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The effect of sewage on uptake of inorganic nitrogen and carbon by natural populations of marine phytoplankton

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ABSTRACT

The short-term effect of sewage effluent on nitrogen and carbon productivity of natural marine phytoplankton obtained near two California outfalls has been studied. Uptake of ammonium was shown to be inhibited at much lower effluent concentrations than was carbon uptake. Since the populations studied were shown to exhibit Michaelis-Menten kinetics for ammonium uptake, a precise measurement of inhibition could be obtained. The results have immediate application since the phytoplankton populations of the area studied have been shown previously to be nitrogen limited.

1. Introduction

The paradoxical qualities of sewage effluent in both enhancing and repressing biological processes and the distribution of these qualities relative to outfall location (and thus effluent concentration) are known generally (e.g. Foyn, 1971; Greene and Sarason, 1974). Municipal sewage contains high concentrations of ammonium relative to ambient seawater levels, making that ion a good tracer for marine outfall discharges and suggesting that nitrogen uptake would provide a good measurement of effluent effects on phytoplankton. The identification of nitrogen as limiting phytoplankton growth in coastal waters (Ryther and Dunstan, 1971; Eppley *et al.*, 1972; Thomas *et al.*, 1974; Goldman, 1976) argues strongly for the need to understand any modifications of nitrogen-uptake processes in phytoplankton exposed to sewage. In this paper, the results of experiments using ¹⁵N to study phytoplankton uptake of inorganic nitrogen contained in the municipal sewage effluents of two coastal California outfalls are described. In these experiments, designed to measure uptake kinetic parameters, ammonium was provided as NH₄Cl or as ammonium contained in sewage effluent. The effect of the sewage effluent on carbon uptake by photosynthesis was measured concurrently using ¹⁴C. The work was done on R.V. *Thompson*

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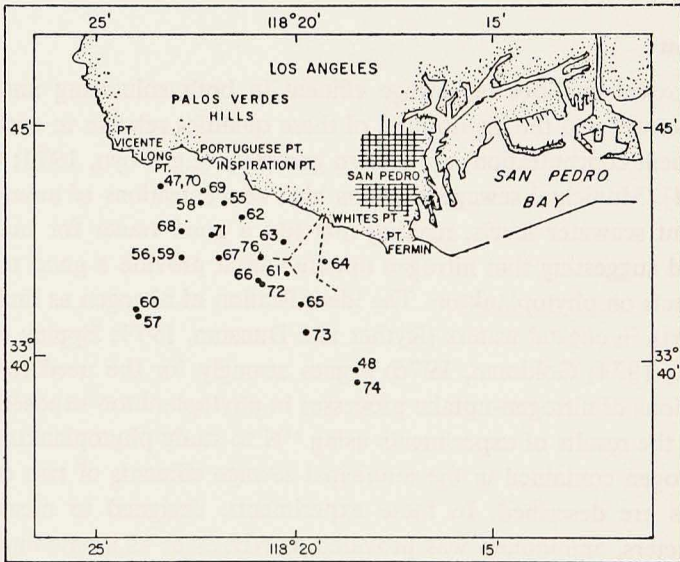
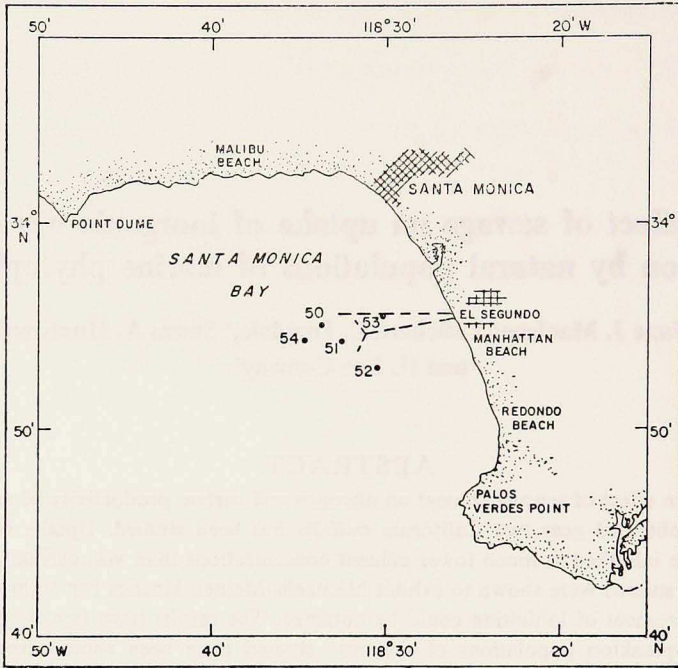


Figure 1. Station and outfall locations during OUTFALL I.

during Cruise 67 (OUTFALL I) in March-April, 1972. Station and outfall locations are shown in Figure 1.

2. Methods

In these experiments, the uptake rates of ammonium and nitrate were measured in natural marine phytoplankton populations using the ^{15}N method described elsewhere (e.g. Neess *et al.*, 1962; MacIsaac and Dugdale, 1972). Seawater samples were collected with a 35-liter nontoxic sampler, mixed in a polyethylene container and distributed into 4-1 Pyrex bottles. The samples were enriched variously with sewage or sterile inorganic nutrient solutions, inoculated with the appropriate ^{15}N labelled compound, and held in seawater-cooled incubators under natural light for 24 hr. The particulate fraction was collected on Hurlbut 984H glass-fiber filters, dried, and retained for mass spectrometry. The analysis was done with an Associated Electrical Industries MS-10 mass spectrometer (180° analyzer, 5 cm radius). The automated Dumas method used to convert samples for mass spectrometry and particulate nitrogen (PN) to N_2 , and calculation of the ^{15}N enrichments are described in Pavlou *et al.*, 1974.

The absolute uptake rate, ρ , with units of $\mu\text{g-atoms } 1^{-1} \text{ time}^{-1}$, was calculated from

$$\rho_{21} = N_T [a_{2(t)} - a_{2(0)}] / a_1 \Delta t$$

following the compartmental notation used by Sheppard (1962), and where:

ρ_{21} is the transport rate of material into the initially unlabelled compartment 2 (PN) from the labelled compartment 1 (ammonium or nitrate in the liquid fraction);

N_T is the PN concentration at the end of the incubation period (units of $\mu\text{g-atoms } 1^{-1}$);

$a_{2(t)}$ is the specific activity of the label (units of atom % ^{15}N) in compartment 2 at time = t ;

$a_{2(0)}$ is the specific activity of the label in compartment 2 at time = 0;

a_1 is the specific activity of the label in compartment 1, assumed to be constant with time; and

Δt is the period of incubation.

The specific uptake rate, V_t , with units of t^{-1} , was calculated as $V_t = \rho_{21} / N_T$. The choice of this method of computing V tends to underestimate the average value of V when high uptake rates result in large increases in PN during incubation. Other values of V may be calculated, but each alternative requires different assumptions (Grunseich *et al.*, 1979). The method described here is consistent with our previously published results of ^{15}N experiments.

Using the method of Pavlou *et al.* (1974), Conway (1974) found means and 95% confidence intervals of ^{15}N enrichment in the particulate fraction of $11.5 \pm 1.1\%$ (9 replicates) and $13.1 \pm 0.6\%$ (10 replicates) for nitrate and ammonium uptake, respectively, with samples from a culture of *Skeletonema costatum*. The means and 95% confidence intervals for PN content in the same samples were 5.32 ± 0.40

and $4.52 \pm 0.17 \mu\text{g-atoms}$, respectively. In a field experiment using the collection and incubation (6 hr only) procedure outlined above, the means and 95% confidence intervals were $6.30 \pm 0.70\%$ for ^{15}N -nitrate enrichment and $15.49 \pm 1.17 \mu\text{g-atoms}$ for PN concentration (Grunseich *et al.*, 1979). In the results to be discussed here, ^{15}N enrichment in the particulate fraction ranged from 0.2-61% in samples over a size range of 13-76 $\mu\text{g-atoms}$ PN.

Detrital nitrogen dilutes the living nitrogen fraction in the particulate sample analyzed with the mass spectrometer and the consequent reduction in ^{15}N enrichment leads to an underestimate of V_t . However, the absolute uptake rate, ρ , is correct since the detrital effect is cancelled in the multiplication of V by PN (Dugdale and Goering, 1967). Primarily, values of ρ are used in the analysis of the data here; however, for interpretation of the results in terms of uptake kinetics, analysis of ^{15}N uptake experiments was done using values of V_t , corrected for the detrital nitrogen added with the sewage. Since no direct analysis was made of the PN in the undiluted sewage, estimates were obtained from the relationship:

$$\Delta\text{PN} = (\rho_{\text{NH}_4} \times \Delta t) + (\text{PN}_s \times \text{VOL})$$

where:

ΔPN is the change in PN in the enriched seawater sample over the incubation period;

PN_s is the concentration of PN in the sewage, all assumed to be detrital; and

VOL is the volume of sewage added 1^{-1} .

Plots were made of $\Delta\text{PN} - (\rho_{\text{NH}_4}) (\Delta t)$ against VOL , and slopes of the resulting straight lines were used to compute the concentration of detrital PN from the sewage. The values obtained were 168 $\mu\text{g-atoms } 1^{-1}$ for the undiluted White's Point Outfall and 116 $\mu\text{g-atoms } 1^{-1}$ for the Hyperion Outfall. Based on these values, the sewage detrital effect was determined to be significant only at the two highest sewage enrichments. Corrections were made for these experiments and are identified wherever they appear in tables.

Table 1. Source nutrient concentrations in the OUTFALL I experiments. Undetectable levels indicated by *u*. Concentrations in $\mu\text{g-atom } 1^{-1}$.

	NH_4^+-N	NO_3^--N	$\text{Si(OH)}_4-\text{Si}$	$\text{PO}_4^{3-}-\text{P}$
White's Point				
Effluent	2300	8	627	262
Hyperion				
Effluent	1145	16	562	265
Station 50				
(4 m)	0.1	<i>u</i>	2.5	0.3
Station 76				
(3 m)	0.1	<i>u</i>	2.4	0.2

Table 2. Dilutions, concentrations, and ammonium uptake rates in the OUTFALL I experiment at Station 50. Concentrations in $\mu\text{g-atoms l}^{-1}$.

Dilution with seawater	NH_4^+-N (added)	Total NH_4^+-N (calculated*)	$^{15}\text{NH}_4^+-\text{N}$ (added)	Approx. Percent ^{15}N	$V_t^{\text{NH}_4}$ hr^{-1}	Total $\text{Si(OH)}_4-\text{Si}$ (by analysis)	$\text{PO}_4^{3-}-\text{P}$
White's Point							
1:13,300	0.17	0.38	0.11	28.9	0.0014	3.48	.37
1: 5,000	0.46	0.77	0.21	27.3	0.0055	3.48	.38
1: 2,500	0.92	1.45	0.43	29.7	0.0112	3.92	.42
1: 250	9.20	13.56	4.26	31.4	0.0163	4.92	1.25
					(.0190)**		
1: 25	92.00	134.20	42.11	31.4	0.0046	26.70	10.70
					(.0124)**		
Hyperion							
1:5,700	0.19	0.40	0.11	27.5	0.0040	3.11	.29
1:2,200	0.49	0.80	0.21	26.3	0.0056	3.86	.35
1:1,100	0.96	1.49	0.43	28.9	0.0095	3.40	.49
1: 110	9.60	13.96	4.26	30.6	0.0137	7.58	2.45
					(.0158)**		
1: 11	96.00	138.20	42.11	30.5	0.0032	49.60	19.90
					(.0107)**		
Inorganic NH_4^+-N							
		0.34	0.25	73.5	0.0107	3.27	.25
		0.84	0.75	89.5	0.0051	3.63	.24
		1.59	1.50	94.3	0.0093	3.64	.23
		15.13	15.04	99.4	0.0258	3.24	.24
	117.00	142.16	25.06	17.6	0.0248	6.80	.40
Inorganic NH_4^+-N , $\text{Si(OH)}_4-\text{Si}$, and $\text{PO}_4^{3-}-\text{P}$							
		0.34	0.25	73.5	0.0039	3.08	.28
		0.84	0.75	89.5	0.0058	3.47	.32
		1.59	1.50	94.3	0.0097	3.35	.36
		15.13	15.04	99.4	0.0278	4.38	1.26
	117.00	142.16	25.06	17.6	0.0270	33.20	17.30

* Includes ambient concentration of $0.1 \mu\text{g-at l}^{-1} \text{NH}_4^+-\text{N}$.

** Corrected for estimated detrital PN content.

Four parallel experiments were set up in which serial enrichments of seawater samples collected at Stations 50 and 76 near the Hyperion and White's Point Outfalls, respectively (Fig. 1), were made with sewage from 1) the Los Angeles County Outfall at White's Point and 2) the Hyperion Outfall serving the city of Los Angeles, 3) with sterile NH_4Cl solution, and 4) with a sterile mixture of NH_4Cl , $\text{NaSi}(\text{OH})_4$, and NaH_2PO_4 . The nutrient concentrations of the effluents and of the water collected from Stations 50 and 76 are shown in Table 1. The dilutions and concentrations of ammonium, silicate, and phosphate added from the various sources were almost identical for the work at both stations. The more detailed work was done at Station 50 and these values are given in Table 2 as representative. The maximum reduction in salinity would have been less than 9%, or from approximately 33.5‰ to 30.5‰. After the addition of ^{15}N , which usually amounted to about 30% of the total amount of the ion species being labelled for the sewage samples and about 80-90% for the inorganic additions (Table 2), subsamples were drawn for chemistry and sometimes for ^{14}C and other measurements. All samples were analyzed at sea for ammonium, nitrate-plus-nitrite, phosphate, and silicate by the automated methods described in Friederich and Whitledge (1972). Carbon fixation was measured in 125 ml bottles with ^{14}C using incubation conditions identical to those of the ^{15}N samples, with the final activity determined by scintillation counter, as described in Huntsman and Barber (1975).

The sewage used represented the phase of processing just prior to discharge into the diffusers and was a composite of hourly subsamples collected over a 24 hr period about a day previous to the experiment at Station 50. The White's Point effluent, with a high industrial load, was chlorinated and received primary treatment; the Hyperion effluent also received primary treatment but was unchlorinated (I. Haydock, personal communication; e.g. Mitchell and McDermott, 1975). The amount of sewage effluent added to each experimental flask was determined by first analyzing the effluent for ammonium concentration and then computing the amount of effluent equivalent to each addition of ammonium as NH_4Cl . Where inorganic silicate and phosphate were added, the enrichments were made to simulate those of the parallel sewage enrichments.

3. Results

The results of the experiments are plotted in Figure 2 where ρ is plotted against concentration of ammonium. The responses of the populations to the various nutrient sources were positive and very similar at low concentrations. However, at the higher enrichments, uptake of ammonium in sewage clearly was suppressed. The highest uptake rate was measured in the presence of the highest ammonium concentration as supplied in the sterile inorganic nutrient mix (Table 2) and not in the sewage additions.

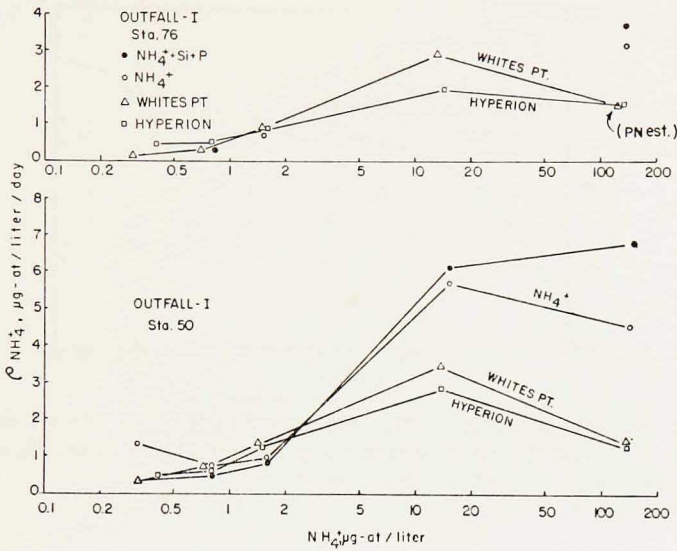


Figure 2. The uptake of ammonium from different sources by phytoplankton from Stations 50 and 76.

The departure of ammonium uptake from normal kinetics in the sewage series can be examined from a theoretical point of view. MacIsaac and Dugdale (1969) have shown that the uptake of ammonium by natural populations of marine phytoplankton can be described by the Michaelis-Menten expression:

$$V = V_{\max} \frac{S}{K_s + S} \text{ where}$$

V = specific uptake velocity, units t^{-1} ;

V_{\max} = maximal uptake velocity, units t^{-1} ;

S = substrate concentration, ammonium in this case, units $\mu\text{g-atoms } l^{-1}$; and

K_s = Michaelis constant, the substrate concentration at which $V = V_{\max}/2$, units $\mu\text{g-atoms } l^{-1}$.

The values of the constants V_{\max} and K_s usually are obtained by various linear transformations of the data. Since the more complete data set was obtained at Station 50, only those results were analyzed in kinetic terms. A Hanes-Woolf (Segal, 1975) linear transformation was applied to the data resulting from the inorganic ammonium additions. In the final analysis, the points associated with the highest and lowest enrichments were excluded on the basis of apparent inhibition in the first case and scatter in the second. The least squares fit of these transformed data gave a correlation coefficient of 0.997. The resulting values of $V_{t_{\max}}$ and K_s , 0.034 hr^{-1} and 4.34 $\mu\text{g-atom } l^{-1}$, respectively, were used to compute the rectangular hyper-

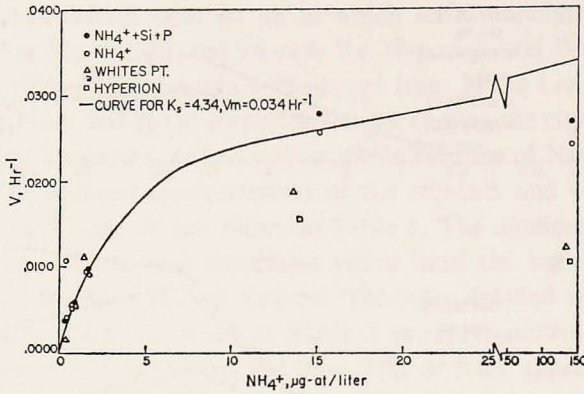


Figure 3. The hyperbola of uptake vs. concentration calculated from selected data ($S = 0.8 - 15.2 \mu\text{g-atom l}^{-1}$) from the inorganic ammonium experiments at Station 50, and its relation to uptake of the sewage ammonium.

bola described by the Michaelis-Menten expression. The calculated hyperbola and all data points are plotted in Figure 3. At the lower enrichments there is good agreement between each of the four experiments and the computed hyperbola. The highest inorganic ammonium concentrations show fairly good agreement with the computed hyperbola also, although the lack of enrichment with phosphate and silicate is apparent. The shortfall in expected uptake by the two sewage series at high concentrations can be expressed by the ratio of expected (uninhibited) to observed (inhibited) uptake velocities. These values are given in Table 3 for the various enrichments. The ratio, $V_{\text{uninhibited}}/V_{\text{inhibited}}$, is independent of substrate concentration in the case of noncompetitive enzyme kinetics (Walter, 1965); a plot of the ratio

Table 3. Ratio of measured ammonium uptake to that calculated from the Michaelis-Menten expression, for Station 50.

Outfall	Sewage Added, ml l^{-1}	$V_i \text{ calc}/V_i \text{ meas}$
White's Point	0.07	1.6
	0.19	0.9
	0.38	0.8
	3.81	1.4
	38.57	2.7
Hyperion	0.17	0.7
	0.43	1.0
	0.83	0.9
	8.33	1.7
	83.81	3.1

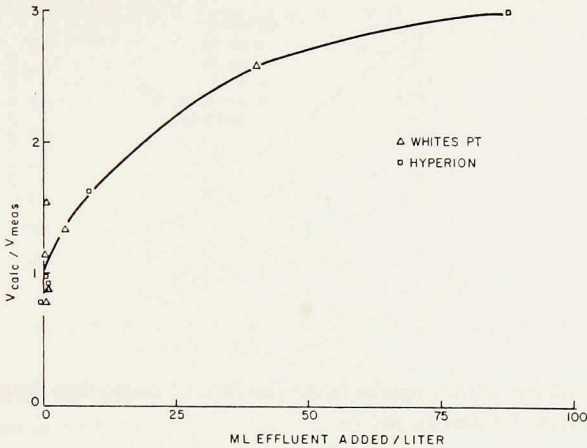


Figure 4. The relationship between sewage concentration and the inhibition of ammonium uptake as expressed by the ratio of $V_{uninhibited}/V_{inhibited}$, in Station 50 phytoplankton.

against inhibitor concentration would give a straight line if noncompetitive kinetics were obeyed. The plot resulting from the data, Figure 4, suggests a curvilinear relationship. The number of data points is, however, insufficient to draw any conclusions other than a positive relationship between sewage added and increase in the ratio, i.e. inhibition with an indication of higher specific inhibition at sewage concentrations of about 4-9 ml l^{-1} than at the highest additions of 40-90 ml l^{-1} . The potential usefulness of the approach with better experimental design is indicated, however. Carbon fixation, Figure 5, was not affected clearly by the enrichments except for the strong suppression observed at the maximum concentrations of both sewage types.

The relative effects on nitrate uptake of ammonium added as NH_4Cl and as sewage effluent were investigated using ^{15}N -labelled nitrate. The results of these experiments, which were designed to reveal any gross differences among the am-

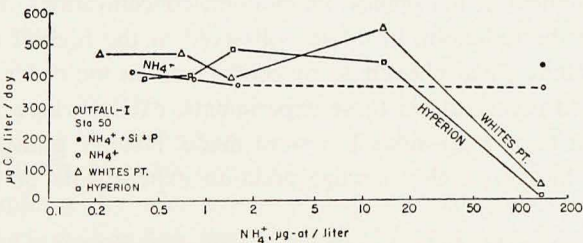


Figure 5. Variation in carbon uptake with source and concentration of ammonium in Station 50 phytoplankton.

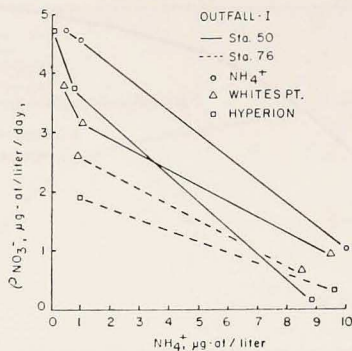


Figure 6. The inhibition of nitrate uptake in the presence of ammonium from different sources, in phytoplankton from Stations 50 and 76.

monium sources, are shown in Figure 6. Nitrate uptake was suppressed strongly by all sources at low concentrations, in accord with a large number of observations made by Conway (1977). The preferential use of ammonium by algae is well known (e.g. Morris, 1974), and the independence of this suppression and the source of the ammonium suggests that the effect in these measurements was related to ammonium concentration only.

4. Discussion

In these experiments, nitrogen productivity was enhanced by the lower enrichments with ammonium from either sewage or sterile inorganic solutions. These results suggest that the phytoplankton populations sampled were nitrogen limited, agreeing with the previously mentioned conclusions of Eppley *et al.* (1972) and Thomas *et al.* (1974) based on California coastal work.

Higher concentrations of sewage ammonium failed to stimulate ammonium uptake to the levels obtained in the presence of equivalent concentrations of inorganic ammonium. With the addition of silicate and phosphate to the inorganic ammonium, uptake was maintained at the highest ammonium concentrations. Phosphate depletion may explain the reduction in uptake observed at the highest concentration of inorganic ammonium. Final phosphate concentrations on the order of $0.1 \mu\text{g-atoms } 1^{-1}$ were observed repeatedly in these experiments, except where initial phosphate enrichments of at least $1 \mu\text{g-atom } 1^{-1}$ were made. Neither phosphate nor silicate was depleted in the two higher sewage addition experiments of each enrichment series.

Carbon fixation usually does not reflect short-term (6-24 hr) fluctuations in nitrogen uptake directly according to MacIsaac and Dugdale (1972). (Recently, however, Falkowski and Stone (1975) observed the temporary suppression of carbon

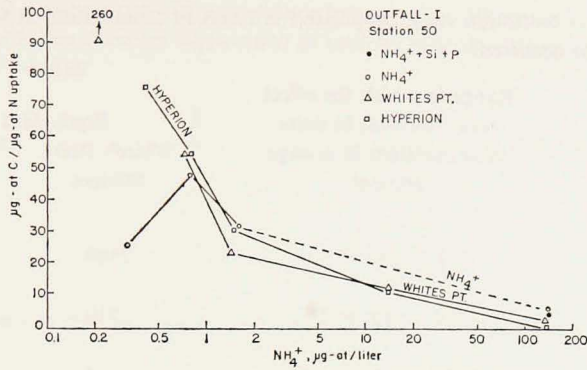


Figure 7. Variation of the ratio of carbon uptake to ammonium uptake with changing sources and concentrations of ammonium in Station 50 phytoplankton.

fixation in field studies for 6-8 hr after nitrate enrichment). The independence of nitrogen and carbon uptake processes in this study can be seen by comparing Figures 2 and 5 where the relative constancy of carbon uptake contrasts with the changes that occurred in ammonium uptake. In Figure 7 the ratio of C to N uptake is plotted against ammonium concentration, revealing the different responses of the two kinds of uptake to the range of experimental conditions. Using the C:N atomic composition ratio of 6.5:1 (Redfield *et al.*, 1963) as an approximation for uptake as well, the ratio was high when the nitrogen supply was low, rapidly decreasing as the nutrient supply increased. The populations enriched with NH_4Cl alone and with inorganic mix showed uptake ratios of 6.6:1 and 5.0:1, respectively, at the highest enrichments. At the highest sewage enrichments, both carbon and nitrogen uptake were very low, but the C:N ratios of 2-3:1 may reflect a more severe effect on photosynthesis. However, the possibility exists that nonbiological sorption of the ^{15}N by the sewage particulates took place in our experiments leading to an overestimate of the nitrogen uptake in these samples. Even if all the uptake in these samples was nonbiological, the rates were so low that the effect would have been insignificant at any of the other sewage concentrations tested.

The results obtained in this study suggest that enhancement and inhibition must be defined in terms of specific responses of phytoplankton. For example, nitrate uptake is inhibited at low concentrations of ammonium, on the order of $1 \mu\text{g-atom l}^{-1}$ or less as observed by Conway (1977) in many parts of the sea and by MacIsaac and Dugdale (1972) in the vicinity of the Keratsini Outfall at Athens, Greece. Urea uptake also is inhibited by ammonium (McCarthy and Eppley, 1972), and since amino acid uptake is slow at best and is enhanced by nitrogen deprivation (Wheeler *et al.*, 1974), there is little to suggest that dissolved organic nitrogen would be utilized to any significant extent in the presence of ammonium. Inhibition of am-

Table 4. Sewage concentration ranges expressed in terms of ammonium, at which various identifiable inhibitions occurred.

Process Inhibited	Range in which the effect was observed, in units of ammonium in sewage effluent	Equivalent Dilution*	
		White's Point Effluent	Hyperion Effluent
Nitrate uptake	$\geq 1 \mu\text{g-atoms l}^{-1}$	2300	1145
Ammonium uptake	$\geq 10 \mu\text{g-atoms l}^{-1}$	230	114
Carbon uptake	$\geq 100 \mu\text{g-atoms l}^{-1}$ (possibly)	23	11

* Computed relative to the concentration of ammonium for White's Point and Hyperion effluent shown in Table 1.

monium uptake, and presumably of growth eventually, occurred in this study when sewage-added ammonium levels were in the range of 10-100 $\mu\text{g-atoms l}^{-1}$; carbon uptake was reduced only at the latter, highest level. The implication of this series of effects is that growth rate may be affected by much lower amounts of effluent than is carbon fixation and that virtual single source (ammonium) dependence is forced even at very low effluent levels. Such dependence may be important if phytoplankton species competition is in part based upon diversity of inorganic species of nitrogen sources. These results are summarized in Table 4.

The extent to which inhibition of ammonium uptake by effluent discharge actually may take place can be estimated from the OUTFALL I data. The ammonium concentration expected in the vicinity of both outfalls can be computed from an assumed initial dilution of 100:1 (Brooks, 1960), and the concentration of ammonium in the effluents used here, 1145 $\mu\text{g-atoms l}^{-1}$ for the Hyperion Outfall and 2300 for the White's Point Outfall. The computed ambient concentrations, 11-23 $\mu\text{g-atoms l}^{-1}$, represent sewage concentrations within inhibitory range for ammonium and nitrate uptake, but not for carbon uptake. Data from the OUTFALL I stations near the White's Point Outfall may be used to assess the probability that sewage concentrations at inhibitory levels are reached in the real world. In Table 5 the depths of maximum ammonium concentrations and the concentrations of ammonium are shown for 4 stations. The range of values is 20-42 $\mu\text{g-atoms l}^{-1}$ and the range of depths is 20-49 m. Values from a station at the same outfall on 6 June 1971 ranged from 18-30 $\mu\text{g-atoms l}^{-1}$ between 10 and 48 m (Institute of Marine Resources, SIO, 1971). The apparent sewage dilution rates computed relative to White's Point effluent ammonium concentrations for the OUTFALL I data also are shown in Table 5 with a range of 55-115.

Table 5. Maximal concentrations of ammonium (greater than 20 $\mu\text{g-atoms l}^{-1}$), depths of occurrence, and effective dilutions represented at stations near the White's Point Outfall during the OUTFALL I cruise.

Station	Z m	NH_4^{+-}N $\mu\text{g-atoms l}^{-1}$	Effective Dilution*
61	49	24.9	92
62	20	42.0	55
64	20	20.0	115
69	20	31.4	73

* Computed relative to the concentration of ammonium for White's Point effluent shown in Table 1.

The upwelling that was in progress during OUTFALL I was shown to carry an ammonium plume, whose core concentration was just over 10 $\mu\text{g-atoms l}^{-1}$, from the area of the White's Point Outfall toward the northeast (Whitledge *et al.*, 1972). Inhibitory concentrations, therefore, were present for a distance of some miles away from the outfall and at depths coinciding with the euphotic zone. The upwelling mechanism is seen to be particularly effective in advecting seawater-effluent mixtures upward and inshore.

Thomas *et al.* (1974) made bioassay experiments on surface waters near various coastal California outfalls, using increase in number of cells or fluorescence over 4 days as a measure of growth. In general, they found inorganic nitrogen to be stimulating, but observed inhibition by White's Point surface water on three occasions; each time the effect was observed in a different assay alga. Stimulation by subsurface water was observed commonly. Sewage from the Point Loma Outfall (San Diego) diluted 1:200 was stimulatory. In other work from the area of the Point Loma Outfall, a series of measurements of ^{15}N -labelled ammonium uptake was made (Institute of Marine Resources, SIO, 1971). In one case the ambient ammonium concentration approached 6 $\mu\text{g-atoms l}^{-1}$ (presumably related to sewage enrichment), and uptake rate in this sample was inhibited relative to rates observed where ambient concentrations fell between approximately 0.5 and 1 $\mu\text{g-atom l}^{-1}$. In continuous culture experiments using diluted-sewage media, toxic qualities of the sewage were suggested as responsible for the growth inhibition observed with the higher sewage concentrations (Dunstan and Menzel, 1971; Goldman, 1976). These results all fit well with the observations reported in this paper and are consistent with the idea that nitrogen limits growth and that above a stimulatory region, sewage properties slow growth through inhibition of ammonium as well as nitrate uptake.

The effect on carbon processes of the toxic qualities observed in these enrichment experiments would not be expected to be widely apparent at the effluent dilutions present in the euphotic zone around the California outfalls, and Eppley *et al.* (1972) described only enhancement of primary production (24 hr experiments) and phyto-

plankton standing stock at their outfall stations there. These observations were confirmed during OUTFALL I in the vicinity of the White's Point Outfall (Whitledge *et al.*, 1972).

There is little to distinguish between the effects of the sewage from the two sources in the results here. Samples enriched with Hyperion effluent did show the lower carbon and nitrogen uptake rates. But this result may reflect the fact that the Hyperion sewage was relatively poor in ammonium, and almost twice as much Hyperion as White's Point sewage was required to achieve the desired ammonium enrichments (Table 1).

When the uptake rates at the highest sewage enrichments are compared to those from the next-highest enrichments (Figs. 2 and 5), the absolute differences are greater in every case for White's Point as opposed to Hyperion effluent. Thus, the White's Point effluent appears to be the more inhibitory, especially considering that smaller volumes of White's Point than of Hyperion effluent were involved. The White's Point Outfall carries a heavy load of industrial sewage and tends to have relatively higher levels of trace metals as well as elevated levels of phenol and cyanide, and in contrast to Hyperion, received chlorination (e.g. Mitchell and McDermott, 1975). In the continuous cultures of Dunstan and Menzel (1971), growth rates were not reduced until sewage concentrations in the influent exceeded 20%, but the undiluted effluent they used contained much lower concentrations at least of trace metals than either effluent used here. The complex mixture of potentially toxic materials in both White's Point and Hyperion effluents and the lack of information on which of these might induce the characteristics observed in these experiments make it impossible to suggest the active agent or agents in the effluents used.

The results reported here suggest the complexity of the effects on phytoplankton of sewage effluent added to the sea through marine diffusers. In this instance, the elucidation of the effects on inorganic nitrogen uptake couples directly with the already existing knowledge that the sea in general and the California coastal region in particular are nitrogen limited. Specifically, while sewage-contained nitrogen ultimately should stimulate production in such a region, the work here demonstrates the existence of sewage qualities that actually impair nitrogen productivity (in 24 hr experiments)—the suppression of nitrate uptake due to elevated ammonium levels and of ammonium uptake due to unspecified properties of the sewage itself. Further similar experiments, designed and analyzed in terms of enzyme kinetics or some other rigorous mathematical framework, should prove useful in building computer models to predict the nature and extent of both eutrophication and inhibition processes resulting from discharge of effluents into the sea.

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