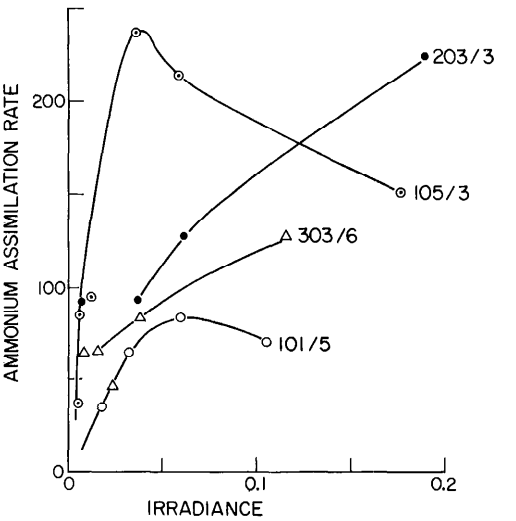

Fig. 6. Ammonium assimilation rate (ng-atom N·μg Chl a^{-1}·d^{-1}) vs. irradiance. Irradiance units are ly·min^{-1}(cal·cm^{-2}·min^{-1}) averaged over 24 h. Lines labeled by station/cruise.

onciled by recalling that McCarthy (1972) found that urea-N assimilation accounted for 28%, on average, of the sum of ammonium, nitrate, and urea-N assimilation. If we assume that urea-N provided 28% in this study as well, we get a value for the first method of 37% for ammonium assimilation as the fraction of total N assimilated. The agreement between the two (37% vs. 34%) is then surprisingly good. If urea-N indeed contributed 28% of phytoplankton N, then these three N sources account for essentially all of the nitrogen required to maintain the observed rate of carbon assimilation and the C:N ratio of the particulate matter. Regenerated forms of nitrogen (ammonium and urea) would provide 62% of the phytoplankton nitrogen and nitrate the remaining 38%.

Modifiers of the rate of ammonium assimilation—Variations with area and depth in ammonium assimilation by the phytoplankton will be considered in the context of factors influencing the process. These modifiers would be expected to include the phytoplankton and ammonium concentrations, temperature, and irradiance which influence growth rate and, as cultures studies have shown, variables such as the C:Chl *a* ratio which are related to the species composition and nutritional status or physiological condition of the phytoplankton.

Examples are readily found in the data showing marked effects of ammonium concentration on its assimilation rate. This was particularly obvious where a wide range of concentrations was found, as in Santa Monica Bay (Fig. 5). Profiles elsewhere showed changes in the uptake rate, when normalized to chlorophyll *a*, consonant with increases or decreases in ammonium concentration with depth, and assimilation rates were saturated at lower ammonium concentrations than in Santa Monica Bay.

Although there is some assimilation of ammonium in laboratory cultures or natural samples incubated in darkness, a positive response to light is often observed (MacIsaac and Dugdale 1972; MacIsaac et al. 1974). Some examples are shown in Fig. 6 for our stations where the ambient concentration of ammonium was fairly uniform over depth. Stations 105, cruise 3, and 101, cruise 5 (Fig. 6), show the most typical response, i.e. a maximum rate at intermediate irradiance and a slight reduction in samples from near the surface that were exposed to the

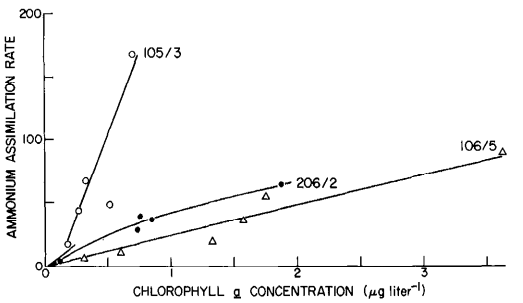

Fig. 7. Ammonium assimilation rate (ng-atom $N \cdot liter^{-1} \cdot d^{-1}$) vs. chlorophyll *a* concentration in three depth profiles with uniform ammonium concentrations. Lines labeled by station/cruise.

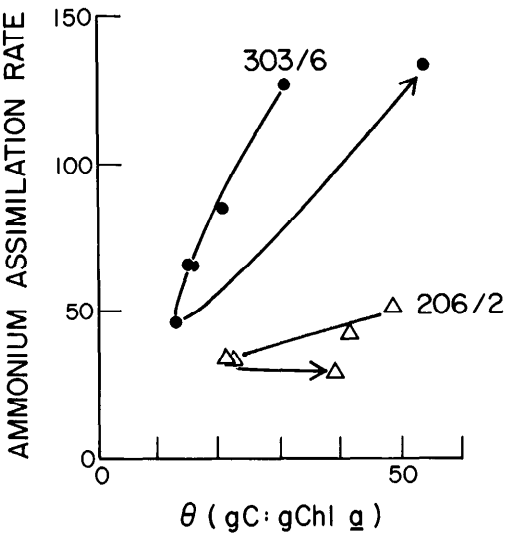

Fig. 8. Ammonium assimilation rate (ng-atom N · μg Chl $a^{-1} \cdot d^{-1}$) vs. θ (C:Chl *a* ratio of the phytoplankton). Lines drawn from shallowest (1 m) sample, with arrows indicating deepest samples at ~1% light depth. Lines labeled by station/cruise.

brightest light. Stations 203, cruise 3, and 303, cruise 6 (Fig. 6), show a continuous increase in rate with irradiance. Both patterns are seen also in photosynthetic rates vs. irradiance. Perhaps related to the reduced assimilation rate at low irradiance is the observation of an increase in ambient ammonium concentration at the 1% light depth on 36 out of 38 occasions at stations 101–103 and 201–203. Presumably the balance between input and uptake rates can shift at very low irradiances, resulting in increased ambient ammonium concentrations at the 1% light depth.

Examples of the variation in rate with the concentration of phytoplankton are shown in Fig. 7. The relationship between uptake rate and chlorophyll *a* concentration is essentially linear in these examples although the slopes of the relation at the three stations are different. These differing slopes reflect in this case differences in the specific growth rates of the phytoplankton assemblages. The specific growth rate at station 105, cruise 3, was $0.9 \cdot d^{-1}$, at 106/5 and 206/2 it averaged about $0.25 \cdot d^{-1}$ over the depth range, as estimated from the carbon assimilation rate and carbon content of the phytoplankton (Eppley et al. 1977*a*).

Other modifiers of ammonium assimilation, identified in laboratory culture studies, are the ratios of C:N and C:Chl *a* of the phytoplankton cells. We consider variations in both ratios to be a result of nutrient deprivation or stress, as in nutrient-limited continuous cultures. We found no clear examples in this study where C:N ratios of particulate matter varied significantly with depth, although they varied somewhat between cruises. Extreme N deficiency is not indicated. However, the phytoplankton C:Chl *a* ratio can vary with the temperature and irradiance prevailing during laboratory growth and among different species of phytoplankton. We noted two examples in the present study where the phytoplankton C:Chl *a* ratio changed progressively over depth and where ambient ammonium concentrations and specific growth rate were fairly uniform (Fig. 8). In both cases the rate of ammonium assimilation per weight of chlorophyll *a* and the C:Chl *a* ratio decreased with depth, then increased abruptly at the depth of lowest light (sta. 303, cruise 6; sta. 206, cruise 3).

Preferential ammonium assimilation—The assimilation of nitrate is inhibited in many phytoplankton species by the presence of ammonium in the medium. The

Table 2. Examples of Relative Preference Index (RPI) for ammonium and nitrate assimilation.

Depth (m)	Light level (% surface)	Ambient concn (μg-atom·liter^{-1})		Assim rate (ng-atom·liter^{-1}·d^{-1})		RPI	
		NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Cruise 2, station 101 during upwelling, inshore diatom-dominated crop							
1	90	0.31	0.70	114	240	0.95	0.88
5	30	0.21	0.92	80	389	0.84	0.93
9	10	0.31	1.44	86	460	0.84	0.97
11	5	0.52	2.34	173	747	0.99	0.96
15	1	0.70	3.59	68	44	3.6	0.46
18	0.5	1.32	7.86	63	48	3.9	0.50
Cruise 2, station 306, offshore							
1	90	0.28	0.18	111	74	0.77	0.80
5	30	0.27	0.13	69	36	0.73	0.79
19	10	0.37	0.45	66	49	1.1	0.68
28	5	0.41	1.36	52	41	2.3	0.54
44	1	0.58	11.1	35	55	7.8	0.64
53	0.5	0.32	14.8	5.4	13	13.8	0.72
Cruise 6, station 301, Santa Monica Bay							
1	90	0.30	0.69	37	25	1.8	0.52
9	30	0.72	0.66	171	95	1.1	0.69
12	18	2.52	3.79	495	86	2.1	0.24
15	12	3.68	6.38	687	83	2.4	0.17

result is that ammonium is assimilated preferentially when both are supplied at equivalent concentrations. Ammonium concentrations as low as 0.5–1 μg-atom·liter^{-1} may partially inhibit nitrate assimilation even when nitrate is present in large excess. McCarthy et al. (1977) have defined a Relative Preference Index (RPI) useful in field studies of this interaction.

$$RPI_{NH_4} = \left(\frac{NH_4 \text{ assim rate}}{\Sigma \text{ N assim rate}}\right) \Big/ \left(\frac{\text{ambient } NH_4 \text{ concn}}{\Sigma \text{ N concn}}\right).$$

In our study the ΣN rate and ΣN concentrations include only ammonium and nitrate while McCarthy et al. (1977) considered urea-N and nitrite as well. When RPI = 1.0, assimilation is equitable to availability. A value of RPI <1 implies rejection, while RPI >1 implies preferential utilization.

Our RPI values for ammonium were about 1 or larger, as expected. The values often increased abruptly with depth at the lowest light levels (Table 2). The ambient nitrate and ammonium concentrations increased less abruptly with depth, suggesting that a factor other than these relative concentrations influenced the RPI. Slight differences in the relationship between the rates of ammonium and nitrate assimilation with irradiance could bring about this result, as noted by MacIsaac and Dugdale (1972). If so, our results suggest that lower irradiance supports a relatively greater rate of ammonium than nitrate assimilation.

The relative preference indices for nitrate uptake were $\leq$1.0. The lowest values, $\leq$0.2, were associated with ammonium concentrations >0.5 μg-atom·liter^{-1} or were found at the lowest light depths (Table 2).

Seasonal and areal variations—We found no seasonal changes in ambient ammonium concentrations. However, the ammonium assimilation rate per chlorophyll *a* followed a seasonal pattern, in parallel with seasonal variation in the photosynthetic rate and C:Chl *a* compositional ratios of the phytoplankton (Eppley et al. 1977*a*). They were highest in summer and lowest in winter, as expected from the seasonal changes in temperature, irradiance, and daylength.

Table 3. Multiple regression analysis of ammonium assimilation rate (ng-atom·liter^{-1}·d^{-1}) according to Eq. 1 (*see text*).

Variable	*F*-values		
	Summer	Winter	All
Phytoplankton concn (P) μg C·liter^{-1}	41.23*	151.41*	129.13*
[NH_4] term	125.91*	14.58*	30.07*
Irradiance term	41.27*	NS†	5.56‡
Temp term	9.19§	NS†	66.09*
C:Chl *a* ratio (θ)	NS†	NS†	NS†

Regression equations

Summer cruise:

$$\log N \text{ assim rate} = 3.86 + 0.364 \log P + 1.31 \log (N \text{ concn term}) + 0.950 \log (\text{Irrad. term})$$

$$F_{(4,53)} = 63.88,\ r^2 = 0.828,\ p < 0.001$$

Winter cruises:

$$\log N \text{ assim rate} = 0.801 + 0.650 \log P + 0.512 \log (N \text{ concn term})$$

$$F_{(2,140)} = 75.74,\ r^2 = 0.520,\ p < 0.001$$

All cruises:

$$\log N \text{ assim rate} = 0.532 + 0.584 \log P + 0.642 \log (N \text{ concn term}) + 0.114\ T + 0.246 \log (\text{Irrad. term})$$

$$F_{(4,196)} = 60.80,\ r^2 = 0.554,\ p < 0.001$$

* $p < 0.001$. † $p > 0.05$. ‡ $p < 0.05$. § $p < 0.01$.

Spatial differences in the ammonium assimilation rate normalized to chlorophyll *a* followed the spatial variations in ambient ammonium concentration (as in Fig. 2). Unnormalized rates, i.e. μg-atoms assimilated per liter and day, followed variations in the phytoplankton standing stock for any particular cruise (given in Eppley et al. 1977*a*), exceeding in magnitude the seasonal effects that were related to the specific growth rate and chemical composition.

Ammonium assimilation was proportionately higher at Santa Monica Bay inshore stations than elsewhere: the ratio of ammonium assimilation to the sum of ammonium plus nitrate assimilation averaged 0.84 ± 0.07 at stations 301 and 302 (n = 6). The ratio averaged 0.47 ± 0.16 (n = 15) at the other inshore stations (101–103, 201–203). A similar ratio was found at the more offshore stations (105–106, 205–206, 305–306) where the average value was 0.50 ± 0.16 (n = 22). No onshore–offshore gradient was apparent in the fraction of regenerated nitrogen assimilated by the phytoplankton, except for the anomolous results for Santa Monica Bay. Station 303 was transitional in terms of this ratio, being high (0.72) on one cruise and low (0.3–0.5) on three others. The influence of the bay didn't extend very far offshore in this respect.

Objective analysis of the variability in ammonium assimilation rate—In the previous sections we gave information on modifiers of ammonium assimilation rate in specific situations in which only one or two of the several modifiers varied and the others were essentially constant over depth. These examples were chosen subjectively, based on experience with both field and laboratory measurements of the process. To provide a more objective view of the variability and the principal modifiers of ammonium assimilation rate we carried out a statistical analysis, omitting data for Santa Monica Bay (sta. 301, 302, 303) because values for several variables, especially ammonium concentration and ammonium assimilation rate, were much higher there than elsewhere.

Ammonium assimilation rate was expected to be a function of phytoplankton concentration (P), ammonium concentration (N), irradiance (I), incubation temperature (T) and the C:Chl *a* ratio in the phytoplankton (θ). We did not expect a simple multiple regression of these variables to explain much of the variability

Table 4. Multiple regression analysis of ammonium assimilation rate per weight of phytoplankton carbon (P) according to Eq. 1. Units—ng-atom·μg C^{-1}·d^{-1}.

Variable	F-values		
	Summer $F_{(1,55)}$	Winter $F_{(1,140)}$	All $F_{(1,197)}$
[NH_4] term	26.31*	21.23*	30.25*
Irradiance term	NS†	NS†	NS†
Temp term	NS†	NS†	94.85*
C:Chl a ratio (θ)	4.16‡	13.75*	9.97§
	Regression equations		
Summer cruise:	log (N assim rate/P) = 1.563 + 1.10 log (N concn term) −0.310 log θ		
	$F_{(2,55)} = 21.60$, $r^2 = 0.440$, $p < 0.001$		
Winter cruises:	log (N assim rate/P) = 0.946 + 0.662 log (N concn term) −0.431 log θ		
	$F_{(2,140)} = 21.88$, $r^2 = 0.238$, $p < 0.001$		
All cruises:	log (N assim rate/P) = 1.209 + 0.714 log (N concn term) −0.316 log θ		
	$F_{(3,197)} = 41.81$, $r^2 = 0.389$, $p < 0.001$		

* $p < 0.001$. † $p > 0.05$. ‡ $p < 0.05$. § $p < 0.01$.

because physiological studies have shown that these factors do not act in a strictly additive fashion. Rather, the form was expected to be more nearly multiplicative:

$$\frac{dN}{dt} \simeq P\left(\frac{N}{K_N + N}\right)\left(\frac{I}{K_I + I}\right) 10^{aT+b}\,\theta. \quad (1)$$

Here θ is used as modifier to perhaps reflect the species composition of the phytoplankton, as this was not determined. Values for half-saturation constants of 0.5 μg-atom·liter^{-1} for K_N and 0.003 ly·min^{-1} for K_I were assumed.

For multiple regression analysis we took logarithms of each of these terms. The analysis was intended to indicate which of these factors, if any, were most important in accounting for the variability observed, and possibly to develop a tool of predictive value. The F-values and slopes of the regression equation (Table 3) suggest the relative importance of the variables. Examination of residuals showed no serious violations of the assumptions of the analysis.

Data for all stations together in the multiple regression analysis indicated that four factors (phytoplankton concentration as carbon, incubation temperature, ammonium concentration, and irradiance) added statistically significant independent contributions to the explained variance. These together accounted for 55% of the variability (196 df, Table 3).

Because ammonium assimilation rates and incubation temperatures were very different between cruise 3 (June) and the cruises 2, 5, and 6 (December–March), these were analyzed separately as a "summer" cruise vs. three "winter" cruises (Table 3). Numbers of observations were 140 for winter cruises and 53 for the summer cruise. For the winter cruises two variables contributed significantly to the explained (52%) variance: phytoplankton concentration and ammonium concentration. For the summer cruise, ammonium concentration was the more important variable, followed in rank by irradiance, phytoplankton concentration, and temperature.

To look more closely at the physiological responses of the phytoplankton to environmental variables, we analyzed the ammonium assimilation rates per weight of phytoplankton carbon in the same manner (Table 4). The ammonium concentration term remained an important variable. The incubation temperature term was important for all cruises together when the full range of seasonal variation in surface temperatures was included, but it was not important within

a season. The C:Chl *a* ratio was of moderate importance. The regression coefficient for the temperature term for all cruises (0.150) is higher than would be expected to result from the seasonal increase in phytoplankton specific growth rate due to temperature (~0.03: Eppley 1972). Thus the coefficient may reflect change related to season and independent of the other variables in the regression equation, but not necessarily only phytoplankton growth rate.

The results of this objective analysis generally support the subjective relations presented earlier. However, the variation in rate of ammonium assimilation with irradiance noted earlier (Fig. 6) either was too infrequent to be significant in the objective analysis or was confounded by the seasonal covariation of irradiance and the C:Chl *a* ratio of the phytoplankton (Eppley et al. 1977*a*).

Discussion

Distribution of ammonium—Ammonium concentrations in southern California coastal waters are low, especially in comparison with the flux of ammonium into the particulate matter and its rate of remineralization. As in the study of heterotrophic uptake of organic matter in the sea, the incorporation of isotopically labeled substrate is more readily and accurately measured than ambient concentrations and the latter provide little insight as to the importance of the substance in the economy of the biota. Low concentrations are not indicative of low importance.

The depth and areal distributions of ammonium (Figs. 2, 4) show two interesting features: concentration maxima at depth, and elevated concentrations in both surface and bottom waters of Santa Monica Bay. In the former category, and excluding Santa Monica Bay, the maximum offshore concentrations at depth within the euphotic zone were low (<1 μg-atom·liter^{-1}) and at mideuphotic depth; inshore they were 3 μg-atoms·liter^{-1} and at the 1% light depth. These maxima may result from the same or from different causes. The offshore observations could result from maximum regeneration rates at middepth or seaward transport of ammonium from local nearshore sources. Attempts to distinguish between these were not successful and the question remains open. At the inshore stations the longshore transport of ammonium from sewage should also be considered, as longshore currents predominate in the nearshore circulation (Hendricks 1977; Winant and Olson 1976). If the benthos were the source of ammonium, then progressive increases in ammonium with depth would be expected rather than a maximum at the 1% light depth. There remains, however, a further possibility that relatively large amounts of ammonium could be released at discrete times and depths by bottom scouring processes of various kinds. We cannot distinguish among the alternatives with the existing information. However, the hypothesis of longshore transport of sewage is attractive because the buoyant jet of sewage at the outfall diffusers is expected to rise from the bottom, mixing with ambient seawater and stabilizing at a depth calculated by the design engineers to be about 40 m. This depth corresponds fairly well with that of the ammonium maximum (cf. Figs. 2 and 4 with Fig. 3).

Ammonium assimilation by the phytoplankton—The expectation that ammonium uptake rate would be related to phytoplankton and ammonium concentrations was supported by the multiple regression analysis. Phytoplankton crops were consistently higher inshore than offshore and were generally highest in Santa Monica Bay (Eppley et al. 1978). The photosynthetic rate and C:Chl *a* ratio of the phytoplankton also vary seasonally, as do surface temperature and irradiance, and these variables also influence the rate of ammonium assimilation. Rates were highest in summer, lower in winter, as would be expected. No onshore–offshore gradient was observed in the ratio of ammonium to nitrate assimilation. However, ammonium assimilation predominated in Santa Monica Bay. The in-

hibition or repression of nitrate assimilation by ammonium as shown by McCarthy's RPI was large both in Santa Monica Bay and at the lowest light depths elsewhere.

Nitrate depth profiles in these waters (except in Santa Monica Bay and elsewhere during strong upwelling) consistently showed a nitrate-depleted surface layer and progressively increasing nitrate concentrations below, the increase beginning middepth within the euphotic zone (Eppley et al. 1979). One expects, since ammonium concentration is more uniform with depth, that regenerated nitrogen would be assimilated predominantly in the surface layer and nitrate at greater depth. This can be seen to a degree in our data, but was less marked than in the eastern tropical Pacific (Goering et al. 1970) or in the central North Pacific (Eppley et al. 1973, 1977*b*). The low RPI values for nitrate assimilation at the 1–3% light depths, the lesser stratification, and the greater vertical diffusion of nitrate in these coastal waters apparently tend to smooth the variation in nitrate and ammonium assimilation with depth. Similar depth profiles of nitrate and ammonium assimilation were noted in these waters by McCarthy (1972).

Variability in ammonium assimilation rate by the phytoplankton—The multiple regression analysis of ammonium assimilation rate as dependent on phytoplankton concentration, an ammonium concentration term, an irradiance term, temperature, and phytoplankton C:Chl *a* ratio indicated that phytoplankton concentration, ammonium concentration, and the C:Chl *a* ratio were important variables within a season. For all seasons temperature was important also. Together the variables accounted for 52, 83, and 55% of the variability observed (r^2 values: Table 3) for winter cruises, a summer cruise, and all cruises together. Except for the summer cruise, insufficient variability is accounted for to tempt one to use the regression equations for predictive purposes. Variability not considered in the regression analysis is inherent in the determination of ammonium concentration, in species differences not reflected in phytoplankton C:Chl *a* ratios, in species differences resulting in the use of inappropriate "constants" in the ammonium concentration and irradiance terms of Eq. 1, in rate variations resulting from incubation of all samples from a depth profile at the surface water temperature, and in analytical error in determining ^{15}N:^{14}N ratios by mass spectrometry. Error in ammonium determination would be large; the average concentration was 0.35 μg-atom·liter^{-1} and the 95% confidence limit would approximate $\pm$0.11 μg-atom·liter^{-1} or $\pm$32%. This error is effectively compounded since the ammonium concentration is used not only in Eq. 1 but also in calculating the ammonium assimilation rate. As noted earlier (McCarthy et al. 1977; Eppley et al. 1977*b*), the determination of ammonium limits the precision and ultimately the understanding of phytoplankton ammonium metabolism in the sea that can be achieved at present.

Comparisons with other areas—Estimates of the contribution of ammonium to the nitrogen assimilation of phytoplankton have been made for a number of ocean areas and the Chesapeake Bay. Similar studies have also been carried out in artificial enclosures containing coastal plankton under the CEPEX program. In these, nitrate was added at intervals to support the continued growth of the enclosed phytoplankton, yet regenerative processes resulted in ammonium production sufficient to account for about 50% of the nitrogen assimilated (Harrison et al. 1977).

Dugdale and Goering (1967) and MacIsaac and Dugdale (1972) have compared available information on ammonium and nitrate assimilation for oligotrophic and eutrophic areas and for oligotrophic areas enriched with sewage. Our data generally agree with their findings. Ammonium assimilation is predominant in oligotrophic areas, accounting for essentially all the inorganic nitrogen assimilated. In such areas, e.g. the central

North Pacific at 28°N, 155°W, nitrate is essentially absent from the euphotic zone and its concentration begins to increase well below the 1% light depth (Eppley et al. 1977*b*). Apparently the major "new production" taking place there is nitrogen fixation in summer (Mague et al. 1977). In eutrophic areas, ammonium usually contributes slightly <50% of the inorganic nitrogen assimilated. In areas enriched by sewage, MacIsaac and Dugdale (1972) reported 80% of the inorganic N assimilated was NH_4^+-N, as we found in Santa Monica Bay near the refinery outfall. Harvey and Caperon (1976) reported 75% in the sewage-enriched portion of Kaneohe Bay, Hawaii. There, urea and ammonium assimilation were nearly equal.

Haines (1973) concluded that regenerated nitrogen accounted for 95% of the N assimilated by primary producers in Georgia shelf waters. This high value is based on budget estimates, unlike the other values cited that were derived from rate measurements. McCarthy et al. (1977: table 6) found that ammonium contributed an average of 70% of the sum of ammonium and nitrate uptake in Chesapeake Bay. MacIsaac et al. (1974) reported values for the contribution of ammonium to the sum of nitrate and ammonium uptake for sectors of the North African upwelling area ranging from 35–71%. The average value of 52% for southern California coastal waters is intermediate, reflects occasional upwelling, continuous diffusive input of nitrate into the euphotic zone from below, localized sewage inputs, and mesoscale intrusions of water into the area that vary in content of plankton and nutrients. As in the CEPEX enclosures and upwelling areas, phytoplankton growth is driven by nitrate additions to the euphotic zone; in fact, over 40% of the temporal and spatial variability in phytoplankton standing stocks is accounted for by the depth of the nitrate concentration gradient within the euphotic zone (Eppley et al. 1979). Regenerative processes act as a multiplier of the nitrate input, resulting in a doubling or tripling of the flux of nitrogen into the phytoplankton. Turnover times for ammonium in the euphotic zone are very brief (1–3 days: McCarthy 1972), and the phytoplankton growth rate is sufficient to replace the total particulate organic carbon and nitrogen in 1–3 weeks (Eppley et. al. 1977*a*). Thus ambient ammonium concentrations are low and do not reflect the significance of ammonium to primary production. The relatively high ammonium concentrations in sewage and the large sewage and refinery effluent inputs ($4 \times \sim 10^9$ liters$\cdot$d^{-1}) represent only a small fraction, of the order of 10%, of the ammonium made available to the phytoplankton by regenerative processes.

The flux of ammonium (and urea) nitrogen through the food web is derived primarily from the metabolic (largely respiratory) activities of animals and bacteria. One can think of this flux as complementary to secondary and higher level production and related to it by the ratio of food nitrogen invested in growth to that lost in catabolic activities. It may ultimately be possible to estimate that production from such simple measures as these.

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