EVIDENCE FOR EUTROPHICATION IN THE SEA NEAR SOUTHERN CALIFORNIA COASTAL SEWAGE OUTFALLS—JULY; 1970¹

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INTRODUCTION

Much of the year surface waters off southern California are depleted of plant nutrients, especially nitrogen, with nitrate undetectable at the surface and ammonium concentrations less than 1 µM (cf. Strickland, ed., 1970, and earlier references cited therein). More recently Ryther and Dunstan (1971) reported nitrogen to be limiting for phytoplankton growth also in waters off Long Island. In local waters natural enrichment takes place periodically, especially in spring and summer, by the upwelling of nutrient-rich water when marked increases in the concentration of nitrate, phosphate, and silicate are seen. A detailed study carried out April through September, 1967, off La Jolla, California, provided comparative data on crop size and nutrient concentrations in both quiescent and upwelling periods (Strickland, ed., 1970). The physical oceanography of upwelling has been reviewed recently (Smith, 1968) and the importance of the processes for local phytoplankton production has been recognized for many years (Moberg, 1928). Nutrient enrichment during upwelling tends to increase the size of the phytoplankton crop in local waters. In some cases, especially offshore, diatoms are the principal components of the resulting blooms (Sverdrup & Allen, 1939; Sargent & Walker, 1948) whereas dinoflagellates often form blooms (red tides) within a few miles of shore (Allen, 1946; Holmes et al., 1967). At present we are unable to predict whether dinoflagellates or diatoms will increase in response to nutrient enrichment nearshore (Strickland, ed., 1970) and further research on the character and mechanisms of species succession is needed.

Enrichment of surface waters results also from sewage disposal off southern California in outfalls serving Ventura, Los Angeles, Orange and San Diego counties. At present we lack sufficient data to compare the nutrient contributions from upwelling and sewage disposal to local surface waters. Very preliminary and approximate estimates suggest that natural upwelling may exceed sewage by an order of magnitude as a source of nitrogen for phytoplankton growth over a year. Fairly accurate estimates of the nitrogen input from sewage can be made but determining the contribution from upwelling would be very costly of ship time and no doubt variable from year to year.

Upwelling provides nitrogen as nitrate while sewage would be expected to supply ammonium as the principle form of nitrogen. Phytoplankton appear to utilize both forms equally well although their chemical composition, especially C/N and C/chlorophyll *a* ratios, may vary with the nitrogen source used for growth (Eppley et al., 1971). Since upwelling is seasonal and intermittent a survey of the region during a quiescent period (no upwelling) would be expected to show the outfall areas as points of nutrient-rich water, with high phytoplankton crops, against a low nutrient, low crop background. This was the case, in part, in July 1–15, 1970, for coastal waters between Los Angeles and San Diego: phytoplankton crops were high only at the outfalls but nutrient concentrations were low everywhere.

Grigg and Kiwala (1970) and Turner, Ebert and Given (1968) provide maps of the White Point and Point Loma outfall areas, respectively, and report studies on benthic organisms.

METHODOLOGY

1. Station Locations

Station locations are depicted in Figure 1. Stations 1, 6, and 10 are located in Scripps Canyon; Stations 12 and 18 at the Point Loma outfall; and Station 19 at the Whites Point outfall.

2. Collection of Water Samples

Water samples were taken with a 50 liter polyvinylchloride sampler and/or an 8 liter Van Dorn sampler for nutrients, dissolved and particulate organic constituents, and for primary productivity measurement; and with a continuous profiling pump for chlorophyll a determinations.

3. Euphotic Zone Depth

The depth of the euphotic zone was taken as three times the Secchi disc depth.

4. Chlorophyll a in micrograms per liter ($\mu g/l$)

Extracts in 90% acetone from filters were measured on a Turner fluorometer standardized against a Beckman spectrophotometer using pure cultures of marine algae and the SCOR-UNESCO equations for evaluating spectrophotometrically, the amount of chlorophyll in an extract.

Continuous depth profiles were made with a modified Turner fluorometer (Lorenzen, 1966); the same fluorometer as was used for extracts but with a flowthrough cell replacement. Four sensitivities could be obtained by changing the size of the illuminated area. This was managed automatically by a servomotor which changes the opening or "door" according to the fluorescence recorded. Thus if, with the widest opening (door 1), the output exceeded 95 units the mecha-

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FIGURE 1. Location of stations.

nism automatically moved the next door, with less area, into position and so on through a total of four doors. If the fluorescence output signal decreased below 20 units the door was again changed, this time to the next largest opening. Factors to convert all door readings to the reading to be expected on door 1 were determined experimentally. Since the amount of fluorescence per weight of chlorophyll varied, to an extent and for reasons yet unknown, this ratio was determined for at least two depths (usually four or five) when making profiles and several times a day when charting surface properties.

5. Phaeophytin a $(\mu g/l)$

This was calculated from fluorometer readings taken before and after acidification of acetone extracts. When calculating a chlorophyll factor for the *in vivo* fluorometer the total pigment (chlorophyll a plus phaeophytin a was used (see above).

6. Surface and Submarine Irradiance

A photometer consisting of a selenium photocell with green filter was used to measure submarine irradiance as percent of that seen by an identical photocell on deck.

Incoming radiation as langlies/day was measured by a bimetallic actinograph (Kahl Scientific Instrument Corporation; El Cajon, Calif.) calibrated against an Eppley pyranometer and kept ashore at the Scripps Institution.

7. Primary Production as milligrams of carbon per cubic meter per day or grams of carbon per square meter per day (mg $C/m^3/day$, g $C/m^2/day$)

Samples were taken at depths corresponding to sunlight irradiances of 87, 43, 20, 7, 4, and 1% of surface irradiance. (These depths were chosen to match the transmission of neutral density filters in the deck incubators.) The light depths for sampling were based on three-times the Secchi disc depth as the 1% light level and the further assumption of a constant attenuation coefficient with depth.

The water samples were passed through 183 micron (μ) netting and placed in 300-ml glass-stoppered bottles. Radiocarbonate solution (5 or 20 microcuries in one ml) was added with rinsing, the contents of the bottles mixed and the bottles placed in incubators on deck in unobstructed sunlight. The incubators provided cooling water at sea surface temperatures.

Samples were incubated for 24 hours. They were then passed through 0.45- μ membrane filters and the filters were dried immediately in a vacuum desiccator over silica gel.

Finally, the radiocarbon of the filtered particulate matter was assayed with a scintillation counter and the counts were corrected for counter efficiency, background radiation and coincidence effects. Carbon assimilation was calculated as mg $C/m^3/day$. These values were integrated over depth to express production as g $C/m^2/day$.

Adenosine triphosphate in nanograms per liter (ng/l)

Water samples were first filtered through a 183- μ nylon net to remove any large particulate matter or zooplankton. Volumes of sample between 0.1 and 1.0 liter depending on phytoplankton population density were then filtered through 25-mm micro-fine glass fiber filters (Reeve Angel 984h). As soon as the filtration was completed the filters were immersed in boiling Tris buffer at 100°C as rapidly as possible to inactivate all enzymes. The concentration of ATP in the extracts was determined by the bioluminescent reaction utilizing firefly luciferin-luciferase (Holm-Hansen and Booth, 1966).

9. Particular Organic Carbon and Nitrogen $(\mu g/l)$

Water samples were first filtered through a 183-µ nylon net. Volumes of sample between 0.3 and 1.0 liter were then filtered through combusted 25-mm glass fiber filters (Whatman GF/C). Carbon was determined by infrared absorption on the CO₂ liberated during wet oxidation of the samples (Holm-Hansen *et al.*, 1967) and organic nitrogen was determined by a colorimetric method with ninhydrin-hydrindantin (Holm-Hansen, 1968).

10. Inorganic Nutrients in microgram-atoms per liter (μg-at/l)

 PO_4^{3-} , SiO_3^{2-} , NO_3^{1-} and NH_3 were determined with the autoanalyzer as described in Strickland and Parsons (1968) with the exception that the ammonia method was modified by using citrate to prevent precipitation (unpublished MS).

11. Dissolved Organic Nutrients (mg/l, µg-at/l, ng/l)

Dissolved organic carbon, nitrogen and phosphorus, and vitamins B_{12} , B_1 (thiamine) and biotin were determined by methods given in Strickland and Parsons (1968).

RESULTS

Measures of the Phytoplankton Crop

Depth profiles of chlorophyll a (discrete sample points) and chlorophyll fluorescence (continuous traces) are shown for two stations off La Jolla (6 and 10) and for the Pt. Loma (Sta. 18) and Whites Pt. (Sta. 19) outfalls (Fig. 2). Values integrated over the depth of the euphotic zone are given in Table 1. Integrated values for the outfall stations approach the highest values observed in the 1967 study during upwelling (81 and 106 mg chl. a/m^2 at two stations) and mark the outfall areas as highly productive of phytoplankton.

Depth profiles of ATP, particulate organic carbon and particulate organic nitrogen (Figs. 3, 4, 5) also suggest high crops at the outfall stations (Sta. 12, 18 and 19). Organic carbon and nitrogen measurements will include a detrital component, but ATP is restricted almost entirely to living organisms (Holm-Hansen and Booth, 1966). That the ATP values represent primarily phytoplankton is suggested by the similarity of ATP/chlorophyll *a* ratios at the outfall stations to values observed in phytoplankton cultures (Holm-Hansen, 1970) in which bacteria, ciliates and other heterotrophic organisms are not present.



FIGURE 2. Concentrations of chlorophyll a in waters from Stations 6 and 10 (non-outfall) and 18 and 19 (outfall). Smooth line with points determined from discrete samples. "Chlorescence" units for continuous profiles are relative chlorophyll concentration.

Phytoplankton Photosynthesis

The outfall stations showed much higher primary productivity than at stations off La Jolla (Fig. 6). Daily production integrated over the euphotic zone (Table 1) was 1.8 and 2.6 gram $C/m^2/day$ at the outfalls, values typical of rich upwelling regions such as



FIGURE 3. Concentrations of ATP (adenosine triphosphate) in waters from Stations 1, 6, and 10 (non-outfall) and 12, 18 and 19 (outfall).



FIGURE 4. Concentrations of particulate organic carbon in waters from Stations 1, 6, and 10 (non-outfall) and 12 and 19 (outfall).

the Peru Current and two to three times higher than seasonal averages off La Jolla (1.0 and 1.1 g $C/m^2/day$ at two stations).

The specific growth rates (μ) of the crops, expressed as doublings of carbon per day, were calculated using 250 times the ATP concentration (Holm-Hansen, 1970) as a measure of the phytoplankton crop as carbon (P) and 24-hour measurements of photosynthetic rate as a measure of the daily carbon increase of the crop (Δ P)

$$\mu = \frac{1}{\text{days}} \log_2 \left[\frac{P + \Delta P}{P} \right]$$
(1)

Rates were relatively low and similar at all stations (Table 1), implying no inhibition of phytoplankton



FIGURE 5. Concentrations of particulate organic nitrogen in waters from Stations 1 and 6 (non-outfall) and 12 and 19 (outfall).



FIGURE 6. Primary production in waters from Stations 1 and 6 (nonoutfall) and 12 and 19 (outfall).

TABLE 1

Comparison of phytoplankton crop and primary production, integrated over the depth of the euphotic zone, for stations near Scripps Canyon, the Pt. Loma (San Diego) and Whites Point (Los Angeles) sewage outfalls.

	Station					
	Pt. Loma		White Pt.	Scripps Canyon		
	12	18	19	1	6	10
Phytoplankton carbon (g/m ²) (as 250 × ATP)	14.9	12.1	5.9	8.4	4.4	3.6
Chlorophyll $a (mg/m^2)_{}$	-	163	83	40	30	29
Primary production (g C/m ² /day)		2.64	1.76	1.37	1.10	0.36
Specific growth rate (doublings/day)	-	0.28	0.37	0.26	0.32	0.13
Depth of euphotic zone (m)	18	21	16.5	33	33	46

growth at the outfall stations. Because of the uncertainties in estimating both standing stock and production, these rates must be regarded as tentative, but nevertheless they provide a basis for intercomparison. Similar rates were indicated from nitrogen assimilation at these stations (McCarthy, 1971). Thomas (1970) reported a 3- to 5-day doubling time for phytoplankton of the nutrient-poor surface waters of the eastern tropical Pacific Ocean. The specific growth rate of phytoplankton in upwelled water off Peru was higher (0.6-0.8 doublings/day when integrated over the euphotic zone). Off Peru nitrate nitrogen was 10-20 micromolar (μM) and we propose that the lower rates measured off southern California are due to nitrogen limitation, as reported for oligotrophic waters of the eastern tropical Pacific Ocean (Thomas and Owen, 1971).

Concentration of Nutrients

Depth profiles of phosphate and silicate (Fig. 7), nitrate and ammonium (Fig. 8) show little difference between outfall stations and other coastal stations in spite of the sewage enrichment.

Nitrate, ammonium, and urea (McCarthy, 1971) were low in the euphotic zone at all stations and ammonium and urea were important sources of nitrogen for plant growth, as shown by the rates of assimilation of 15 N-labelled compounds (McCarthy, 1971).

Water at the outfall stations contained higher concentrations of silicate, relative to phosphate and nitrate, then elsewhere. This is best seen by plotting a graph of phosphate vs silicate or nitrate vs silicate (see, for example, Strickland, Solórzano and Eppley, 1970). Graphs of nitrate vs silicate for water off La Jolla, California, show a similar high silicate/nitrate ratio at an inshore station (approximately 1 km from the beach) but not for stations farther offshore. The higher inshore silicate/nitrate ratio could not be attributed to lack of diatoms in the phytoplankton (Strickland, ed., 1970) but seems to be characteristic of nearshore water.

Dissolved Organic Constituents of the Seawater

Dissolved organic carbon (DOC) and dissolved organic phosphorus (DOP) (Table 2) and dissolved organic nitrogen (DON, Fig. 8) did not show significant differences between outfall and other stations. At the Whites Pt. outfall (Sta. 19) the vertical distributions of DOC, DON and DOP were remarkably

TABLE	2
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Dissolved organic carbon (DOC) and phosphorus (DOP) at Stations 1 and 6 (non-outfall) and Stations 18 and 19 (outfalls).

	Depth (m)	DOC (mg/l)	DOP (µg-at/l)
Station 1	1	1.12	0.48
	5	0.66	0.59
	12	0.90	0.33
	18	0.98	0.63
	25	1.38	0.63
	33	0.63	0.77
Station 6	2	1.42	0.89
	7	1.25	1.49
	13	0.86	1.11
	16	0.91	0.86
	23	0.85	0.88
	30	0.39	1.19
Station 18	1	1.40	0.47
	4	1.23	0.59
	7	1.61	0.57
	12	2.30	0.61
	15	1.10	0.38
	21	-	0.40
	30	1.34	0.15
	40	0.75	0.08
	55	1.00	0.12
Station 19	1	1.26	0.35
	3	1.50	0.47
	6	1.26	0.59
	9	1.22	0.74
	12	1.12	0.50
	15	1.22	0.65
	30	1.12	0.23
	50	1.46	
	80	1.05	0.33
1		•	r

uniform even though there were considerable gradients in the inorganic nutrients. This situation suggests rapid bacterial oxidation of the dissolved organic fraction at this station.

Dissolved vitamin B_{12} profiles likewise showed no marked differences between outfall and other stations (Fig. 9). Biotin concentration was elevated at the Whites Pt. outfall, but not at the Pt. Loma outfall (Fig. 9). Vitamin B_1 (thiamine) showed little difference between stations (Table 3).

DISCUSSION

The timing of this work in July, 1970, was propitious for showing the eutrophication around southern California sewage outfalls where phytoplankton crops and primary production exceed typical levels off this coast and approach values characteristic of upwelling periods.

Surprisingly, to us, concentrations of nutrients (except silicate) and dissolved organic materials were not

and 6 (non-outfall) and St	ations 18 and 19	9 (outfalls).
Station	Depth (m)	Vitamin B ₁ (ng/l)
1	1 5	26 20
	12 18 25	5 12 10
6	33 2 7	18 36 8
	13 16 23	40 12 11
18	30 1	13 16 20
	4 7 12 15	16 8 5
	21 30 40	5 11 5
19	55	10 11
	3 6 9	16 5 10
	12 15 30 50	6 30
	80	26

TABLE 3 Dissolved vitamin B1 (thiamine) in waters from Stations 1 and 6 (non-outfall) and Stations 18 and 19 (outfalls).

elevated in samples taken at the outfall stations even when bottle casts were taken directly over outfalls (noted on the ships fathometer). This would seem to imply that the hydraulic diffusers were effective in diluting the wastes prior to emission and/or that the phytoplankton growth was sufficiently vigorous to deplete the waters of excess nutrients.

If a value for inorganic nitrogen (nitrate and ammonium) of 2 millimolar (mM) is taken for sewage (Weibul, 1969), with a 200-fold dilution with sea water prior to emission, the final nitrogen concentration would be about 10 µM. This concentration of nitrogen would produce a phytoplankton crop equivalent to about 10 μ g/l of chlorophyll *a*, using conversion factors found in this laboratory. Maximum observed chlorophyll a concentrations were 10 to 17 μ g/l at the two outfall stations, in reasonable agreement with the hypothesis that the phytoplankton crop was assimilating the inorganic nitrogen of the effluent as fast as it was released. Such calculations can only provide a rough guide to reality because of advection and the unknown nature of the local food chain (see, for example, Smith, 1969).

Nutrient (nitrate and phosphate) concentrations found in this study were much lower than in eutrophic estuaries of the eastern coast of the United States (Ketchum, 1969; Carpenter, Pritchard and Whaley, 1969; Ryther and Dunstan, 1971) and fall within the range of values observed elsewhere along the southern California coast (Strickland, ed., 1970). Chlorophyll a and phosphate concentrations resemble those from Long Island Sound rather than polluted estuaries (see Fig. 4, in Ketchum, 1969). Particulate nitrogen and carbon were high at the outfall stations primarily as constituents of the phytoplankton crop. The fate of these large crops was not studied and their contents of toxicants (heavy metals, pesticides) are unknown. Recently fish caught near Los Angeles have been impounded by the authorities because of excess DDT content (San Diego Union, 1 Jan., 1971) and this underscores the need for studies of pelagic and benthic food chains in relation to such poisons.

The calculation of phytoplankton specific growth rate requires some explanation, particularly in respect to methods used to estimate the phytoplankton standing stock as carbon. In the past phytoplankton carbon was computed from laborious cell counts and measures of cell volume (Reid, Fuglister and Jordan, 1970). In this study the carbon of the living cells was computed as 250 times the ATP content of the samples. The conversion factor, 250, is based upon laboratory cultures of bacteria (Hamilton and Holm-Hansen, 1967) and phytoplankton (Holm-Hansen, 1970). The two methods were compared with good agreement for several stations (Holm-Hansen, 1969). The ATP measured, of course, includes that contained in all living cells and is not unique to phytoplankton. And one wonders if the quantity and proportion of heterotrophic organisms may not differ among the stations. The ratio $(250 \times \text{ATP/chlorophyll } a)$ tended to be low at the sewage outfall stations and similar to values observed in natural phytoplankton cultures aboard ship (Eppley et al., 1971). Higher values of the ratio were observed at the Scripps Canyon stations and may reflect a greater contribution of heterotrophs or higher earbon/chlorophyll a ratios in phytoplankton resulting from higher mean irradiance, nutritional status, or species composition of the phytoplankton community.

Specific growth rates so computed were as high, or higher, at the outfall stations than off Scripps Canyon. No toxic effects of sewage effluent on phytoplankton growth were noted. Rather, if one considers that the crops at outfall stations were high, and that specific growth rates could well be a crop density-dependent function (Sutcliffe, Sheldon and Prakash, 1970) then the sewage-enriched waters could be regarded as stimulatory with respect to phytoplankton growth rate, as would be expected from the nutrient enrichment.

In the absence of measurements over the seasons it cannot be assumed that our measurements of the phytoplankton crop and growth rate represent a steadystate. Advective processes, variation in the quantity and quality of wastes discharged, and periodic upwelling would complicate mathematical modeling but efforts in this direction would be interesting and perhaps useful (Dugdale and Whitledge, 1970). On the other hand, the outfalls provide appealing sites for study, especially in respect to tracing food chain pathways of pesticides, heavy metals, and other toxicants or when large phytoplankton crops facilitate evaluation of shipboard analytical methods.



FIGURE 7. Concentrations of silicate and phosphorus in waters from Stations 1 and 6 (non-outfall) and 18 and 19 (outfall).





SUMMARY

Eutrophication of seawater at the Point Loma and Whites Point sewage outfalls off southern California has been demonstrated by measurments of primary productivity two to three times the normal seasonal averages along the coast. Associated with this enhanced phytoplankton production were high concentrations of chlorophyll a, ATP, and particulate organic carbon and nitrogen. These parameters provide evidence for high phytoplankton standing stock. However, there were no appreciable differences in the nutrients or dissolved organic constitutents between outfall areas and the normal coastal background. This latter condition suggests rapid uptake of nutrients and/or effective dispersal from the outfall origins.

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