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Size structure of phytoplankton biomass and photosynthesis in subtropical Hawaiian waters *

M. Takahashi [1] and P. K. Bienfang [2]

[1] Institute of Biological Sciences, University of Tsukuba; Sakura-mura, Ibaraki 305, Japan
[2] The Oceanic Institute; Waimanalo, Hawaii 96795, USA

Abstract

In a subtropical Hawaiian ecosystem, phytoplankton size structure analyses (November–December, 1980) showed that ultraplankton ($< 3\,\mu m$), nanoplankton ($< 20\,\mu m$) and netplankton ($> 20\,\mu m$) accounted for ca. 80, 98, and 2% of total chlorophyll standing stock, respectively, on the basis of chlorophyll. Similar trends were evident for other biomass indices (e.g. cell numbers, total cell volume, ATP, particulate organic carbon, particulate organic nitrogen). The ultraplankton fraction consisted primarily of small flagellates (1 to $3\,\mu m$ diam) and coccoid cells ($\cong 1\,\mu m$ diam); the 3 to $20\,\mu m$ fraction was represented by dinoflagellates, coccolithophores, diatoms, and chrysophytes; and the netplankton fraction consisted principally of dinoflagellates and centric diatoms. Community photosynthesis had a size distribution similar to that of biomass. Sinking rates for the $< 3\,\mu m$, 3 to $20\,\mu m$, and $> 20\,\mu m$ fractions averaged 0.0, 0.09, and 0.29 m d^{-1}, respectively. The absence of measurable sinking rates for the ultraplankton, together with the relative abundance of biomass in this fraction, result in very small phytoplankton losses due to sinking in such subtropical surface waters.

Introduction

The size of primary producers is an important ecological attribute of marine environments. The chemical and physical character of a given ecosystem is believed to be reflected in the size of its initial energy-fixers (Eppley *et al.*, 1969; Semina, 1972; Parsons and Takahashi, 1973; Margalef, 1974; Parsons *et al.*, 1978; Turpin and Harrison, 1980). The size distribution of phytoplankton may, in turn, exert a pronounced influence on the food-chain dynamics (e.g. Parsons and LeBrasseur, 1970; Walsh, 1976; Landry, 1977), biological structure and/or the ecological efficiency of oceanic ecosystems (Ryther, 1969; Greve and Parsons, 1977; Steele and Frost, 1977; Jackson, 1980; Malone, 1980).

Traditionally, phytoplankton have been categorized according to size as either netplankton (i.e., $< 20\,\mu m$) or nanoplankton (i.e., $> 20\,\mu m$). The quantitative importance of the latter has been described for a variety of environments (e.g. Takahashi *et al.*, 1972; Burnison, 1975; Throndsen, 1976, 1979; Malone, 1980; Taguchi, 1980; Bienfang and Szyper, 1981; Hallegraeff, 1981). Predominance of the nanoplankton biomass component has been well established for the oceanic waters of the central gyres (Hulburt *et al.*, 1959; Beers *et al.*, 1975; Fryxell *et al.*, 1979; Taguchi, 1980). Recent research has focused attention on even smaller size classes of photoautotrophic organisms. Throndsen (1979) and Waterbury *et al.* (1979) described the importance of ultraplankton (i.e., $< 5\,\mu m$) at high latitudes and in the open ocean. Bienfang (1980) and Bienfang and Szyper (1981) have shown that the $< 5\,\mu m$ size fraction accounts for about 80% of the total chlorophyll standing stock in oligotrophic, Hawaiian waters. The widespread occurrence of a substantial biomass in the $< 2\,\mu m$ component has been demonstrated by Sieburth *et al.* (1978) and Sieburth (1979).

Size fractionation is a way of separating phytoplankton assemblages into various taxonomic groups. Floristic studies frequently describe a high level of species diversity within natural phytoplankton communities inhabiting oceanic, subtropical waters: unfortunately, the principal phyletic categories addressed in such floristic examinations usually include only groups with species $> 5\,\mu m$ in diameter (e.g. dinoflagellates, coccolithophores, diatoms and blue-green algae). The fact that nearly all the components of these classes are $> 5\,\mu m$ implies that a major biomass component has been ignored, and negates the correlative value between floristic and biomass information. In contrast, separation of phytoplankton assemblages on the

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basis of size permits more complete evaluations of ecosystem ecology and phytoplankton autoecology.

The purpose of the present work was to describe the phytoplankton community structure in a subtropical oceanic system. This was done by examining the size-distribution of phytoplankton biomass and photosynthetic activity in a number of (small) size categories together with determinations of sedimentation rates and the predominant species (or higher taxonomic groups) in those size classes.

Materials and methods

All water samples were collected from the oligotrophic coastal waters at a site (21°26'N; 157°39'W) located about 1.5 km off the island of Oahu, Hawaii, where water depth exceeded 125 m. The area is well-flushed and communicates freely with the open ocean. Experiments were conducted in November and December, 1980. Surface (1 m) samples were collected at about 09.00 hrs and returned to the laboratory within 30 min. Sample handling and size fractionation took place in the laboratory at ambient water temperature ($T=25$ °C). For the various experiments, fractionations of 20, 40, and 212 μm intervals were done using Nitex screen; separations at 1, 3, 5 and 8 μm intervals employed Nuclepore polycarbonate filter papers. All filtrations for size-fractionation studies were done using only gravity pressure.

For floristic analyses, we used a Nikon inverted microscope (according to Utermöhl, 1958), and allowed samples to settle for > 5 d. The floristic authorities employed for the identification of the dinoflagellates and diatoms were Taylor (1976) and Cupp (1943), respectively. Chlorophyll *a* and phaeopigments were measured with a Turner 111 fluorometer, according to the procedures described in Strickland and Parsons (1972) for extracted samples. All analyses were done on triplicate samples. The ATP determinations were done in triplicate, and the extracts were analyzed according to Karl and Holm-Hansen (1978). Photosynthesis rates were determined by the ^{14}C method described in Strickland and Parsons (1972). All samples were incubated at a temperature of 25 °C and a light intensity of 250 μE m^{-2} s^{-1} ($=1.5\times10^{16}$ quanta cm^{-2} s^{-1}); the incubation period ranged from 4 to 6 h. Standard deviations (SD) for the P:B ratios were calculated from the equation

$$SD_{P/B}=(P/B)\,[p/P)^2+(b/B)^2\,]^{1/2},$$

where p and b represent the standard deviations of the means for productivity (P) and biomass (B), respectively; all analyses were performed in triplicate.

Phytoplankton sinking rates were determined in duplicate by a homogeneous sample method (Bienfang, 1981). This procedure, called SETCOL, involves the use of settling columns, initially containing a uniform distribution of cells, and the calculation of the population's mean sinking rate based upon the change in vertical distribution of biomass after a given time.

The method of Gordon (1969) was used to analyse particulate carbon and nitrogen; samples were analyzed on a Hewlett Packard 185B CHN analyzer. The CHN samples for the various size fractions were first filtered through Nuclepore filters; the collected material was then washed free and collected on glass-fiber filters for combustion. In consideration of transfer efficiency in this process, the washed Nuclepore filters were eluted in acetone and the residual chlorophyll compared to totals for each fraction that had been acquired by separate determinations; the calculated transfer efficiency data were then used to correct the measured CHN values.

Results

The size distribution of chlorophyll *a* (Fig. 1) shows that the majority of phytoplankton biomass in subtropical waters resides in very small particles. The amount of chlorophyll retained by filters, ranging in pore size from 0.4 to 20 μm, declines sharply with increasing pore size. The volume filtered in these trials ranged from 750 ml (for the 0.4 μm pore) to 4 liters (for the 20 μm pore). In percentage terms, the amount of chlorophyll in fractions greater than 1, 3, 5, 8 and 20 μm was 47, 23, 13 and 3%, respectively.

Phaeopigments show a similar distribution with size, and a general trend of increasing phaeopigment:chlorophyll ratios with increasing size. Ancillary measurements showed that the amount of chlorophyll in the > 40 μm fraction accounts for about 0.9 to 1.6% of the total; therefore, the 40 μm prescreening (used in later experiments to remove herbivorous components) removed only a very small fraction of the phytoplankton. The predomi-

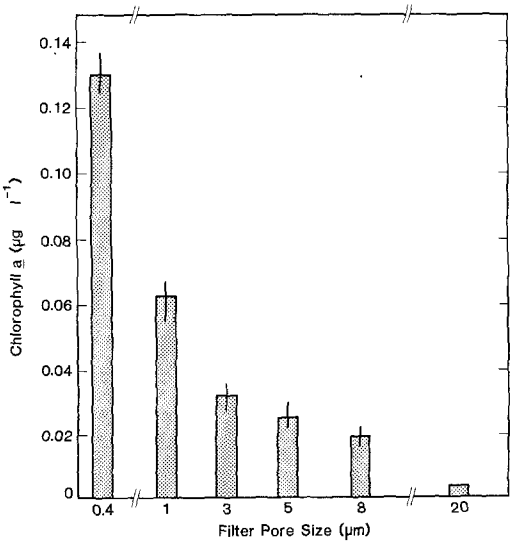

Fig. 1. Size distribution of chlorophyll in subtropical waters off Oahu, Hawaii. Error bars give standard deviations about the means acquired by filtration of triplicate samples through filters of various pore sizes. Note that the % total chlorophyll in the < 3 μm, 3 – 20 μm and > 20 μm fractions were 67, 20 and 3%, respectively

Table 1. Phytoplankton cellular biomass in each size class. Phytoplankton cell volume was estimated by microscopic measurements. Total phytoplankton cell volume in the sample was 21 502 μm^3 ml^{-1}

Size fraction	% of total cell volume	Major algal groups
$< 3\,\mu m$	40.9	Small flagellates and monads
$3-20\,\mu m$	53.9	Small dinoflagellates Coccolithophorids Small diatoms
$> 20\,\mu m$	5.1	Large dinoflagellates Centric diatoms Large pennate diatoms

nance of biomass as small cells was also described by floristic analysis based on both numerical abundance and cell volume analyses. Table 1 shows the biomass distribution in terms of percentage of total cell volume in three size classes and identifies the phyletic groups in each. These data show general agreement with the biomass values based on chlorophyll. We think that the value for the $< 3\,\mu m$ fraction (Table 1) is an underestimation due to: (a) the incomplete concentration of $< 3\,\mu m$ cells in the Utermöhl chambers (which arises from the very slow sinking rate of these particles); (b) difficulties of cell recognition in microscopic examinations; and (c) possible cell losses following preservation/settling. Such errors would both decrease counts for the $< 3\,\mu m$ fraction and increase the subsequent percentage values for the larger fractions.

Fractionations also gave rise to phyletic separations into groups which were quite invariant among trials. The $< 3\,\mu m$ fraction was dominated by small flagellates (1 to $3\,\mu m$ diam) and unflagellated coccoid cells ($\cong 1\,\mu m$ diam). The 3 to $20\,\mu m$ fraction was dominated (on a cell volume basis) by small dinoflagellates followed by coccolithophorids, centric and pennate diatoms (in roughly equal proportions) and to a lesser degree flagellates (Chrysophytes), and blue-green algae. The $> 20\,\mu m$ fraction was dominated by large dinoflagellates followed by centric diatoms and to a lesser amount pennate diatoms and blue-green algae (e.g. *Oscillatoria* sp.).

Table 2 gives a floristic breakdown of the phytoplankton community composition in these waters. The larger cells were concentrated about 150 times by gentle sieving with Nitex nettings; even with these increased concentrations, data for the larger organisms were more variable because of their scarcity. Coccolithophorids were fixed with formalin, neutralized with hexamine, and all others were fixed with Lugol's solution. Cell volume for each cell type was approximated with cylinder, sphere and cone formulae. Notwithstanding the probable underestimations of the $< 3\,\mu m$ components, the data show that these organisms comprise the majority of the phytoplankton numbers and total cell volume. The data also illustrate the great species diversity within the larger dinoflagellate and diatom components. Fig. 2 shows the size distribution of various phyletic groups/species in these waters. For each component the bar boundaries represent the maximum and minimum cell dimensions.

Table 3 presents the distribution of several biomass indices in 5 size fractions, and shows the variation of several parameter ratios within these size classes. The size distributions of ATP, particulate organic carbon, and particulate organic nitrogen were similar to that of chlorophyll, but revealed somewhat larger percentages in the larger size fractions. The 3 to $40\,\mu m$ fraction contained about 32, 58 and 64% of the total ATP, carbon and nitrogen, respectively, compared with a 22% for chlorophyll. The chlorophyll:ATP ratios of the 0.4 to $40\,\mu m$ fraction appeared to be somewhat higher than ratios of the larger fractions (e.g. 8 to $40\,\mu m$). The C:N ratios of the particulate material were fairly similar for all but the largest size class. Carbon:ATP and carbon:chlorophyll ratios tended to increase progressively with size. The values of both indices are nearly 100 times the values obtained from cultured cells and probably resulted from the presence of considerable amounts of detritus in the system.

The photosynthetic activities of various size fractions are summarized in Table 4. Experiments 2 and 6, which compared three size fractions, show the distribution of photosynthetic activity to be similar to that observed for biomass. Experiment 2 shows that 82, 17 and 1.2% of the total photosynthetic activity took place in the $< 3\,\mu m$, 3 to $20\,\mu m$ and $> 20\,\mu m$ size fractions, respectively. Experiment 6 produced values of 77, 21 and 2% for these same fractions. Both trials show highest P:B values in the 3 to $20\,\mu m$ fraction and lowest P:B values in the $> 20\,\mu m$ fraction; P:B values for the $< 3\,\mu m$ fraction in these trials were similar to, but slightly lower than, those of the 3 to $20\,\mu m$ fraction. The P:B data within each size fraction also show considerable variability from trial to trial. This variation with time is thought to be real and associated with changes in sea state occurring throughout the 6 wk period of investigation (Bienfang and Takahashi, 1983). Samples for Experiments 6, 11 and 12 were taken during periods of extremely calm weather; large populations of gelatinous herbivores were observed during sampling and their excretions are thought to have affected the photosynthesis prevailing in these populations. Ammonium levels during Experiment 2 were observed to be about $0.15\,\mu M$ as compared with values in excess of $1\,\mu M$ for Experiments 6, 7, 11 and 12.

Table 5 summarizes the sinking rate data for these three size classes. Sinking rates for the $< 3\,\mu m$, 3 to $20\,\mu m$ and $> 20\,\mu m$ fractions averaged 0.0, 0.09, and 0.29 m d^{-1}, respectively. Within each size class, rates are generally low and suggest that sinking becomes faster as cell size increases. Negligible sinking rates were measured for the $< 3\,\mu m$ fraction, which constitutes most of the phytoplankton biomass in these waters.

An estimate of chlorophyll flux (F chl) from a given depth may be derived from the sum of the products of the

Table 2. Phytoplankton community composition in a water sample collected at the surface on 9 December 1980 off Oahu, Hawaii. ND: no data available

Species	Cell dimensions (μm) (minimum) × (maximum) cell length	Cell volume (μm^3 $cell^{-1}$)	Total cell number (cells l^{-1})	Total cell volume (μm^3 ml^{-1})
Small flagellates and monads				
~1 μm diam	–	0.0655	497 000	32.6
1~2 μm diam	–	1.77	298 000	527
2~3 μm diam	–	8.18	190 000	1 550
3~5 μm diam	–	33.5	100 000	3 350
5~7 μm diam	–	113	27 000	3 050
Chrysophyceae	(12.5) × (17.5)	716	400	290
Dinophyceae				
Prorocentrum sp. 1	(21.9±2.4) × (22.1±2.5)	8 320	ND	ND
Prorocentrum sp. 2	(19.4±4.3)	3 820	200	760
Amphisolenia bidentata Schröder	(20±0) × (850±50)	267 000	ND	ND
Ornithocercus skogsbergii Abé	(73.5±18) × (106±31)	33 500	0.6	20
Ceratium declinatum (Karsten) Jörgensen	(82±9) × (163±9)	62 800	0.84	53
C. furca (Ehrenberg) Claparéde et Lachmann	(30±4) × (208±11)	49 000	0.7	34
C. fusus (Ehrenberg) Dujardin	(21.3±7.8) × (624±212)	74 000	ND	ND
C. macroceros (Ehrenberg) Cleve var. *gallicum* (Kofoid) Sournia	(234±52) × (350±76)	65 000	0.1	7
C. pentagonum Gourrett	(57±8) × (276±83)	102 000	0.84	86
C. schrankii Kofoid	(166±21) × (343±91)	331 000	ND	ND
C. teres Kofoid	(39±8) × (213±23)	75 000	0.7	53
Peridinium tenuissimum Kofoid	(29±9) × (59±1)	39 000	1.3	51
P. trochoideum (Stein) Lemmermann	(14) × (18)	2 800	ND	ND
Peridinium spp.	(21.7±3.1) × (23.1±2.4)	8 500	3.6	31
Golnyaulax glyptorhynchus Murray et Whitting	(46.8±14.3) × (96.2±31.4)	55 000	0.4	22
Oxytoxum challengeroides Kofoid	(19.2±2.7) × (91.1±20.0)	8 800	0.98	8.6
Amphidinium sp. 1	(8.3±1.3) × (24.6±8.9)	440	2 600	1 140
Amphidinium sp. 2	(20.9±9) × (52±11)	5 900	100	590
Gymnodinium spp.	(9.7±2.4) × (12.9±3.1)	950	8 750	ND
Unidentified athecate dinoflagellates	(32.8±9) × (35.2±2.2)	2 900	10	29
Bacillariophyceae				
Coscinodiscus nitidus Gregory	(28±1)	6 160	1.1	6.8
Coscinodiscus spp.	(54.5±1.8)	46 700	ND	ND
Chaetoceros convolutus Castracane	(270±150) × (350±230)	974	7	6.8
Chaetoceros spp.	(62) × (125)	1 570	0.7	1.0
Rhizosolenia spp.	(6.6±4.4) × (252±176)	8 620	0.6	5.2
Hemiaulus hauckii Grunow	(15±2) × (98±41)	17 300	4	69
H. sinensis Greville	(55±29) × (73±49)	173 000	ND	ND
Eucampia zoodiacus Ehrenberg	(25) × (50)	24 500	0.3	7.4
Leptocylindrus danicus Cleve	(5.8±3.6) × (207±150)	5 470	4	22
Asterolampra marylandica Ehrenberg	(52±0)	42 500	ND	ND
Climacodium frauenfeldianum Grunow	(14±6) × (128±46)	6 570	ND	ND
Gyrosigma sp.	(25±0) × (111±2)	18 200	0.4	7.3
Navicula spp.	(4.2±1.5) × (27±14.3)	129	500	65
Asterionella japonica Cleve	(15±0) × (108±0)	6 360	0.1	0.6
A. kariana Grunow	(12±0) × (106±0)	4 520	ND	ND
Nitzschia closterium (Ehrenberg) W. Smith	(3.1±1.2) × (59.6±28.1)	150	2	0.3
Lichmophora sp.	(99±37) × (23±0)	13 700	0.1	1.4
Striatella unipunctata (Lyngbye) Agardh	(73±30) × (99±28)	72 300	0.3	22
Unidentified pennate	(17.5) × (45.0)	3 600	0.84	3.0
Haptophyceae				
Discophaera tubifer	(18.3±1.4)	3 210	100	320
Unidentified coccolithophorids	(8.5±3.0)	322	3 000	970

sinking rate (ψ) and biomass (B) parameters for the larger two size fractions, i.e.,

$$F\,\mathrm{chl} = [(\psi \times B)_{3-20\,\mu m} + (\psi \times B)_{>20\,\mu m}].$$

Based on data in Fig. 1 and Table 5, the chlorophyll flux in such waters is Fchl $= [(0.09 \times 0.026) + (0.29 \times 0.004)]$ $= 0.0035$ mg chlorophyll m^{-2} d^{-1}. The F chl number is small because (a) the total standing stock is small, and (b) the $< 3\,\mu$m fraction, containing the majority of chlorophyll present, does not contribute to the sinking loss. We also examined the short-term buoyancy response to nutrient enrichment ($+6\,\mu M$ NH_4^+ and $3\,\mu M$ PO_4^{-3}) by the

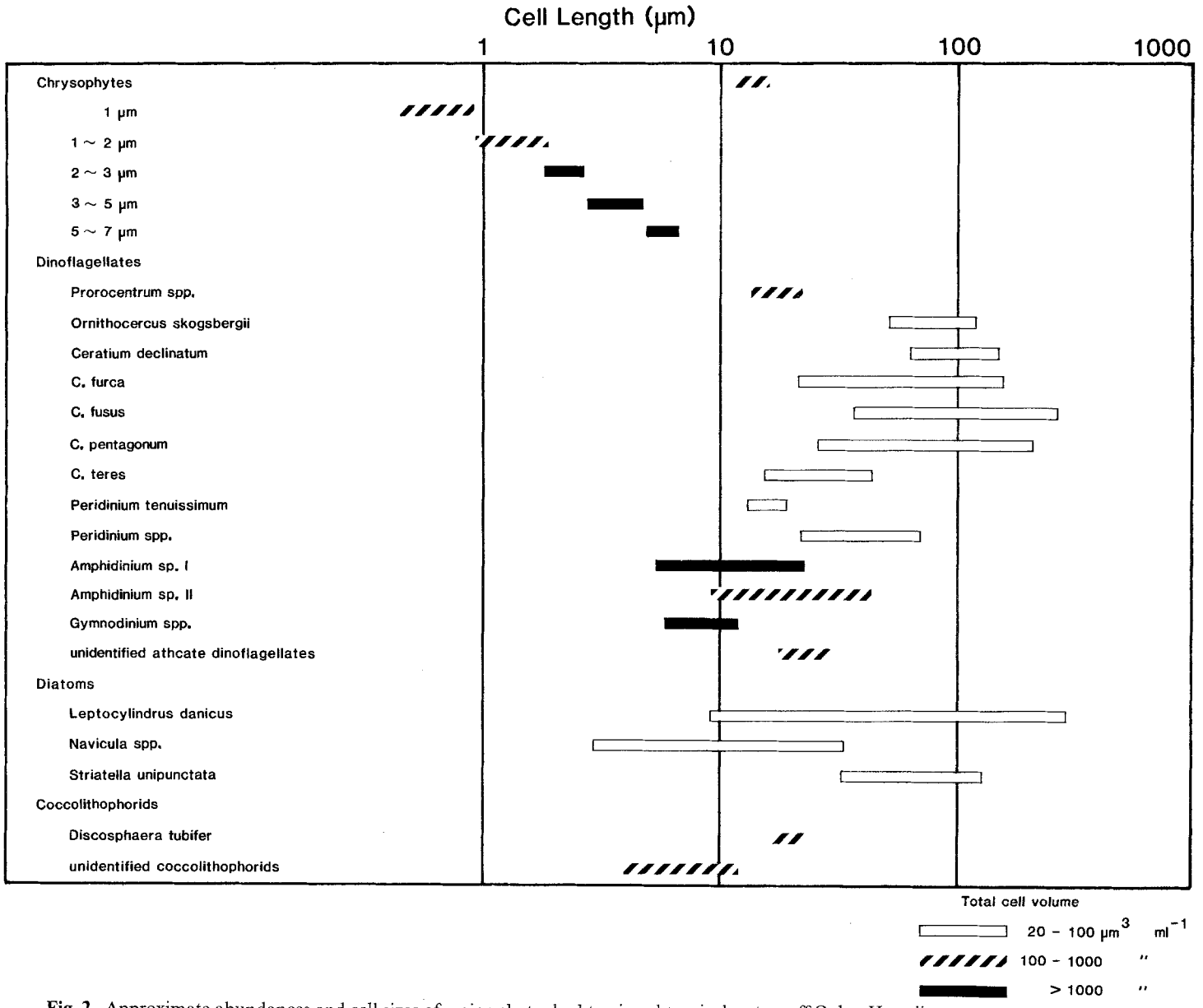

Fig. 2. Approximate abundances and cell sizes of major phytoplankton in subtropical waters off Oahu, Hawaii

3 to 20 μm and > 20 μm size fractions. Both size fractions showed lower sinking rates following enrichment. The enriched 3 to 20 μm samples sank at rates which were 90% of the unenriched samples (based on means of duplicate measurements). The enriched > 20 μm samples sank at rates which were only 29% of the unenriched samples. The sinking rate response to nutrient enrichment was considerably more pronounced for the > 20 μm size fraction whose P:B ratios suggest the strongest nutrient-limited condition.

Discussion

These results demonstrate that a considerable proportion of the phytoplankton biomass in subtropical waters comprises extremely small cells. The relative predominance of ultraplankton was reflected in all the biomass indices examined (i.e., cell numbers, total cell volume, chlorophyll *a*, and ATP). In terms of chlorophyll *a*, the most commonly measured biomass parameter, the < 3 μm fraction accounted for 75 to 80% of the total standing stock.

The quantitative importance of nanoplankton (< 20 μm) in subtropical environments, has been shown by the microscopic observations of Throndsen (1979) and the chlorophyll, ATP, carbon, and floristic data of Beers *et al.* (1975), who made observations throughout the entire photic zone. Recent work has extended the implications of such results to even smaller size categories. Bienfang (1980) and Bienfang and Szyper (1981) found significant proportions (65 to 80%) of the total chlorophyll biomass in the < 3 and < 5 μm size fractions in subtropical Hawaiian

Table 3. Size distribution of various biomass indices ($\mu g\ l^{-1}$) and parameter ratios in subtropical waters off Oahu, Hawaii. Confidence limits give standard deviations about the means of triplicate samples, except for carbon and nitrogen values for the 20 to 40 μm fraction which represents a single sample. Values in parentheses give percentage contribution of the fraction to the total (as represented by the 0.4 to 40 μm samples)

Size fraction	Biomass indices:					Parameter ratios:			
	chlorophyll *a* ($\mu g\ l^{-1}$)	phaeopigments ($\mu g\ l^{-1}$)	ATP ($10^{-3}\ \mu g\ l^{-1}$)	carbon ($\mu g\ l^{-1}$)	nitrogen ($\mu g\ l^{-1}$)	$\frac{\text{chlorophyll } a}{\text{ATP}}$	$\frac{\text{carbon}}{\text{nitrogen}}$	$\frac{\text{carbon}}{\text{ATP}}$	$\frac{\text{carbon}}{\text{chlorophyll } a}$
0.40 – 40 μm	0.116 ± 0.013 (100)	0.063 ± 0.020 (100)	17.80 ± 1.59 (100)	63.92 ± 19.52 (100)	6.31 ± 0.00 (100)	6.52 ± 0.93	10.10 ± 3.11	3 592 ± 1 159	551 ± 182
1 – 40 μm	0.047 ± 0.019 (40.4)	0.065 ± 0.029 (100)	9.80 ± 1.62 (55)	42.70 ± 4.47 (67)	5.04 ± 0.11 (81)	6.03 ± 2.63	8.50 ± 0.71	4 357 ± 853	909 ± 380
3 – 40 μm	0.025 ± 0.006 (21.6)	0.031 ± 0.012 (48.7)	5.69 ± 0.50 (32)	36.90 ± 2.64 (58)	4.02 ± 0.18 (64)	4.39 ± 1.12	9.20 ± 0.28	6 485 ± 735	1 476 ± 370
8 – 40 μm	0.012 ± 0.003 (10.1)	0.031 ± 0.018 (49.1)	3.76 ± 0.39 (21)	26.5 ± 0.14 (41)	3.07 ± 0.09 (41)	3.19 ± 0.86	8.65 ± 0.35	7 048 ± 732	2 208 ± 552
20 – 40 μm	0.006 ± 0.001 (4.8)	0.005 ± 0.002 (7.0)	1.86 ± 0.66 (10)	14.72 (23)	2.75 (44)	3.23 ± 1.22	5.3	7 913 ± 2 808	2 453 ± 327

waters, and data from a large number of stations located in a transect between 20 °N and 6 °S (Bienfang, unpublished data) have shown that similar contributions of the < 5 μm biomass prevail over extensive geographical areas of the Pacific. The present results, together with such previous findings, point out the need of phytoplankton ecologists working in such oligotrophic waters to focus attention on phytoplankton components which are smaller than those commonly addressed in other environments.

Temperate coastal waters, in contrast, are characterized by high standing stocks, more frequently dominated by large-celled organisms. The biomass levels in such systems may be 10× to 100× greater than those found in subtropical systems; large centric diatoms commonly are the primary constituent of the phytoplankton biomass, and ultraplankton comprise a relatively small component of the total standing stock. Compared with phytoplankton biomass in temperate coastal waters, the biomass levels of nanoplankton and netplankton in subtropical waters are much more reduced than that of the ultraplankton size fraction. Differences in the phytoplankton size distribution of these two systems are as dramatic as differences in standing stock levels. In this work we found that not only did netplankton account for only 2 to 3% of the biomass, but nearly 90% of the total biomass comprised cells < 8 μm.

The biomass size-structure described for such oligotrophic waters precludes the predominance of several phyletic groups commonly associated with phytoplankton, e.g. dinoflagellates, coccolithophorids, and diatoms. Indeed, these classes are always present and represented by high species diversity; however, the abundance of ultraplankton (< 5 μm) biomass discounts the importance of netplankton (and even much of the nanoplankton size class) as principal constituents of the photoautotrophic biomass in oligotrophic systems. Our floristic data (Fig. 2, Table 2) show small flagellates and coccoid cells to comprise the bulk of the phytoplankton standing stock. Other microscopic observations of ultraplankton have shown *Micromonas pusilla* to be widely distributed in significant numbers throughout tropical, temperate, and arctic waters (Throndsen, 1979). Recently, wide distributions of small (< 1 μm) procaryotic, blue-green algae, e.g. *Synechococcus* sp., were described for the Atlantic Ocean (Johnson and Sieburth, 1979; Waterbury *et al.* 1979).

The predominance of ultraplankton biomass merits several comments regarding the appropriateness of methods to be used in such waters. For floristic research, focus on an extended size range (down to the 0.5 μm level) is desirable. Unless attention is given to the ultraplankton organisms, the floristic data cannot be compared directly to the biomass and photosynthesis data collected on the 0.4 μm membrane filters. Secondly, the relative contribution of < 1 μm biomass (Fig. 1, Table 3 and other, unpublished, data) indicate that serious underestimations of chlorophyll and/or photosynthesis may result from the use of glass-fiber filters rather than membrane or polycarbonate filters in such waters. Thirdly, most of the

Table 4. Size distribution of photosynthetic activity in subtropical waters off Oahu, Hawaii. Data from 6 separate experiments. Confidence limits give standard deviations about the means of triplicate samples

Size fraction	Expt No.	Chlorophyll *a* ($\mu g\ l^{-1}$)	Photosynthesis ($\mu g\ C\ l^{-1}\ h^{-1}$)	P:B ($\mu g\ C\ \mu g^{-1}$ chl *a* h^{-1})
$< 3\ \mu m$	2	0.098±0.009	0.317±0.003	3.24±0.30
	4[a]	10.31±0.846	51.97±7.38	5.04±0.83
	6	0.078±0.003	0.745±0.09	9.57±0.61
	11	0.096±0.029	1.389±0.024	14.51±4.39
3 – 20 μm	2	0.014±0.001	0.066±0.003	4.76±0.39
	4[a]	7.17 ±0.846	13.46±1.68	1.88±0.32
	6	0.015±0.001	0.206±0.025	14.01±1.98
20 – 40 μm	2	0.009±0.000	0.005±0.000	0.53±0.05
	6	0.022±0.00	0.020±0.007	0.88±0.31
40 – 212 μm	7[a]	0.448±0.231	1.487±0.311	3.32±1.85
	12[a]	0.286±0.065	1.063±0.074	3.72±0.88

[a] Fractions concentrated before incubation

Table 5. Mean sinking rates (ψ) for various size classes of heterogeneous phytoplankton from subtropical waters off Oahu, Hawaii. Confidence limits give standard deviations about the means of *n* individual assessments for each size fraction

Size fraction	ψ ($m\ d^{-1}$)	*n*
$< 3\ \mu m$	0.00±0.01	5
3 – 20 μm	0.09±0.02	4
20 – 212 μm	0.29±0.13	6

Table 6. Comparison of pre- vs post-incubation fractionation on size distribution of photosynthetic activity. Values based on analysis of triplicate samples for each fraction. Range estimates derived on basis of one standard deviation about the mean

Treatment	Size fraction	% of total primary production	
		Mean	Range
Pre-incubation fractionation	$< 3\ \mu m$	88	39 – 100
	3 – 20 μm	4.8	1 – 9
	20 – 40 μm	7.1	1 – 13
Post-incubation fractionation	$< 3\ \mu m$	82	81 – 83
	3 – 20 μm	17	16 – 18
	20 – 40 μm	1.2	1 – 2

previous *in vitro* kinetic studies, describing growth in relation to nutrient or photic regimes, have examined organisms which are dissimilar to those predominating in subtropical waters. The absence of such kinetic information for ultraplanktonic flora brings uncertainty to the application of such data to oligotrophic environments. There is need for the isolation, culture and kinetic studies of ultraplanktonic organisms.

The size distribution of photosynthetic activity (Table 4) is similar to that observed for biomass. In addition to comprising most of the phytoplankton biomass, the $< 3\ \mu m$ fraction also accounts for most of the energy entering the food web of subtropical systems. Data showing that the $< 3\ \mu m$ fraction: (1) contains chlorophyll; (2) assimilates inorganic nutrients (Bienfang and Takahashi, 1983); (3) fixes carbon; and (4) evolves oxygen (P. J. LeB. Williams, unpublished data) leave little doubt that this fraction contains organisms which are photosynthetic.

Most of the size fraction experiments for photosynthesis were done by performing the size separations after the incubations. This minimized the stress due to preincubation handling. Differential damage to cells of various size would impart artifacts to the size distribution attributed to the photosynthesis rate data. We performed a set of trials to compare effects of pre- versus post-incubation separation to the size structure of photosynthetic activity in the waters under study (Table 6). Irrespective of the experimental design, more than 80% of the total community photosynthesis took place in the $< 3\ \mu m$ fraction. There were indications of small variations in the relative distributions within the 3 to 20 μm and $> 20\ \mu m$ fractions, but these differences were not significant ($P > 0.10$), and range estimates for percentage total photosynthesis in these size fractions were similar between the two procedures. Thus, the experimental techniques evidently introduced little bias.

The photosynthetic activity in each size class varied considerably among experiments, particularly in the $< 20\ \mu m$ size fraction (Table 4). Chlorophyll and ambient nutrient concentrations also varied by 2× to 3× during the 6 wk experimental period. This variability is attributed to changes in environmental conditions during this time interval, which was marked by periods of extremely calm seas. Very dense populations of gelatinous zooplankton were observed in the surface layer during some sample collections; nutrient excretions from these populations are

Table 7. Summary of P:B ratios for several size classes of heterogeneous phytoplankton from subtropical waters off Oahu, Hawaii. Confidence limits give standard deviations about the means of n determinations. Data collected during a 6 wk period in November – December 1980

Size fraction	Average P:B ratio (μg C μg^{-1} chl a h^{-1})	n
$< 3\,\mu$m	8.09 ± 5.04	4
$3 - 20\,\mu$m	6.88 ± 6.34	3
$> 20\,\mu$m	2.11 ± 1.64	4

thought to be related to the high nutrient levels and photosynthetic rates that we measured. The overall averages of photosynthetic activity obtained from the various experiments for the three size classes (Table 7) show a general trend of decreasing activity with increasing cell size. The coincident temporal variability during the period of examination increased the standard deviations for each size class; under more typical conditions, smaller standard deviations would be expected. The P:B data for the $> 20\,\mu$m fraction is similar to that for oligotrophic samples examined by Ichimura and Aruga (1964); however, those for the smaller size fractions were very high and within the range for eutrophic waters. Similarly high P:B ratios have been reported occasionally in oligotrophic oceanic samples (e.g. Takahashi *et al.*, 1972; S. A. Cattell and D. Gordon, unpublished data).

By monitoring chlorophyll increase with time, Bienfang and Takahashi (1983) determined specific growth rate as high as 0.07 h^{-1} for the $< 3\,\mu$m fraction. The relationship between specific growth rates and P:B ratios was non-linear over the entire range of values, but showed direct proportionality for P:B values $< 10\,\mu$g C μg^{-1} chl h^{-1}. Using that proportionality, we obtained specific growth rates of 0.056, 0.048 and 0.015 h^{-1}, which were implied by the average P:B data (Table 7) for the $< 3\,\mu$m, 3 to 20 μm, and $> 20\,\mu$m fractions, respectively. Because growth was constant for the first 10 h and leveled off thereafter, daily growth rates of 0.56, 0.48, and 0.15 d^{-1} were estimated for the respective size classes. The $\mu = 0.15$ d^{-1} value for the $> 20\,\mu$m size class is in the same range as the average *in situ* growth rates determined by microscopic procedures for the larger *Ceratium* sp. (Weiler, 1980). Such daily growth estimates are about 2.5 times greater than rates reported by Eppley *et al.* (1973) for subtropical waters. We believe that under more typical environmental conditions our growth rate estimates would correspond more closely with those of Eppley *et al.* (1973).

Because the phytoplankton size structure described here is fairly constant with time and prevalent over extensive areas of oligotrophic seas, there must be forces at work to maintain the predominance of ultraplankton. The prevailing nutrient regime, grazing constraints and sedimentation considerations all seem to favor a phytoplankton assemblage dominated by small-celled organisms. The low ambient concentrations of nutrients and frequent but small inputs of excretory nutrients favor assimilation by small cells having a high surface area:volume ratio which (1) promotes diffusive uptake; (2) results in a high proportion of uptake sites among the surface components; and (3) minimizes transport energy to internal sites of utilization. Nutrient uptake rates of the $< 3\,\mu$m fraction are much more rapid than those of the larger population components (Bienfang and Takahashi, 1983). The small size of ultraplankton prohibits their ingestion and/or efficient utilization by many forms of herbivores. Grazing upon ultraplankton is probably restricted to microherbivores and gelatinous zooplankton having particle-collection properties which are not size-selective. The negligible settling rate of the ultraplankton (Table 5) indicates that there should be virtually no loss of ultraplankton biomass from the photic zone due to sedimentation. This minimal resource loss could well be an important factor for the maintenance of predominance in environments such as oligotrophic seas which are intrinsically conservative.

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