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Effect of Sewage Enrichment on the Phytoplankton Population of a Subtropical Estuary[1]

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ABSTRACT: Phytoplankton primary production; concentrations of chlorophyll *a*, particulate carbon and nitrogen, adenosine triphosphate, inorganic nitrogen and phosphorus; and secchi depths were measured at four stations in Kaneohe Bay, Oahu, on a biweekly basis for 20 months prior to diversion of sewage discharges from the bay. Nutrient enrichment experiments designed to determine biomass limitation indicated that phytoplankton biomass, as measured by chlorophyll *a*, was nitrogen-limited in all parts of the bay, and that phosphorus was simultaneously limiting in the sector of the bay furthest from the sewer outfalls. Mean light-saturated productivity indices in all parts of the bay were about 11–12 mg C · mg^{-1} chl *a* · hr^{-1}, values close to the maximum reported for phytoplankton in eutrophic marine environments. Based on the results of dawn-to-dusk C-14 incubations and an estimated phytoplankton C: chl *a* ratio of 50 by weight, phytoplankton growth rates were estimated to fall in the range of 4–6 percent per hour in all parts of the bay. Such growth rates are close to the maximum growth rates reported for marine phytoplankton grown on light-dark cycles in continuous culture, suggesting that phytoplankton growth rates (as opposed to biomass) were limited primarily by suboptimal or supraoptimal light intensities rather than by nutrients. Based on these growth rates and an assumed phytoplankton C : N ratio of 5.68 by weight, nitrogen recycling was estimated to account for 80 percent of phytoplankton nitrogen uptake in the part of the bay receiving direct sewage inputs, and for over 90 percent of phytoplankton nitrogen uptake in the other sectors of the bay. Estimates of living and detrital particulate carbon were made based on an assumed C : ATP ratio in living organisms of 285 by weight. From this partitioning, living carbon was found to vary by a factor of 3–4 between the sewage-enriched and unenriched sectors of the bay. However, estimated detrital carbon concentrations were uniform throughout the bay, as were the measured concentrations of inorganic nitrogen. These results are consistent with the interpretation that the population of microorganisms, both bacteria and phytoplankton, are substrate-limited in all sectors of the bay.

KANEOHE BAY is a subtropical embayment located on the northeast side of the island of Oahu in the Hawaiian Islands (Figure 1). The human population of the bay's watershed has grown rapidly over the past 60 years, from about 3000 persons in 1920 to roughly 60,000 in 1978 (Smith, Laws, and Hirota 1978). This rapid development of the watershed has been associated with an increasing stress on the bay's ecosystem due to the discharge of treated sewage and urban runoff.

The first sewage treatment plant (STP) in the watershed was constructed to serve the Kaneohe Marine Corps Air Station on Mokapu Peninsula during World War II. A municipal STP for the town of Kaneohe was built in 1963. The outfalls of both these plants

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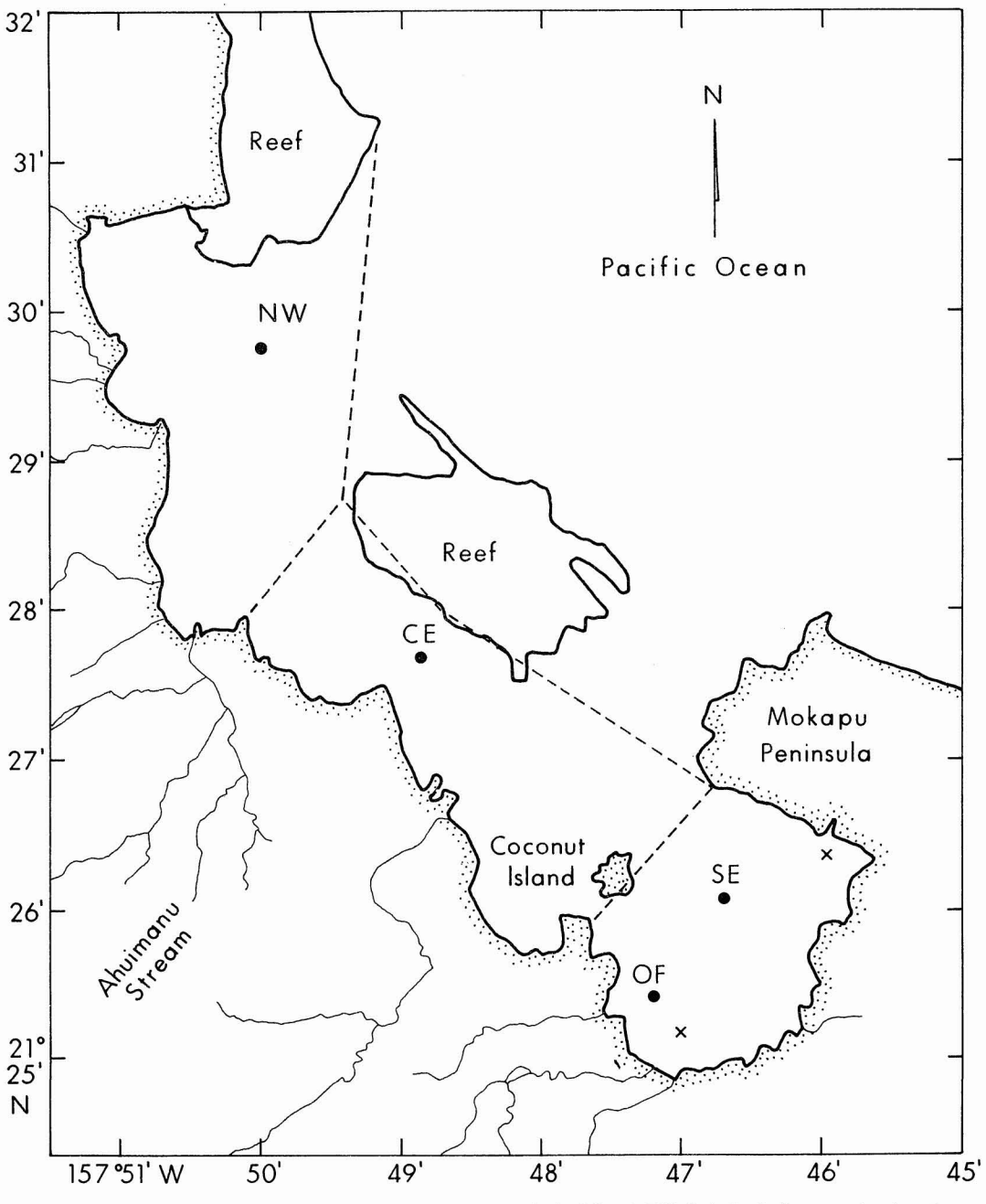

FIGURE 1. Map of Kaneohe Bay showing sector divisions (dashed lines). Filled circles indicate station locations.

are located in the southeast sector of the bay as indicated by the crosses (×) in Figure 1. Both plants presently provide secondary treatment by means of trickling filters. A third and relatively small tertiary treatment plant was built in 1969 and discharges into Ahuimanu Stream (Figure 1). About 97 percent of the watershed's population is presently served by one or another of these three plants, while the remainder of the population

is served by cesspools. As of 1977, the secondary STPs were discharging about 17,300 m^3/day of effluent into the southeast sector of the bay, while the tertiary plant was discharging about 1000 m^3/day of effluent into Ahuimanu Stream (Smith et al. 1978).

The large amount of sewage discharged into the southeast sector has created an artificial enrichment gradient proceeding from the eutrophic southeast sector to the relatively oligotrophic northwest region of the bay (Figure 1). The effects of this enrichment gradient on the plankton and coral reef communities in the bay have been the subject of several investigations during the last 10 years (Banner 1974, Caperon, Cattell, and Krasnick 1971, Caperon, Harvey, and Steinhilper 1976, Harvey and Caperon 1976, Hirota and Szyper 1976, Szyper et al. 1976). Published studies of the bay's phytoplankton community have been based on data collected over periods of no more than 4 months at a time. Beginning in February 1976, an intensive study of Kaneohe Bay was begun to assess the status of the ecosystem prior to anticipated diversion of the secondary-treated sewage from the bay. In conjunction with this study, sampling of the bay's phytoplankton community has been conducted on a biweekly basis since 17 February 1976. This report summarizes the results of that phytoplankton study until the time of the initial sewage diversion in November 1977.

THE STUDY AREA

Kaneohe Bay is a well-mixed subtropical embayment with salinities generally in the range of 33–35 ‰. Circulation with the Pacific Ocean is somewhat restricted by a barrier reef system that extends along most of the 8.8-km mouth of the bay. A lagoon behind the barrier reef contains numerous patch reefs and several small islands. Fringing reefs are found along most of the landward shoreline. Although the mean depth of the bay is only about 6 m, the mean depth of the southeast sector is about twice this value, and a ship channel dredged to a depth of about 12 m extends along the principal axis of the bay. The southeast sector of the bay is partially isolated from the rest of the bay by Coconut Island (Figure 1) and a system of shallow reefs. As a result, circulation in the southeast sector is somewhat sluggish; the mean residence time of water in the southeast sector has been estimated to be about 2 weeks, while the residence time of water in the rest of the bay is about 10 days (Smith et al. 1978). For purposes of the phytoplankton study, sampling areas were initially established in the southeast sector (SE), and in the central (CE) and northwest (NW) parts of the bay (Figure 1). In September 1976, a fourth station was added near the Kaneohe municipal sewer outfall (OF).

MATERIALS AND METHODS

Water samples were taken using a modified bilge pump fitted with an ordinary garden hose. Primary production measurements on water samples before and after passing through the pump were found not to be significantly different ($p > 0.05$). The water from the pump was screened through 102-μm-mesh nylon gauze in order to remove the larger zooplankton. Samples were taken from depths of 1, 5, and 10 m at the NW, CE, and SE stations, and from 1, 3, and 5 m at the shallower OF station. Water samples to be analyzed for nutrients and particulate concentrations were stored in polyethylene bottles which had been acid-cleaned and rinsed with distilled water. These samples were stored in the dark at ambient water temperature until they were filtered. All filtering operations were generally completed within 4–5 hr of the time the samples were collected. Samples (200 ml) for particulate carbon (PC) and particulate nitrogen (PN) analysis were filtered onto precombusted GFC filters and stored at $-20°$ C until analysis on a Hewlett-Packard model 185B CHN analyzer. Particulate organic carbon (POC) and particulate inorganic carbon (PIC) were determined by precombusting samples at 500° C for 4–6 hr according to the method of Hirota and Szyper (1975). Samples (2 liter) for chlorophyll analysis were filtered onto GFC filters

and analyzed following the procedures recommended by Jeffrey (1974) and Jeffrey and Humphrey (1975). Phaeo-pigment corrections were made following the procedures in Strickland and Parsons (1968: 193).

For adenosine triphosphate (ATP) extractions, either 200- or 400-ml samples were filtered onto Nucleopore 0.4-μm polycarbonate filters, and the particulate ATP extracted in boiling Tris buffer at a *p*H of 7.7. The ATP was assayed with an AMINCO photometer by the procedure of Holm-Hansen and Booth (1966) but employing the luciferase enzyme preparation of Karl and La Rock (1975). Initially, the assay was performed by the integration method (Holm-Hansen and Booth 1966), but after June 1977, the peak height method was employed. This change in procedure resulted from the discovery that the integration method fails to distinguish between ATP and other nucleotide triphosphates, particularly guanosine triphosphate (GTP), and hence may seriously overestimate the amount of ATP in a sample (Karl 1978, Karl and Holm-Hansen 1978). Comparison of ATP results before and after this change in methodology indicated that the integration method overestimated ATP concentrations by about 50 percent at the NW, CE, and SE stations, and by about 20 percent at the OF station.

Samples for nutrient analysis were filtered and stored at $-20°$ C until analysis on a Technicon AutoAnalyzer. Analysis for nitrate plus nitrite was done using the cadmium–copper reduction method of Wood, Armstrong, and Richards (1967). Phosphate and ammonium analyses were performed according to the methods of Murphy and Riley (1962) and Solórzano (1969), respectively.

Primary production measurements were made using C-14 according to the basic procedures in Strickland and Parsons (1968: 267–271), except that 60-ml BOD bottles were used to incubate the samples and the assimilated C-14 radioactivity was determined by counting on a Searle Analytic Delta 300 liquid scintillation counter. The activity of C-14 spikes was determined following the recommendations of Iverson, Bittaker, and Meyers (1976). The BOD bottles were incubated in situ between approximately 0930 and 1230 hr. On three occasions sunrise-to-sunset incubations were done in conjunction with short-term incubations to determine the relationship between daily and short-term (i.e., 0930–1230 hr) production.

Nutrient enrichment experiments were performed 5–7 times at each of the bay stations over the course of the 20-month study period. Approximately 500-ml aliquots of prescreened (102-μm-mesh) water were poured into glass flasks which had been acid-cleaned and rinsed with distilled water. A control flask from each station received no nutrient additions, while other flasks received additions of four of the following five essential nutrients or nutrient mixtures:

1. Nitrate, 500 μm
2. Phosphate, 50 μm
3. Silicate, 250 μm
4. Metal mixture: zinc, 1.7×10^{-3} μm; copper, 1.0×10^{-3} μm; cobalt, 1.1×10^{-3} μm; molybdenum, 1.0×10^{-3} μm; iron, 1.9 μm; manganese, 1.2 μm; $Na_2 \cdot EDTA \cdot 2H_2O$, 5 mg/liter
5. Vitamins: vitamin B_{12}, 1 μg/liter; biotin, 1 μg/liter; thiamine–HCl, 10 μg/liter

A final flask received a complete quota of nutrient additions. The flasks were incubated under continuous light of about 20 μeinstein $\cdot$ $m^{-2} \cdot sec^{-1}$ (400–700 nm radiation) provided by cool-white fluorescent lamps and at a temperature of 22–24° C. The flasks were usually monitored every 2–3 days for a period of not more than 1 month for chlorophyll *a* fluorescence using a Turner fluorometer. Fluorometer readings were converted to chlorophyll *a* concentrations using the in vivo fluorescence yield for chlorophyll *a* determined from a mixed population of marine phytoplankton cultures as recommended by Strickland and Parsons (1968: 202). The highest chlorophyll *a* concentration determined by this method in each flask at any time during the 1-month period was taken to be the yield in that flask. This approach to analyzing nutrient enrichment results avoids the problems associated with short-term C-14

bioassays discussed by Gerhart and Likens (1975). Since the completion of this work, Slovacek and Hannan (1977) have pointed out that the in vivo fluorescence yield of chlorophyll *a* may vary with nutrient availability as well as between species. Such variability may have quantitatively affected our enrichment yields, but we feel it is unlikely that statistical tests on the yield results were qualitatively affected by this problem.

Secchi disk readings were made at each station from the sun side of the boat (a Boston Whaler) using a 30-cm diameter white disk (Tyler 1968). On one occasion a survey of numerous stations in the bay was made, combining secchi measurements with measurements of surface and subsurface photosynthetically active radiation (400–700 nm) using a quantum radiometer equipped with a cosine collector (Lambda Instruments model LI-185A). This survey provided a check of the correlation between secchi depth and light extinction coefficient.

RESULTS

Nutrient Limitation

Two different types of experiments were used to study nutrient limitation in the bay. The first set of experiments consisted of the nutrient enrichment experiments previously described. Of the first nine such experiments, three were performed at the CE station and two each at the OF, SE, and NW stations. Analysis of these results revealed that (1) yields in the flasks enriched with all nutrients except silica, metals, or vitamins were greater than yields in the control flasks in all nine cases ($p < 0.002$); and (2) yields in the flasks enriched with all nutrients except silica, metals, or vitamins were not significantly different ($p > 0.10$) from yields in the flask that received all nutrient additions, based on a *t*-test of the mean of paired differences.

We therefore concluded that silica, metals, and vitamins were not limiting phytoplankton biomass in Kaneohe Bay. The remaining experiments concentrated exclusively on the question of inorganic nitrogen (N_i) and inorganic phosphorus (P_i) limitation. Figure 2 summarizes the results of the enrichment experiments relevant to the question of N_i and P_i limitation.

Based on a *t*-test of the mean of paired differences, the following conclusions could be reached from these results: (1) There was no difference at any of the stations between yields in the control flasks and the flasks with no nitrates. (2) There was a significant ($p < 0.01$) difference between yields in the control flask and the flask with no phosphates, and between yields in the no-phosphate and no-nitrate flasks at the OF, SE, and CE stations. (3) There was no significant difference between yields in the control, no-phosphate, and no-nitrate flasks at the NW station.

On the basis of these experiments, we concluded that nitrogen was limiting phytoplankton biomass in all parts of the bay, but that phosphorus was simultaneously limiting (or nearly so) with nitrogen at the NW station.

As a check on nutrient limitation in the southeast sector, a series of stations was set up on a transect beginning near the Kaneohe municipal sewer outfall and extending away from the outfall in roughly a NNE direction to the center of the southeast sector. Samples from approximately 1 m depth were taken for P_i and N_i analysis along this transect on 18 occasions between September 1976 and March 1977. Nutrient concentrations near the outfall were of course rather high, but declined steadily with increasing distance from the outfall due to the combined effects of mixing (dilution) and nutrient uptake by the phytoplankton. By plotting the concentrations of N_i against P_i, it was possible to estimate which nutrient would be exhausted first. This technique is analogous to the method used by Edmondson (1970), who plotted nitrate concentration versus phosphate concentration during the spring bloom in Lake Washington, but in this case we made use of a spatial rather than temporal variation in nutrient concentrations. The data points were fit by a straight line using the geometric mean model II regression technique suggested by Ricker (1973). The cor-

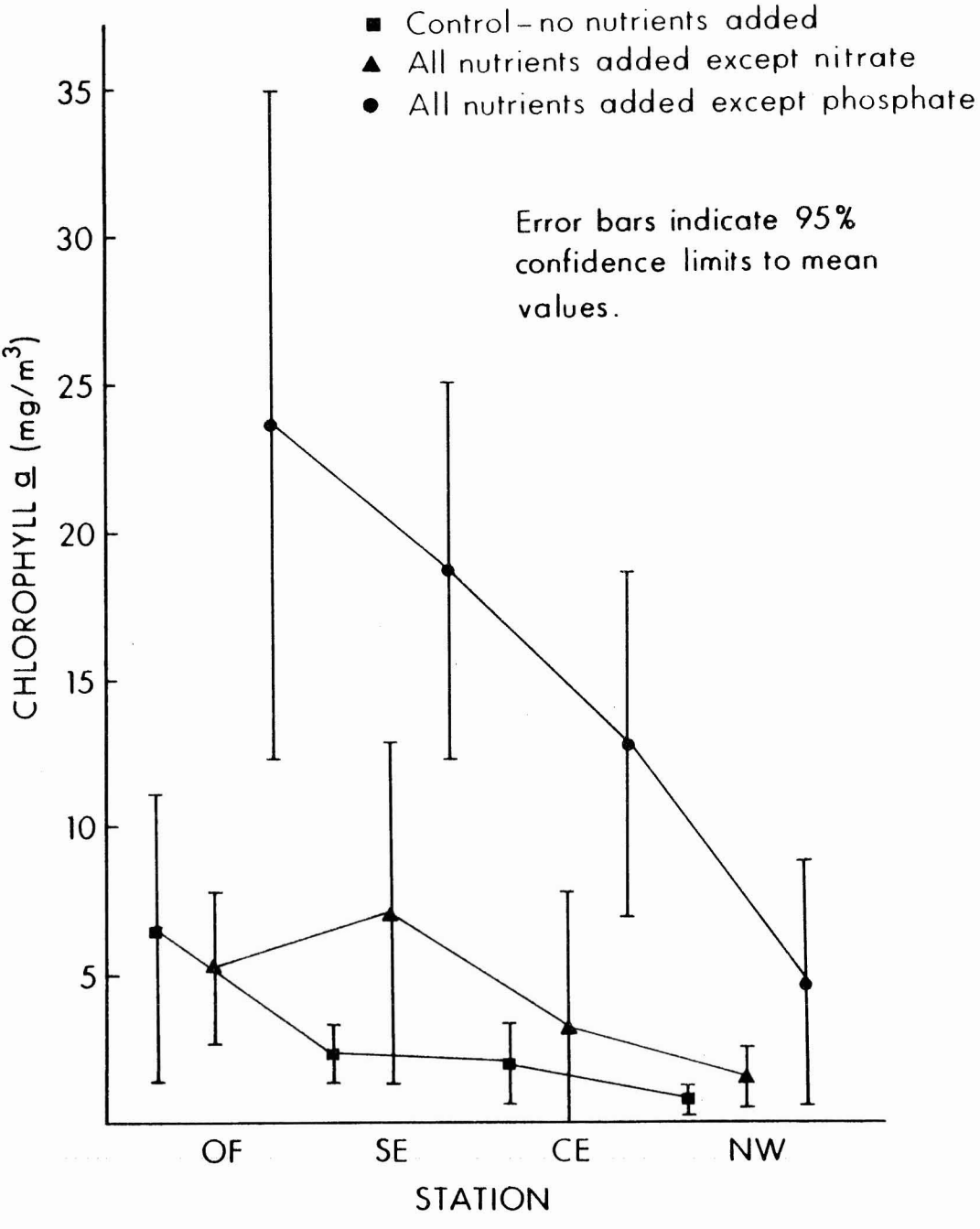

FIGURE 2. Mean yields in nutrient enrichment flasks.

relation coefficient, 0.824, was significant at $p < 0.01$. The least-squares line intersected the P_i axis at a value of 0.17 μM, significantly different from zero at $p < 0.01$. Thus, phytoplankton in the southeast sector were evidently taking up N_i and P_i in relative amounts sufficient to exhaust N_i before P_i. Hence, both the nutrient enrichment experiments

and the nutrient gradient study indicated that N_i was more limiting than P_i to phytoplankton biomass in the southeast sector.

Light Limitation

The photosynthetic rate of phytoplankton can be expressed as the product of the phytoplankton chlorophyll *a* concentration times the photosynthetic rate per unit of chlorophyll *a*, i.e., the productivity index. Although it is evident from the previous discussion that nitrogen primarily limited the phytoplankton biomass as measured by chlorophyll *a* in Kaneohe Bay, it is logical to expect from previous work (Platt and Jassby 1976, Steemann Nielsen and Jørgensen 1968) that productivity indices would be strongly influenced by the supply of light. In general, one expects productivity indices to vary linearly with light intensity at low light levels, to saturate at some optimal light level, and to decline at higher light levels due to photoinhibition. A simple analytical model expressing this relationship has been worked out by Steele (1965: 389), who adopted an empirical equation of the form

$$P = \frac{P_m}{I_m} \cdot I \cdot \exp\left(1 - \frac{I}{I_m}\right) \qquad (1)$$

where P is the productivity index, I is light intensity, and I_m and P_m are empirical parameters. Clearly, P_m is to be interpreted as the light-saturated value of P and I_m is the corresponding light intensity. In applying equation (1) to our field data, we assumed that light intensity varied exponentially in the water column and that the extinction coefficient of light could be estimated from the commonly assumed expression

$$K = \frac{1.7}{D} \qquad (2)$$

where K is the extinction coefficient (m^{-1}) and D is the secchi depth (m) (Sverdrup, Johnson, and Fleming 1942: 82). A check on the validity of equation (2) was possible from the set of secchi depths and visible light (400–700 nm) intensity profiles made at various stations in the bay on 9 August 1977. Extinction coefficients were estimated from the natural logarithm of the ratio of surface light intensity to the light intensity at a given depth. Extinction coefficients calculated in this way showed little variation with depth in most parts of the bay and were described quite well by equation (2). However, at the OF station, significant stratification of the water column was apparent, causing the extinction coefficient to change by about a factor of 2 in the first few meters. Analysis of particulate concentrations (see following results) and salinity (Smith et al. 1978) indicated that this stratification was a persistent feature near the OF station, due to the tendency of the sewage plume to rise to the surface. Because we had no detailed information on the variation of light intensity with depth at the OF station, it was impossible to examine the photosynthesis versus light relationships at that station. At the other bay stations, we used equation (2) to rewrite equation (1) in the form

$$P = P_m \cdot \frac{I_0}{I_m} \exp\left(-\frac{1.7x}{D}\right) \cdot \exp\left[1 - \frac{I_0}{I_m} \cdot \exp\left(-\frac{1.7x}{D}\right)\right] \qquad (3)$$

where I_0 is the surface light intensity and x is the depth at which P is measured. Equation (3) contains only two independent empirical parameters, P_m and the ratio I_m/I_0. Since at each station we measured photosynthetic rates and chlorophyll *a* concentrations at three depths on each sampling day, it was necessary to use a least-squares method to find the values of P_m and I_m/I_0 that gave the best fit to the data on each day and at each station. Because P_m appears as a linear parameter in equation (3) it was necessary only to search for the best value of I_m/I_0, since the optimum value of P_m could be determined in a straightforward manner for a given value of I_m/I_0. From a total of 129 such analyses, we found 3 cases that gave unreasonable parameter values and we simply ignored these in analyzing our results. Because of the gradient in trophic status of our stations in the order OF (eutrophic), SE, CE,

TABLE 1

MEAN PARAMETER VALUES FROM THE LIGHT LIMITATION STUDY

STATION	P_m (mg C · mg^{-1} chl a · hr^{-1}) ±95% CONFIDENCE LIMITS*		I_m/I_0 ±95% CONFIDENCE LIMITS
OF	—	(12.2 ± 2.3)	—
SE	13.7 ± 3.0	(11.1 ± 2.1)	0.57 ± 0.12
CE	13.1 ± 2.0	(12.3 ± 2.4)	0.57 ± 0.15
NW	12.7 ± 1.7	(11.7 ± 1.7)	0.49 ± 0.07

*Values in parentheses are means of the maximum productivity index measured at any depth on each sampling day.

and NW (oligotrophic), we felt that it was interesting to compare the mean values of P_m and I_m/I_0 at each station. Table 1 summarizes these results. For all stations, we have included the mean of the maximum productivity index measured at any depth on each day in order to provide a comparison with the OF station, where P_m could not be estimated. We did not attempt to measure mean values for I_0 at each station during the C-14 incubations, and therefore could not determine I_m in absolute units. However, it is known that phytoplankton adapt to different light levels in a time scale on the order of 1 day or less (Steemann Nielsen, Hansen, and Jørgensen 1962), and thus large variations in I_m could be expected due to changes in solar insolation. Hence, we expected less scatter in the parameter I_m/I_0 than in I_m itself.

Standing Stocks and Production

Because of the shallowness of Kaneohe Bay and the persistence of the tradewinds, the water column is generally well mixed. With the exception of primary production rates, which declined significantly with depth at the SE and OF stations due to the turbidity of the water, most parameters measured during this study therefore showed little significant variation with depth. In general, mean particulate concentrations tended to increase slightly with depth at the SE, CE, and NW stations due to settling during calm weather, but decreased with depth at the OF station due to the tendency of the sewage plume to rise to the surface. For example, mean chlorophyll a concentrations (±95% confidence limits) at 1, 5, and 10 m were 0.54 ± 0.11, 0.61 ± 0.12, and 0.71 ± 0.14 mg/m^3, respectively, at the NW station. Comparable values at 1, 3, and 5 m at the OF station were 5.07 ± 0.92, 4.10 ± 0.61, and 3.22 ± 0.46 mg/m^3, respectively. For purposes of comparing the four stations, we have simply averaged each parameter value over the depth of sampling on each sampling day and then taken the mean of this integral average over all sampling days. These results are summarized in Table 2. We have included in this table the mean at each depth of the primary production rates and productivity indices as well as the mean of the integral averages. Based on the comparisons of sunrise-to-sunset C-14 uptake with short-term C-14 uptakes, mean daytime integral production rates would be about 8.4 ± 1.0 (mean ± the standard error for $n = 3$) times the hourly integral rates listed in Table 2.

It is noteworthy here that Caperon et al. (1976) previously found a steady increase with time of particulate organic carbon (POC), particulate nitrogen (PN), and chlorophyll a (chl a) in the southeast sector, based on data collected in 1970, 1972, and 1974. Examination of our data revealed evidence of seasonal periodicity in the depth-averaged values of some parameters, and since the data were collected over a nonintegral (1.66) number of years, it seemed best to filter out the effects of seasonal periodicity before examining the data for temporal trends. In order to filter out seasonality, we fit the raw data with a truncated Fourier series consisting of a constant term and sine and cosine terms having periods of 1 year. A least-squares straight line was then fit to the differences between the raw data and the truncated Fourier series, and the significance of the

TABLE 2

MEAN VALUES ±95% CONFIDENCE LIMITS OF PARAMETERS BASED ON NUMBER OF SAMPLES IN PARENTHESES

	OF	SE	CE	NW
PN, mg/m^3	86.5 ± 9.5 (34)	49.1 ± 3.3 (49)	26.0 ± 2.4 (43)	20.8 ± 1.8 (43)
POC, mg/m^3	400 ± 53 (34)	260 ± 28 (49)	183 ± 34 (43)	181 ± 27 (43)
PIC, mg/m^3	83 ± 12 (35)	75 ± 10 (49)	53 ± 21 (44)	48 ± 8 (44)
ATP, mg/m^3	1.00 ± 0.37 (14)	0.43 ± 0.07 (14)	0.20 ± 0.04 (9)	0.13 ± 0.04 (9)
Chl *a*, mg/m^3	4.40 ± 0.65 (25)	1.78 ± 0.21 (50)	0.71 ± 0.10 (44)	0.61 ± 0.10 (44)
C-14, mg $C/m^3/hr$*	41.2 ± 9.8 (35)	10.9 ± 1.5 (50)	5.3 ± 0.54 (44)	5.1 ± 0.70 (43)
	65.6 ± 19.7 [1 m]	15.6 ± 3.0 [1 m]	5.6 ± 0.8 [1 m]	5.2 ± 0.9 [1 m]
	28.1 ± 6.4 [3 m]	10.7 ± 1.5 [5 m]	5.1 ± 0.6 [5 m]	5.4 ± 0.9 [5 m]
	11.1 ± 3.0 [5 m]	4.9 ± 1.1 [10 m]	5.2 ± 1.1 [10 m]	4.2 ± 0.9 [10 m]
Productivity index, mg $C \cdot mg^{-1}$ chl $a \cdot hr^{-1}$*	9.4 ± 1.6 (35)	6.9 ± 0.96 (50)	8.4 ± 1.02 (44)	9.4 ± 1.4 (43)
	12.1 ± 1.1 [1 m]	10.8 ± 2.1 [1 m]	10.8 ± 1.4 [1 m]	9.8 ± 1.2 [1 m]
	7.8 ± 1.6 [3 m]	7.3 ± 1.0 [5 m]	9.9 ± 2.3 [5 m]	10.4 ± 1.6 [5 m]
	4.0 ± 1.1 [5 m]	2.9 ± 0.6 [10 m]	6.4 ± 1.0 [10 m]	6.6 ± 1.3 [10 m]
$NO_3 + NO_2$, μM	0.60 ± 0.12 (35)	0.31 ± 0.05 (50)	0.30 ± 0.07 (44)	0.66 ± 0.11 (44)
NH_4, μM	2.01 ± 0.65 (28)	1.49 ± 0.46 (40)	1.16 ± 0.28 (34)	1.31 ± 0.29 (34)
N_i, μM	2.57 ± 0.72 (28)	1.81 ± 0.47 (40)	1.47 ± 0.28 (34)	1.99 ± 0.30 (34)
P_i, μM	0.77 ± 0.12 (35)	0.43 ± 0.05 (50)	0.21 ± 0.04 (44)	0.20 ± 0.04 (44)
Secchi depth, m	3.1 ± 0.3 (35)	5.0 ± 0.5 (50)	7.3 ± 0.8 (44)	6.7 ± 0.5 (44)

*Mean values at depth in brackets.

linear regression tested using the correlation coefficient. Based on this analysis, we found a significant positive correlation between primary production rate and time at the OF and SE stations ($p < 0.05$ and $p < 0.01$, respectively), and a significant positive correlation between chl *a* concentration and time at the SE station ($p < 0.05$). However, we found no significant temporal correlations for any of the other parameters listed in Table 2. A similar analysis of monthly mean sewage discharge rates from the Kaneohe municipal outfall also revealed no significant temporal correlation.

DISCUSSION

Nutrient Limitation

The results of our nutrient limitation studies are in qualitative agreement with the earlier findings of Ryther and Dunstan (1971), who performed nutrient enrichment experiments in sewage-polluted Long Island bays and along transects radiating from the New York bight into the Atlantic Ocean. They found nitrogen to be the only nutrient limiting phytoplankton biomass in the Long Island bays and at stations near New York harbor, but with increasing distance from the New York bight the P_i concentration in the water steadily declined, and at stations beyond the continental shelf addition of only N_i or P_i to enrichment flasks produced yields little different from controls. That N_i should be primarily limiting to phytoplankton biomass in sewage-enriched systems is hardly surprising; the ratio of N_i to P_i in sewage is about 6 by atoms (6.1 for the Kaneohe municipal sewage effluent), whereas phytoplankton appear to take up N_i and P_i in a ratio of about 10 : 15 when both nutrients are present in abundance (Edmondson 1970, Ryther and Dunstan 1971).

Some nutrient enrichment experiments (e.g., Thomas 1969) have indicated that N_i is primarily limiting to phytoplankton biomass in certain parts of the open ocean as well as in sewage-enriched coastal areas. Ryther and Dunstan (1971) have reasoned that N_i might be more limiting than P_i in open ocean areas due to the slower regeneration rate of N_i from detritus, but their argument, based on a study of the decomposition of a mixed plankton population by Vaccaro (1965:381), has been found to be in error (Ryther, personal communication). They ignored the initial concentration of P_i in the water containing the plankton, and therefore overestimated the regeneration rate of P_i relative to N_i. A least-squares regression of Vaccaro's (1965) P_i and N_i data versus time gives a $N_i : P_i$ regeneration ratio of 14.6 by atoms, within the preferred range of 10 : 15 noted by Ryther and Dunstan (1971). Therefore, there is nothing in Vaccaro's (1965) data to indicate that remineralization rates would favor either P_i or N_i limitation in the open ocean. However, in a similar study of decaying plankton, Antia et al. (1963) found very little (about 10 percent) regeneration of nitrogen over a 75-day period, though over half the particulate phosphorus was remineralized in 2 weeks. The cause of the discrepancy between the results of Antia et al. (1963) and Vaccaro (1965) is not clear, but may be due to differences in the bacterial populations present in the plankton cultures. More recently, Perry (1972) has found evidence of P_i limitation (as well as N_i limitation) in the oligotrophic Central North Pacific, based both on the results of nutrient enrichment experiments and on the presence of alkaline phosphatase activity in the seawater. Her results are consistent with the findings of this work as well as with the nutrient enrichment experiments performed by Ryther and Dunstan (1971) off the Atlantic continental shelf, in the sense that all three studies showed evidence of simultaneous N_i and P_i limitation in marine areas removed from the direct influence of sewer outfalls or sludge dumping. Edmondson (1969, 1972) has noted that land runoff sometimes contains N_i and P_i in a ratio considerably greater than 15 by atoms, and hence P_i limitation may be favored in some coastal systems where land runoff is a significant source of nutrients. In this respect it is noteworthy that the $N_i : P_i$ ratio in stream runoff to Kaneohe Bay is about 21 by atoms,

and that the northwest sector of the bay receives about 59 percent of that runoff (Smith et al. 1978). This runoff is probably the cause of the significantly elevated nitrate plus nitrite concentrations at the NW station as compared to the CE and SE stations (Table 2), and undoubtedly contributes to the tendency for phytoplankton biomass at the NW station to be simultaneously limited by N_i and P_i.

Light Limitation

A comparison of mean productivity indices as a function of depth at the four bay stations (Table 2) indicates that primary production in the SE sector is significantly reduced due to light attenuation, whereas at the CE and NW stations productivity indices are insignificantly different at 1 and 5 m, and decline by only about 35 percent at a depth of 10 m. In fact, productivity indices were higher at either 5 or 10 m than at 1 m on 54 and 41 percent of the sampling days at the NW and CE stations, respectively, indicating that primary production was often repressed at these stations due to photoinhibition (McAllister 1961, Ryther 1956). By contrast, productivity indices were highest at depths below 1 m on only 19 and 7 percent of the sampling days at the SE and OF stations, respectively. Thus, primary production appears to have been close to light saturation thoughout much of the water column in the central and northwest sectors of the bay, but light-limited below the first few meters in the southeast sector.

Considering the gradient in trophic status and light limitation between the four bay stations, the similarity in the mean values of P_m and I_m/I_0 in Table 1 may be surprising. Based on the work of Steemann Nielsen and Hansen (1959*a*:362) and Steemann Nielsen and Jørgensen (1968), one would expect phytoplankton in the more turbid southeast sector of the bay to have lower P_m and I_m/I_0 values than phytoplankton in the central and northwest sectors. However, given the mean secchi depths in Table 2, and assuming that conditions at the SE station are fairly representative of most of the southeast sector, the mean water column light intensity in the southeast sector is only about 25 percent less than that of the central and northwest sectors. This difference in light conditioning is evidently insufficient to produce a significant change in P_m and I_m/I_0, or else the effect is masked by other factors. Curl and Small (1965) have argued that P_m values are correlated with the degree of growth limitation by nutrient depletion, with P_m values in the range 0–3 indicative of oligotrophy and values in the range 5–10 indicative of eutrophy. More recently, Thomas (1970:384) measured P_m values in nitrogen-poor and nitrogen-rich waters of the eastern tropical Pacific, and reported mean values of 3.15 and 4.95 mg $C \cdot mg^{-1}$ chl $a \cdot hr^{-1}$, respectively. Judging from these two studies, phytoplankton production in all parts of Kaneohe Bay would be considered nutrient-saturated. In this respect it is noteworthy that Caperon et al. (1971:603) reported mean P_m values of 11.5, 11.5, and 13.0 mg $C \cdot mg^{-1}$ chl $a \cdot hr^{-1}$ in the southeast, central, and northwest sectors of the bay, respectively, in 1970. Although sewage input to the bay has increased by over 20 percent since that time, there has evidently been no significant change in P_m values (see Table 1), suggesting that phytoplankton production has been nutrient-saturated for several years in all parts of the bay. Indeed, the mean N_i concentrations in Table 2 are a factor of 4 or more higher than N_i concentrations measured in N_i-limited chemostat cultures, even for cultures grown at close to maximum growth rates (Caperon and Meyer 1972, Eppley and Renger 1974, Eppley et al. 1971).

An estimate of phytoplankton growth rates at each of the four stations can be made from the productivity indices and chl *a* values in Table 2, given the chl *a* : C ratio in the phytoplankton and the dark respiration rate. In general, the chl *a* : C ratio in marine phytoplankton is believed to fall in the approximate range 0.01–0.04 by weight (Strickland and Parsons 1968:185), and has been found to correlate positively with nutrient-limited growth rates (Caperon and Meyer 1972, Perry 1976, Thomas and Dodson 1972). Respiration rates in natural marine phytoplank-

ton communities probably rarely exceed 15 percent of photosynthetic rates (Steemann Nielsen and Hansen 1959*b*) except at very low growth rates. Following the methodology of Steele and Baird 1965), Caperon et al. (1976) estimated the chl *a* : C ratio of phytoplankton in the southeast sector to be about 0.02 by weight based on the slope of a regression of chl *a* against POC. Assuming this ratio to be typical of phytoplankton in all parts of the bay, taking a dark respiration rate equal to 15 percent of the daytime net photosynthetic rate and a mean daytime production rate equal to 8.4 times the hourly production rates in Table 2, we conclude that phytoplankton at the OF, SE, CE, and NW stations are growing at 5.8, 4.1, 5, and 5.6 percent per hour, respectively. These rates are 5–7 times higher than the estimated mean growth rate of phytoplankton in the oligotrophic central gyre of the North Pacific (Eppley and Sharp 1975:985) and well within the range of maximum growth rates observed for phytoplankton grown on light-dark cycles (Caperon and Ziemann 1976, Paasche 1967, 1968).

Thus, we conclude that the phytoplankton in all parts of Kaneohe Bay are growing in close to a nutrient-saturated environment, with either suboptimal or supraoptimal light intensities being perhaps the principal factor limiting growth rates. Although the phytoplankton biomass itself is evidently limited by the availability of nitrogen, the nitrogen pool seems to be turning over rapidly; our own data (see the following discussion) indicate that even in the southeast sector, which receives most of the sewage input, nitrogen recycling accounts for about 80 percent of phytoplankton nitrogen uptake.

Standing Stocks

Of the various particulate parameters measured, only ATP and chl *a* provide direct measures of the size of living populations. Particulate ATP is found only in living organisms, since hydrolysis of ATP to ADP occurs rapidly upon death. The ratio of carbon to ATP in living microorganisms is variable, but a ratio of about 285:1 by weight appears to represent a good working mean (Holm-Hansen 1970). Using this ratio and the previously noted estimate of 0.02 for the chl *a* : C ratio in the phytoplankton, it is possible to use the mean concentrations of POC, ATP, and chl *a* in Table 2 to make a breakdown of the particulate carbon pools at each station (Table 3). This analysis indicates that the percentage of living POC steadily decreases from the most productive to the least productive sector of the bay. However, the percentage of living carbon as phytoplankton shows no clear correlation with productivity.

TABLE 3

PARTITIONING OF POC BASED ON ASSUMED POC:chl *a* AND POC:ATP RATIOS

	OF	SE	CE	NW
Detrital POC, mg/m^3	115	137	126	144
Living POC, mg/m^3	285	123	57	37
Phytoplankton POC, mg/m^3	220	89	35.5	30.5
$\frac{\text{Living POC}}{\text{Total POC}} \times 100\%$	71%	47%	31%	20%
$\frac{\text{Phytoplankton POC}}{\text{Living POC}} \times 100\%$	77%	72%	62%	82%

That living carbon should constitute a higher percentage of the total POC in the more eutrophic part of the bay is logically appealing if one assumes that the sewer outfalls provide the principal source of nutrients to the phytoplankton in the southeast sector, while in the less productive sectors of the bay recycling of nutrients from detritus is more important than external nutrient inputs. However, an analysis of our data indicates that this explanation is probably an oversimplification. Table 4 summarizes a simple calculation to estimate the relative importance of sewage and stream inputs versus recycling as sources of nitrogen to the phytoplankton in the three sectors of the bay. In estimating the phytoplankton biomass in terms of nitrogen, we have converted the mean chl *a* concentrations in Table 2 to phytoplankton nitrogen by assuming a chl *a* : C ratio of 0.02 by weight and a C : N ratio of 5.68 by weight (Riley and Chester 1971: 173). From these calculations, we conclude that at least 80 percent of phytoplankton

TABLE 4

CALCULATION OF RELATIVE IMPORTANCE OF EXTERNAL NUTRIENT INPUTS AND RECYCLING TO PHYTOPLANKTON N_i UPTAKE

	SE	CE	NW
Phytoplankton PN, mg/m^3	15.7	6.25	5.37
kg/sector	1,250	751	343
Phytoplankton N_i uptake, kg/day	1,230	901	461
N_i input, kg/day	248	2.8	38.8
Recycled N_i, kg/day	982	898	423
mg/m^3/day	12.3	7.5	6.6
$\frac{N_i \text{ input}}{\text{Phytoplankton } N_i \text{ uptake}} \times 100\%$	20.2%	0.3%	8.4%

nitrogen uptake in all parts of the bay is to be accounted for by recycling. This conclusion is qualitatively consistent with the earlier study of Harvey and Caperon (1976), who estimated that recycling accounted for about 95 percent of phytoplankton nitrogen uptake in the southeast sector. The principal cause of the discrepancy between their estimate of 95 percent and our estimate of 80 percent (Table 4) appears to be due to the fact that they overestimated the biomass of phytoplankton in the southeast sector because their sampling stations were "biased" by being located "in the more eutrophic part of the southern basin" (Caperon et al. 1976:324). In any case, it is apparent that the relative abundance of living and detrital POC in the three sectors is not to be accounted for on the basis of the relative importance of external nutrient inputs and recycling, since external inputs apparently account for only a small percentage of phytoplankton uptake in all parts of the bay. The almost eightfold decline in living POC between the OF and NW stations (Table 3) undoubtedly reflects the effect of dilution with oligotrophic offshore water. The lack of any corresponding trend in detrital POC is noteworthy, since one would expect the rate of detritus production to correlate positively with living biomass. Furthermore, Table 4 indicates a decreasing trend in the rate of N_i recycling per unit volume from the southeast to the northwest sector. These observations suggest that the detrital POC is rather refractory and is not directly linked with nitrogen recycling. Following this line of reasoning, it seems likely that the detritivore population is substrate-limited, and that easily biodegradable detritus is rapidly consumed. Hence, actual nutrient regeneration rates would be expected to correlate with the biomass of detritivores rather than with detrital carbon. In this respect it is noteworthy that the percentage of nonphytoplankton living POC (perhaps largely protozoans, bacteria, and other detritivores) is largest at the CE station, where external nutrient inputs are negligible, and smallest at the OF and NW stations, which receive the greatest external nutrient inputs.

Indices of Eutrophication

Government agencies charged with the responsibility of setting water quality standards must inevitably wrestle with the problem of choosing parameters that are both sensitive to the problem of concern and applicable to many different types of aquatic systems. The mean parameter values in Table 2 provide a good check of the sensitivity of various water quality criteria to eutrophication in Kaneohe Bay. In order to rank the various parameters as to sensitivity, we have simply taken the ratio of the parameter value at the OF and SE stations to the mean of the values at the CE and NW stations. These ratios are listed in Table 5. Using these ratios as criteria, chl *a* concentration is clearly the most sensitive indicator of sewage enrichment, followed by P_i, ATP, and PN. Of particular interest is the very insensitive nature of the inorganic nitrogen concentrations. Ryther and Dunstan (1971) have

TABLE 5

RELATIVE SENSITIVITY OF PARAMETERS AS INDICATORS OF EUTROPHICATION

		OF	SE
		1/2(CE + NW)	1/2(CE + NW)
Most sensitive	Chl *a*	6.7	2.7
Sensitive	P_i	3.8	2.1
	PN	3.7	2.1
	ATP	3.0	1.3
Insensitive	1/secchi	2.3	1.4
	POC	2.2	1.4
Very insensitive	PIC	1.6	1.5
	NH_4	1.6	1.2
	N_i	1.5	1.0
	$NO_3 + NO_2$	1.3	0.7
	P_m	1.0	0.9

NOTE: P_m ratios are based on values in parentheses in Table 1.

previously noted that in N_i-limited systems inorganic nitrogen concentrations are kept uniformly low by phytoplankton uptake, and in such systems P_i provides a much better tracer of eutrophication. Our results are in agreement with this thesis. Following similar reasoning, it seems likely that in P_i-limited systems, inorganic nitrogen concentrations would provide a good indicator of enrichment, whereas P_i would not. In short, in nutrient-enriched systems much of the added nutrient is likely to become incorporated into seston due to rapid uptake by phytoplankton, and therefore particulate concentrations such as chl *a*, ATP, and PN are likely to provide more sensitive and more widely applicable indices of eutrophication than do inorganic nutrient concentrations.

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