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Picoplankton Growth Rates in Subtropical Hawaiian Embayments[1]

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ABSTRACT: The size structure of phytoplankton biomass and productivity and the specific growth rates (μ) of the picoplankton (i.e., <3 μm fraction) were examined in six Hawaiian embayments. The portion of total phytoplankton chlorophyll present in the <10 μm and <3 μm fractions ranged from 38 to 62 percent and 16 to 52 percent, respectively. Picoplankton accounted for between 34 and 63 percent of total community photosynthesis. Picoplankton growth rates ranged from 0.056 to 0.202/h (0.97 to 3.62 doublings/day). The rapid growth rates in these aquatic environments probably result from inputs of terrestrially derived nutrients; the μ values for the picoplankton fraction are thought to represent upper limits for growth rates of the total population.

THE ECOLOGICAL RESPONSE OF subtropical island embayments to urbanization, coastal development, and various forms of nutrient loading is extremely important because these waters are nurseries for the juvenile stages of many neritic fish populations. Such embayments represent aquatic environments very different from the oceanic waters surrounding them. The oceanic waters are characterized by low nutrient supply rates whereas coastal embayments receive considerably higher allochthonous nutrient supplies from terrestrial runoff, surrounding human activities, and remineralization from the benthos. Greater nutrient supply in the embayments, together with restricted flushing, results in higher phytoplankton standing stocks.

Subtropical oceanic waters support phytoplankton populations having low standing stocks and a predominance of very small-celled organisms; this predominance of small cells is a distinguishing feature of these ecosystems. Recent works have shown that 60–80 percent of the phytoplankton biomass occurs in the picoplankton (i.e., <3 μm) fraction (Bienfang 1980, Bienfang and Szyper 1981, Takahashi and Bienfang 1983), and that the specific growth rate (μ) of this biomass can be estimated (Bienfang and Takahashi 1983). The μ measurement is based on the rate of increase of chlorophyll biomass following isolation of the <3 μm fraction from herbivores. This technique assumes that (a) a close coupling between phytoplankton growth and contemporaneous grazing accounts for the lack of temporal (i.e., throughout the light period) biomass changes commonly observed in these waters; (b) separation at the 3 μm size interval isolates the photoautotrophic picoplankton fraction from herbivorous organisms; and (c) the rate of biomass (chlorophyll) synthesis in the incubation bottles is unaffected by the removal of the >3 μm fraction.

The purpose of this study was to examine the size structure of phytoplankton biomass and the specific growth rates of picoplankton biomass within a number of Hawaiian embayments. The intent was to determine whether and to what extent the size structure of phytoplankton biomass and photosynthesis in these nutrient-enriched water bodies is different from the surrounding nutrient-poor oceanic waters. The growth rate information provides estimates of the rates at which the primary trophic level puts chemical energy into the

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systems; this in turn should provide insight on the rates at which these embayments may be expected to respond to various forms of perturbations.

Four of the six embayments studied are located on the leeward side of Oahu; Kaneohe Bay is located on the windward side of Oahu; and Honokohau is located on the leeward side of the island of Hawaii. All receive substantial amounts of surface runoff except for Honokohau, which receives large amounts of nutrients via groundwater intrusion.

MATERIALS AND METHODS

The chronology of samplings was: Honokohau Harbor (August 1982), Kaneohe Bay (September 1982), Honolulu Harbor and Keehi Lagoon (March 1983), East Loch and Middle Loch of Pearl Harbor (April and June 1983). All samples were collected from the surface (1 m) between 0800–1000 hours and were processed immediately.

Photosynthetic rates were assessed with the ^{14}C method (Strickland and Parsons 1972); samples were incubated in situ for ca. 4 hours. Two sets of triplicate samples were run for each embayment to determine total photosynthesis and the proportion occurring in the $<3\,\mu m$ fraction. Total photosynthesis was determined by filtration through $0.45\,\mu m$ Millipore HA filters. Photosynthesis in the $<3\,\mu m$ fraction was determined by passing samples through $3\,\mu m$ pore Nuclepore polycarbonate filters prior to incubations, and subsequent filtration through $0.45\,\mu m$ HA filters. Filters were acidified to expel dissolved ^{14}C (Lean and Burnison 1979), then admixed with cocktail and counted on a liquid scintillation counter. Sample counts were corrected using zero-time blanks (Berman and Williams 1972), and working activities were standardized with the method of Iverson, Bittaker, and Myers (1976). Nutrient samples were filtered and frozen prior to analysis on a Technicon AutoAnalyzer II system using automated methods described in Strickland and Parsons (1972) and Technicon, Inc. (1977).

Chlorophyll (chl) and phaeopigments were measured in triplicate according to fluorometric methods for extracted samples (Strickland and Parsons 1972). Samples for total chlorophyll biomass were collected on $0.45\,\mu m$ HA filters under 1/3 atm vacuum. Chlorophyll biomass in the $<3\,\mu m$ and $<10\,\mu m$ fractions was determined by filtrations through Nuclepore polycarbonate filters using only gravity pressure. Previous studies employing microscopic examinations demonstrated that size fractionations through polycarbonate filters having various pore sizes is a valid means of describing particulate size structure, and for partitioning picoplankton into size classes available to suspension-feeding herbivores (Runge and Ohman 1982, Takahashi and Bienfang 1983).

Growth rate (μ) was measured by isolating the $<3\,\mu m$ fraction and monitoring the rate of change of chlorophyll biomass in the absence of grazers (Bienfang and Takahashi 1983). To isolate the $<3\,\mu m$ fraction samples were passed initially through large (147 mm diameter) Nuclepore polycarbonate filters ($3\,\mu m$ pore size) using only gravity pressure. Samples containing the $<3\,\mu m$ fraction were placed in clear polycarbonate bottles and incubated in situ at 1 m. Immediately after isolation and at 2 hour intervals for 6–8 hours, triplicate subsamples were removed and processed to describe the time-series increase in chlorophyll biomass. These data were least squares fit to an exponential equation, $B(t) = B(o)*e[\exp(\mu*t)]$, where μ, t, and B represent the specific growth rate, time, and biomass, respectively. The μ values (in units of inverse hours) were calculated over the time period for which biomass displayed continual increase. Multiplying μ by 12, and dividing the product by 0.693 converted specific growth rate (μ) to divisions per day (k). The rationale for multiplying μ by 12 rather than 24 is that chlorophyll (the biomass parameter monitored in the time series from which μ is calculated) is produced primarily during daylight hours, although k implies a 24-hour time unit. The assumption that chlorophyll synthesis occurs primarily during the day is consistent with a number of laboratory studies (Jorgensen 1966, Eppley and Coatsworth 1966, Eppley, Holmes, and Paasche 1967, Paasche 1968, Laval-Martin,

TABLE 1

SUMMARY OF THE PHYTOPLANKTON AND NUTRIENT CONDITIONS IN SIX HAWAIIAN EMBAYMENTS

	PHYTOPLANKTON PARAMETERS			AMBIENT NUTRIENTS			
LOCATION	TOTAL CHLOROPHYLL A	TOTAL PHOTOSYNTHESIS	P/B RATIO	NITRATE (μM)	AMMONIUM (μM)	PHOSPHATE (μM)	SILICATE (μM)
Honokohau	15.94	292.58	18.34	.54	.11	.26	
Harbor	(2.65)	(29.39)		(0.77)		(0.05)	
Kaneohe	2.2	19.41	8.82	.20	.15	.08	
Bay	(0.0)	(8.82)					
Honolulu	.90	9.71	10.79	.65	.45	.38	10.96
Harbor	(0.15)	(0.75)		(0.14)	(0.15)	(0.03)	(0.21)
Keehi	1.08	4.42	4.09	.51	.49	.30	8.65
Lagoon	(0.15)	(0.56)		(0.03)	(0.19)	(0.03)	(0.11)
Pearl Harbor	5.5	7.33	3.24	.39	0.0	.23	74.45
Middle Loch	(1.50)	(0.39)		(0.02)	(0.0)	(0.04)	(0.32)
Pearl Harbor	2.23	11.21	5.03	.70	0.0	.33	43.16
East Loch	(0.26)	(0.31)		(0.02)	(0.0)	(0.02)	(8.30)

NOTE: Data are given in the following units: chlorophyll (μg/liter), photosynthesis (μgC/liter*h), P/B ratio (μgC/μg chl*h), nutrients (μM). Numbers in parentheses are the standard deviations of the analyses.

Schuch, and Edmonds 1979) and field studies (Bienfang, unpublished). Note, however, that some evidence (e.g., Laws and Bannister 1980) indicates that this diurnal pattern may not be common to all species.

RESULTS

Table 1 presents the phytoplankton biomass, productivity, and nutrient conditions prevailing in the six embayments at the time of their examination. The phytoplankton data show standing stocks (μg chlorophyll/liter), photosynthesis rates (μgC/liter*h), and production/biomass (P/B) ratios (μgC/ug chlorophyll*h) which are considerably higher than those common to the oligotrophic coastal waters adjacent these areas. The generally low ambient concentrations of nitrate, ammonium, and phosphate indicate phytoplankton demand approximated the nutrient supply rates to these systems. Elevated silicate levels reflect the primary requirement for nitrogen and phosphorus.

The size distributions of phytoplankton biomass (Figure 1) indicate that the $<10\,\mu$m fraction represented 38–62 percent of total standing stocks. The picoplankton (i.e., $<3\,\mu$m) components accounted for 16–52 percent of the phytoplankton biomass but represented 34–63 percent of total primary production in these embayments. The relative contribution of the picoplankton to total primary production was about $1.8\times$ greater than its contribution to total biomass. This indicates specific rates of growth for the picoplankton which were more rapid than those of the larger components of these populations.

Figure 2 shows the time-series of chlorophyll biomass used to calculate specific growth rates (μ) of the $<3\,\mu$m fraction in four of the embayments. Chlorophyll biomass in the $<3\,\mu$m fraction displayed continual increase over 8–10 hours following isolation from larger planktonic components. Curves drawn through the points represent least-squares fit of the data to the exponential growth equation $Bt = Bo*e[\exp(\mu*t)]$. The values for specific growth rates (μ) and population division rates calculated from these distributions ranged between $\mu = 0.056$–0.202/h, and $k = 0.97$–3.62 doublings/d. Table 2 summarizes the μ and k values of the picoplankton component and its relative contribution to total phytoplankton biomass.

DISCUSSION

The results indicate that in such embayments picoplankton biomass demonstrates a

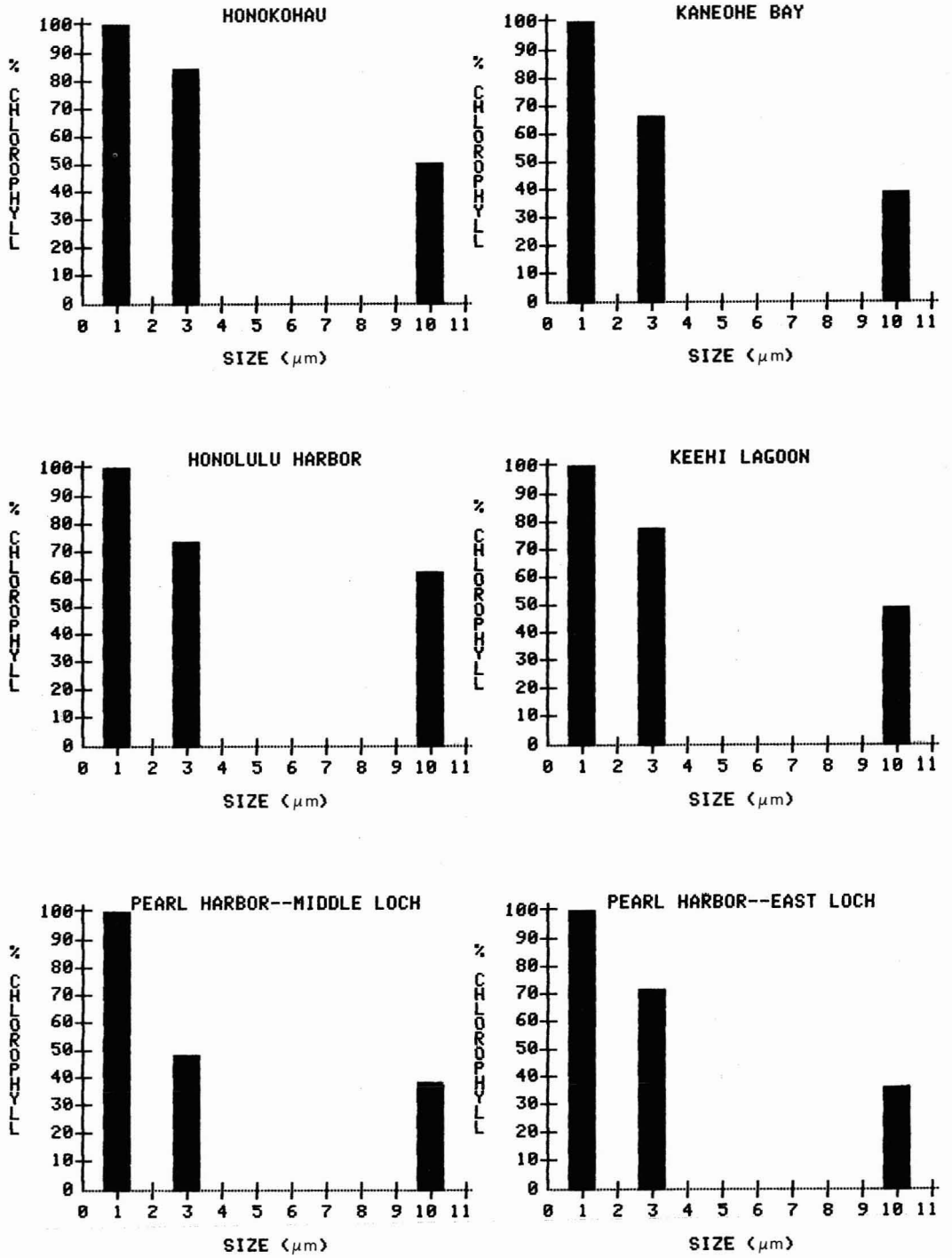

FIGURE 1. Phytoplankton size structure in six Hawaiian embayments. Figures show the relative (percent) contribution of total chlorophyll biomass in the fraction greater than the size interval indicated; values are based on total chlorophyll levels indicated in Table 1.

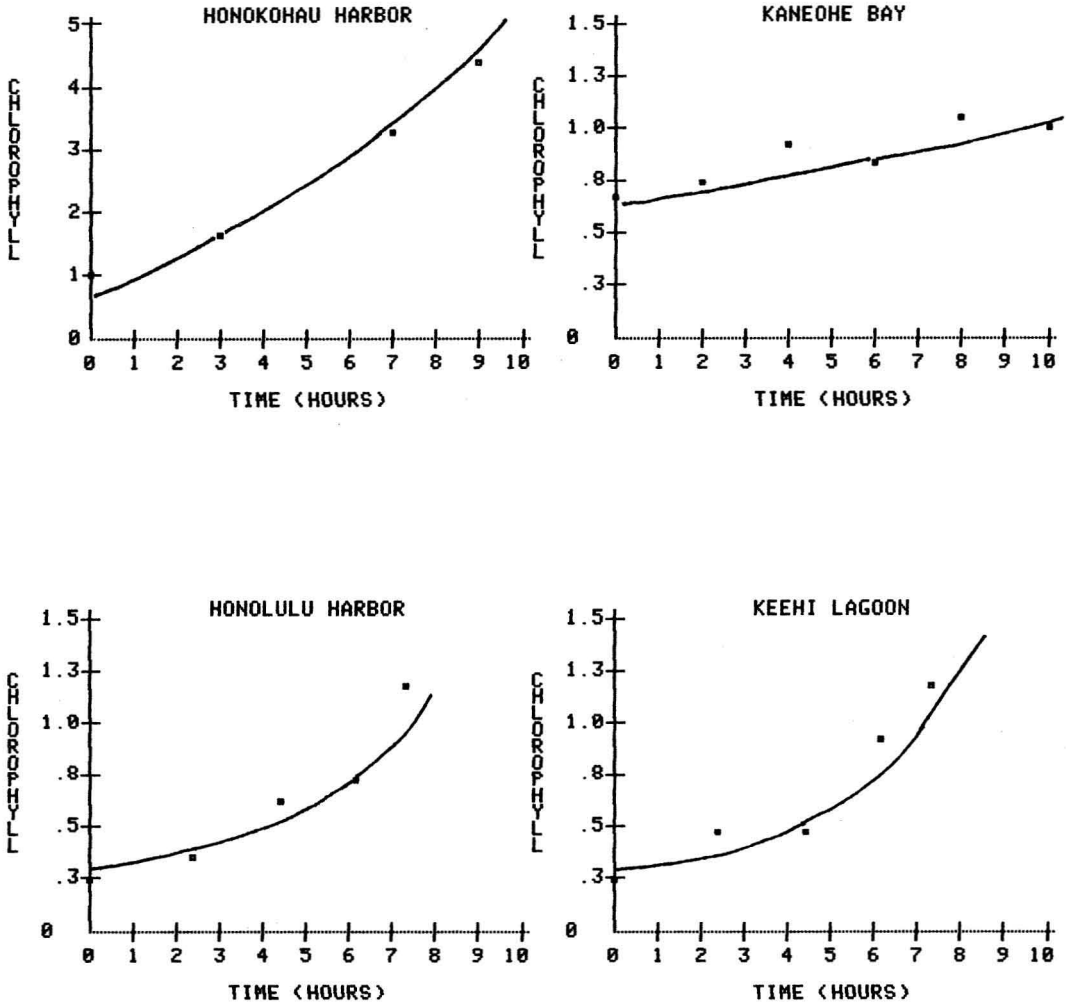

FIGURE 2. Time-series distributions showing the change of chlorophyll biomass in the $<3\,\mu m$ fraction after isolation from larger components. Curves through the points represent least squares fits of the data to the exponential growth equation. Specific growth rates and doubling times computed from these data are summarized in Table 2.

capacity for rapid growth rate and constitutes a considerable portion (16–52 percent) of total phytoplankton. The relative contribution of the picoplankton fraction to total community biomass is lower in these nutrient-rich waters than in the oligotrophic waters surrounding the islands. Previous work has shown that the picoplankton fraction accounts for about 70 percent of the photoautotrophic biomass and for an even greater share of primary production (Bienfang 1980, Bienfang and Szyper 1981, Bienfang and Takahashi 1983, and Takahashi and Bienfang 1983). The reduced importance of this size fraction in these semienclosed waters is probably related to the characteristically higher nutrient availability. The nutrient regime of the subtropical, oceanic waters is characterized by small infrequent allochthonous nutrient inputs and persistently low ambient concentrations; these conditions are exploited most efficiently by small cells having high surface area : volume ratios and low sinking rates. All of the embayments examined re-

TABLE 2

SUMMARY OF PICOPLANKTON KINETIC DATA FROM SIX HAWAIIAN EMBAYMENTS

LOCATION	B (% 3 μm)	P (% 3 μm)	μ (/h)	k (DIV/D)
Honokohau Harbor	16	40	.162	2.81
Kaneohe Bay	31	63	.056	.97
Honolulu Harbor	27	53	.209	3.62
Keehi Lagoon	23	34	.202	3.5
Pearl Harbor Middle Loch	52	51		
Pearl Harbor East Loch	28	55		

NOTE: Data give the relative contribution of the < 3μm fraction to total community chlorophyll (B) and photosynthesis (P), and the specific growth rates (μ) and the number of doublings per day (k), calculated from the time series shown in Figure 2.

ceive substantial amounts of terrestrially derived nutrients from surface runoff and groundwater sources, and remineralized nutrients from the benthos. Such allochthonous nutrients are reflected primarily in the phytoplankton standing stocks rather than in the ambient nutrient concentrations (Laws and Redalje 1979), and these nutrients create a regime more favorable to larger cells (Turpin and Harrison 1980).

The picoplankton biomass in these embayments displayed rapid growth rates; the data indicate division rates ranging from 0.97–3.62/d. Available data on the size distribution of P/B ratios (Bienfang and Takahashi 1983) suggest that activity rates of the $< 3\ \mu m$ fraction are typically greater than those of the larger population components. To the extent this is valid, the calculated μ and k values from these experiments represent an upper limit for the entire population at any given time. Using such values to estimate total population growth rates in environmental impact analyses would likely embrace the worst-case condition and yield a conservative estimate of the ecosystem's response to a particular perturbation.

Despite repeated attempts, we did not acquire growth rate estimates for either of the Pearl Harbor locations. In all of the samplings, we were unable to get reliable time series which fit the exponential growth model upon which the technique is based. In contrast to a steady increase over the incubation period, chlorophyll concentrations were constant for 4–5 hours, and then increased precipitously in the early afternoon. We doubt that we were observing the effects of a diurnal synchrony leading to phased cell division because one would expect a similar behavior in the nearby water bodies (Keehi Lagoon and Honolulu Harbor), and this was not the case. The latter bodies of water are dominated by dinoflagellate populations which were so dense they discolored the water. We speculate that the predominance of dinoflagellates relates to the peculiar growth response of the picoplankton component within Pearl Harbor. Dinoflagellates are known to release exogenous substances which can influence other cells. Possibly, the growth of picoplankton organisms was being constrained in situ, and the growth spurt observed several hours after the isolation was a response to separation from the dinoflagellates. We have, however, no chemical evidence for this high allelopathic effect of the dinoflagellates on the picoplankton component.

In this work, growth rates were calculated from measurements of biomass increase with time in incubated samples. Chlorophyll was chosen as the biomass index because of its analytical convenience and its direct reflection of the photoautotrophic population; similar strategies have used ATP, DNA, and cell numbers (Sheldon and Sutcliffe 1978, Falkowski and Owens 1982). With any of these indices, the calculated increase rates will best reflect cell division rates per se when the cellular content of the index is constant over the incubation period. When using chlorophyll, the principal concern is avoiding photic

conditions during the incubation which would cause shade-adaptation (increase chlorophyll per cell) to occur. We believe using in situ incubations at the surface, where the samples were collected, avoided this artifact.

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