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Comparison of Bacterial and Algal Utilization of Orthophosphate in an Estuarine Environment

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Abstract

Bacterial utilization of orthophosphate in an estuarine environment has been differentiated from algal utilization by using flow-filters of 5.0, 1.2 and 0.45 µm pore-size. Examination by light microscopy showed that most of the bacterial population passed through a 5.0-µm filter, whereas most algae were retained. In all experiments, bacterial and algal cell numbers and biomass were estimated. P-uptake by algae and bacteria was closely correlated with cell biomass. P-uptake by algae was high only in the summer months, whereas P-uptake by bacteria was high throughout the year. Neither algal nor bacterial P-uptake, however, was correlated with temperature or dissolved orthophosphate, total organic phosphate or total phosphate concentrations. Cell biomass of algae at a given time had a high correlation with dissolved organic phosphate levels in 2 weeks prior to sampling (r = 0.830) and a low correlation in the 2 weeks following sampling (r = 0.0005). Algal cell numbers had a high correlation with bacterial cell numbers (r = 0.950). The biomass of algae and bacteria also had a high correlation (r = 0.902). The rate of P-uptake from the water by algae and bacteria varied with season and with the species composition of the natural population.

Introduction

One of the major difficulties in establishing the path of phosphorus cycling in the estuarine environment is the lack of information about the role of various microorganisms in this process. The role of algae in phosphorus uptake (P-uptake) was emphasized by Fuhs and Canelli (1970) and Taft (1974), who found marked differences in P-uptake by algal species. Some investigators thought that both algae and bacteria were important (Harris, 1957; Lean, 1973), and others looked upon bacteria as the major microorganisms responsible for primary assimilation of this element (Johannes, 1964, 1965; Correll *et al.*, 1975). Two investigators excluded direct P-uptake by zooplankton (Harris, 1957), including filter-feeding protozoans (Hutner and Provasoli, 1955).

The lack of concensus stems from the fact that most investigators have not attempted to distinguish between algal and bacterial P-uptake. The fractionation procedure employed was either coarse filtration, which allowed both types of organism to flow through, or very small pore-size filters, which retained both organisms (Rigler, 1956; Harris, 1957; Watt and Hayes, 1963; Johannes, 1964, 1965; Lean, 1973; Taft, 1974).

In this study, our objective was to use more controlled filtration and microscopy to distinguish between bacterial and algal utilization of inorganic phosphate in the estuarine habitat. Such a filtration technique has been employed previously by Correll *et al.* (1975) for phosphorus and by Derenbach and Le B. Williams (1974) for carbon utilization.

In addition to such size fractionations of natural populations of microorganisms, there are other types of evidence that bacteria may be the principal assimilators of phosphorus. P-uptake was similar in light and dark bottles (Taft, 1974; Correll *et al.*, 1975), indicating that orthophosphate was taken up by heterotrophic rather than by autotrophic cells. This fact strongly indicates bacterial involvement, but cannot be accepted as positive proof. Although algal P-uptake is generally regarded to be

photosynthetic in many species, algae also can take up P in the dark under some conditions (Ketchum, 1939; Kuenzler and Ketchum, 1962). Several algae are either obligate heterotrophs (Faust and Gantt, 1973) or facultative heterotrophs if the necessary carbon source is available (Hutner and Provasoli, 1951, 1955), and inorganic P-uptake of algae is generally stimulated by, but not dependent on, light (Healey, 1973).

It appears that, under certain conditions, bacteria may be more important than algae in P-uptake. Bacteria-accelerated phosphate return from the sediment to lake water (Hayes and Phillips, 1958) thus appeared to be responsible for the rapid turnover of phosphorus (Rigler, 1956). In high P media, three strains of soil bacteria competed successfully with algae but failed to do so at low P levels (1-3 µg-at m/l; Fuhs *et al.*, 1972). Even though most laboratory experiments were carefully designed, Johannes (1964) warned that laboratory experiments in which bacteria competed for phosphorus were unnatural, since bacterial densities were several orders of magnitude greater than those attained in the sea.

It appeared to us that the role of bacteria in P-uptake in estuaries was not clear, and that further study of this phenomenon was warranted.

Materials and Methods

Sampling Site

Monthly sampling was carried out in the main basin of the Rhode River subestuary, an arm of Chesapeake Bay, from March 1973 through February 1974. The sampling site in the subestuary (Latitude 38° 53' 02" N; Longitude 76° 32' 00" W) has a mean depth of 4 m. The salinity varied from 4.7‰ in the spring to 12‰ in the fall of 1973. Freshwater enters the study area from the Muddy Creek watershed, which has the following land uses: forest, brushland, marshes, nonpasture grassland, pasture, cropland. Human populations are low (0.3/hectare) in this watershed. Salt water enters the system from the Chesapeake Bay.

Plankton Bottle Experiments

Clear glass cylindrical bottles of 250 ml capacity were equipped with rubber stoppers through which two 16-gauge syringe needles had been placed. One needle was short and one extended well into the bottle. The bottle was attached to a float by 1 m of line. Bottles were dipped in black epoxy paint to darken them. The initial sample of plankton was taken from a depth of 1 m with a peristaltic pump (Correll *et al.*, 1975). Subsamples of this water were also taken for chemical analysis. ^{32}P-orthophosphate (carrier-free) was then added to the bottle of plankton (2 µC/250 ml) and the contents were mixed thoroughly, the two syringe needles capped, and the bottle incubated at a depth of 1 m. At appropriate time intervals (2, 4, 6, 10, 15, 20, 25, 30, 40, 50, 60 min) the bottle was recovered, and the sample was mixed by swirling. The needle caps were removed, and a 10-ml aliquot was obtained through the long needle with a 20-ml glass syringe.

About 10 ml of air was pulled into the syringe. The sample was then passed through a series of filters in 25-mm diameter Swinex filter holders (Millipore Corp., Bedford, Mass.). The first holder contained Nitex screening with a pore size of 5 µm (Tobler, Ernst & Traber, Inc., Elmsford, N.Y.), which was coated around the edges with silicone rubber to facilitate sealing. The second and third holders contained 1.2 and 0.45 µm pore-size membrane filters (Millipore Corp., Bedford, Mass.), respectively. Enough air was forced through after each sample to remove any chance of excess moisture on the filters. Each filter and 1-ml aliquot of the final filtrate were counted. Metabolically-inhibited controls were run by dissolving iodoacetic acid (IAA), to a final concentration of 0.05 M, 5 min prior to adding the ^{32}P-orthophosphate.

Radioisotope Counting and Uptake Calculations

Samples were counted in a Packard, Model 3320, liquid scintillation spectrometer as described previously (Correll *et al.*, 1975). Uptake of counts/min (cpm) on various filters was plotted against time. An intercept and slope were calculated from the uptake data points in the time range in which uptake appeared to be approximately linear, by a linear least-squares regression calculation. This time interval usually varied from 6 to 30 min. Uptake after this point was usually not linear, and the results were considered at least partially due to recycling and were not used in the calculations. The initial specific activity of P was calculated from the orthophosphate concentration of the water after filtration through a 0.45-µm pore-size filter, and the cpm/ml of whole water. The particulate uptake, expressed as

µg P/h/l, was then calculated from the slope and the specific activity of the available P. Cells retained on the 0.5-µm pore-size filter were considered for uptake calculations to be algal, and those retained on the 1.2 and 0.45-µm filters to be bacterial. Rates of uptake into specific size classes were converted to percentages. Total P-uptake rates were also calculated from the rate of disappearance of radioactivity from the 0.45-µm filtrate (Correll *et al.*, 1975). Total uptake rates (from disappearance of radioactivity in filtrate) were then multiplied by the percentages found in each class of particulates to obtain better estimates of absolute rates of uptake by that class. Uptake was also expressed as µg P/mm^3 h^{-1} of algal biomass or of bacterial biomass.

Water Chemistry

Water samples for phosphorus analysis were taken as described under plankton bottle experiments. Analysis for orthophosphate was conducted colorimetrically by testing the samples' reaction with ammonium molybdate and reduction with stannous chloride. After potassium persulfate digestion (American Public Health Association, 1971), samples were analyzed for total phosphorus by the same procedure. Analysis was conducted both before and after filtration through 0.45-µm pore-size filters. The total organic phosphate fraction was calculated as the total P minus orthophosphate in unfiltered water.

The results of water analyses, made at weekly intervals between plankton experiments, were also used in the analysis of conditions prior to and subsequent to plankton experiments.

Biomass Determinations

Cell numbers of bacteria (Rodina, 1972) and algae (Campbell, 1973) were determined by direct-count procedures on samples fixed to a final concentration of 0.4% glutaraldehyde and 0.01 M sodium phosphate buffer, pH 7.0. Live material was also collected for the identification of algae. Fixed samples were then processed in the laboratory. First they were centrifuged for 20 min at 2000 x *g*. The pellet was resuspended and washed two more times with distilled water and resuspended in 3 ml final volume. Each sample was then sonicated for 5 to 10 sec to break up any clumps of cells (Model W 140C, Heat Systems Co., at 60 W output). Cell suspensions were applied to clean microscope slides. Bacteria made visible by the gram-staining technique were measured and counted on slides. Rod-shaped forms and cocci were counted separately, and the number of cells in different size classifications was registered. Algal cells were counted similarly, but without staining.

Different quantities of cell suspension were used to determine bacterial and algal counts. For the bacterial count, 0.01 ml of the fixed 3-ml suspension was used, and this volume was spread on a 400-mm^2 area. The number of bacteria were counted on 100 microscopic fields of each sample at 1000 X magnification. Cell numbers of algae were estimated using several 0.05-ml aliquots from the 3 ml final suspension spread under a cover slip of known area. The number of algae were counted on 500 to 800 microscopic fields of each sample. From this known area and known volume, calculations of the number of cells per unit volume were made for each species. At the same time, the species were examined and identified. From the mean diameters and lengths of each size group and the number of microorganisms present, the biomass was calculated as described by Rodina (1972). Taking the dilution into account, the biomass per unit of water was then determined.

Electron Microscopy

Water samples were taken as described under plankton bottle experiments. The samples were fixed with 4% gluteraldehyde in 0.1 M phosphate buffer, pH 7.0, final concentration. Fixed cells were dehydrated in alcohol and embedded in epon (Gantt *et al.*, 1971). Thin sections were examined in a Philips, model 300, electron microscope.

Results

Association of Microbial Cells with Particulates

The association of algae and bacteria with particulates, as found in estuarine-water samples, is illustrated in transmission electron micrographs (Fig. 1). Some cells were free of particulates, but the majority were surrounded by particulates adhering to specialized structures of the microbial cell surface (Fig. 1a). The adhesive material on these cells varied in structure. Some consisted of fibrous strands of various lengths forming a thin layer similar to that seen on a blue-green algal cell sur-

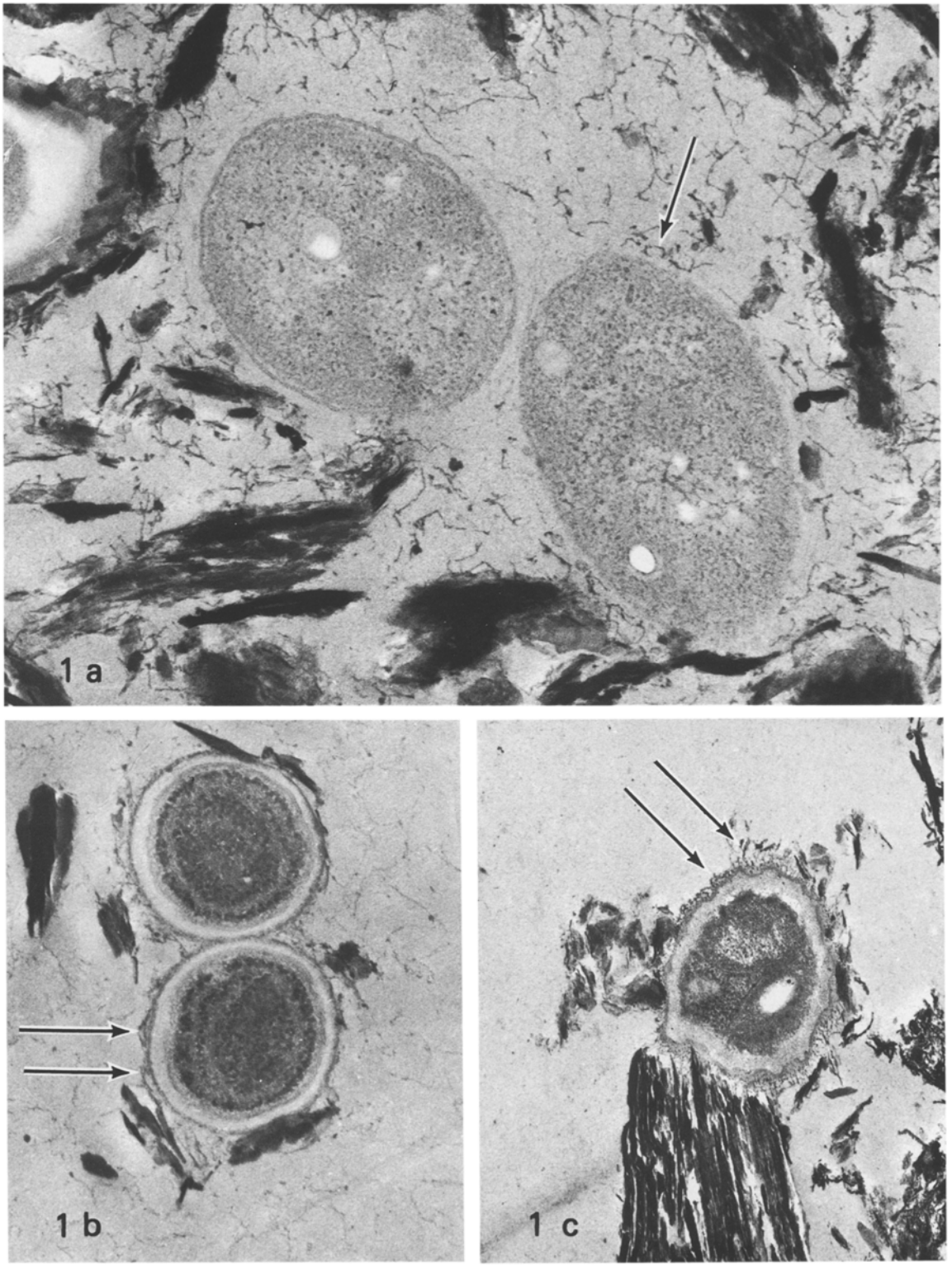

Fig. 1. Microorganisms associated with clay particulates in water samples. Sections typical of cell-clay particulate associations, showing 2 Gram-negative bacteria (a) x 60100, 2 blue-green algae (b) x 34600, and a cyst-like organism (c) x 27200. The cells are surrounded by clay particles of various shapes and sizes as dark-layered or flaky amorphous structures. Some clay particles are in close association with cell surface, connected by fibrous polymeric strands. Fibrous strands of various lengths form a thin layer on the blue-green algal cell surface (b), shorter fibers are protruding on the surface of the cyst-like organism (c), and a mesh of fibrous strands surrounds the Gram-negative bacteria (a). (Fibrous strands and fibers are indicated by arrows.)

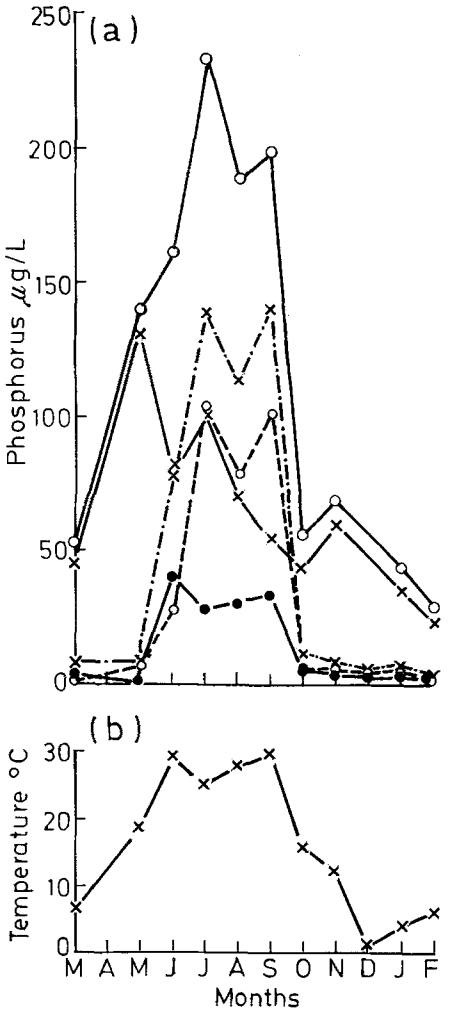

Fig. 2. Seasonal changes in phosphorus concentrations (a) and water temperature (b) at Rhode River sampling station at 1-m depth. Open circles and solid line: total-P; crosses and solid line: total organic-P; crosses and broken line: total ortho-P; open circles and dashed line: dissolved ortho-P; closed circles and solid line: particulate orthophosphate

face (arrows, Fig. 1b); some was in the form of short dense fibers such as those shown on the surface of the cyst-like organism illustrated in Fig. 1b, c. Particulates also varied in shape and size, and some were closely associated with the cell surface.

When the retention of radioactivity by filters is used to measure phosphorus assimilation, various non-living particles, which may adsorb ^{32}P-containing compounds, are also filtered out, and the adsorption by the non-living particulates has to be estimated. The adsorption of ^{32}P to particulates was measured by adding IAA to the sample 5 min before ^{32}P was added. This compound effectively inhibits biological uptake, and the zero time intercept of a linear least-squares regression indicates the amount of phosphate bound to particulates (Correll *et al.*, 1975). This intercept was about equal for light, dark, and inhibited experiments, indicating that this deviation from a zero intercept was due to adsorption to particulates rather than biological uptake.

Availability of Phosphorus in the Water

The total phosphorus rapidly increased during the months of May, June and July, remaining high until September, and rapidly decreased to low levels thereafter (Fig. 2a). The various inorganic-P fractions all followed the same pattern, but at lower magnitudes. The total organic phosphorus pattern was significantly different. It was high during two algal blooms in May and July, and slightly increased in November. Comparison of water temperature (Fig. 2b) and the various fractions of inorganic P indicated that there was some correlation between the inorganic-P concentrations of the water and water temperatures (for the dissolved ortho-P, $r = 0.560$; for particulate ortho-P, $r = 0.750$). In contrast, the correlation between temperature and total organic-P in the water was low ($r = 0.165$).

^{32}P-Uptake into Particulates

^{32}P incorporation into particulates retained on 5.0-µm pore-size filters was 1 to 6% of the total, except in June and July (Table 1). ^{32}P incorporation into particulates over 5 µm size was always less than for smaller-sized particulates. Direct microscopic observations of the 1.2 and 0.45-µm filters indicated that chlorophyll-containing algal cells were retained only occasionally on 1.2 and 0.45-µm pore-size filters. This led to the conclusion that bacteria were the only significant biological component retained on these two filters. The proportions of ^{32}P retained on various filters changed little during the year, indicating that when changes occurred in the biota influencing the total uptake, the numbers of both algae and bacteria changed in a similar manner.

The total amount of P incorporated into particulates (µg P/l h^{-1}) varied with sampling time (Table 1). Total P-uptake was the highest (480 to 500 µg P/l h^{-1}) in May during a *Prorocentrum mariae - lebouriae* bloom; next highest (39 to 42 µg P/l h^{-1}) in November, during a *Katodinium rotundatum* bloom, and the

Table 1. Seasonal changes in P-uptake by plankton in the Rhode River at 1-m depth

Date (1973-1974)	Treatment	Apparent P-uptake into particles by accumulation into particles				Total P-uptake into particles by disappearance from solution (μgP/l/h)	P-uptake into particles (Columns 4 & 6 times 7; μgP/l/h)	
		>5 μm		<5 μm			>5 μm	<5 μm
		(μgP/l/h)	%	(μgP/l/h)	%			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Mar. 28	L[a]	0.01	0.4	2.2	99.6	4.4	0.02	4.4
	D[b]	0.01	0.3	3.1	99.7	4.7	0.01	4.7
May 16	L	2.10	5.0	39.0	95.0	500.0	25.00	470.0
	D	2.70	6.0	42.0	94.0	480.0	29.00	450.0
June 14	L	0.73	8.8	7.6	91.2	10.0	0.80	9.1
	D	0.46	15.4	2.6	84.6	10.0	1.50	8.4
July 9	L	1.50	41.9	2.1	58.1	8.5	3.60	4.9
	D	0.28	7.0	3.7	93.0	3.2	0.22	3.0
Aug. 7	L	0.06	2.9	2.0	97.1	15.0	0.43	15.0
	D	0.04	2.3	1.7	97.7	22.0	0.50	21.0
Sept. 6	L	0.06	3.2	1.8	96.8	7.9	0.25	7.6
	D	0.01	0.7	1.5	99.3	26.0	0.17	26.0
Oct. 3	L	0.08	3.8	2.0	96.2	5.9	0.22	5.7
	D	0.02	0.7	2.7	99.3	5.3	0.03	5.3
Oct. 31	L	0.02	1.6	1.2	98.4	2.5	0.04	2.4
	D	0.02	1.2	1.7	98.8	2.1	0.02	2.1
Nov. 26	L	0.46	3.0	15.0	97.0	42.0	1.30	41.0
	D	0.27	1.5	18.0	98.5	39.0	0.58	38.0
Dec. 18	L	0.03	3.2	0.9	96.8	1.6	0.05	1.5
	D	0.02	2.8	0.7	97.2	0.6	0.02	0.6
Jan. 29	L	0.01	1.6	0.6	98.4	0.6	0.01	0.6
	D	0.01	2.0	0.5	98.0	0.6	0.01	0.5
Feb. 28	L	0.01	1.2	0.8	98.8	1.2	0.01	1.2
	D	0.01	1.4	0.7	98.6	2.0	0.02	2.0

[a] L: light bottle experiments.

[b] D: dark bottle experiments.

lowest during the winter months. Observed total P-uptake rates calculated by ^{32}P disappearance from the water varied by three orders of magnitude. P-uptake was also expressed per biomass of algae and of bacteria (μg P/mm^3 h^{-1}) for samples at various times of the year (Figs. 3, 4). High correlation was found between P-uptake by particulates larger than 5 μm in size and algal biomass (Fig. 3). A linear least-squares regression calculation gave the equation: μg P-uptake h^{-1} = 0.34 + (0.033)(mm^3 of algal biomass/l). The correlation coefficient was 0.99. The mean uptake (ng P/mm^3 h^{-1}) into this size class was 0.14 ± 0.25 in the dark or 0.17 ± 0.18 in the light. A high correlation was also found between P-uptake by smaller particulates and bacterial biomass (Fig. 4). A linear least-squares regression calculation gave the equation: μg P-uptake/l h^{-1} = 49+(27)(mm^3 of bacterial biomass/l). The correlation coefficient was 0.880. The mean P-uptake (μg P/mm^3 h^{-1}) into this size class was 6.4 ± 8.2 in the light and 6.8 ± 7.9 in the dark. Although high correlation existed between total P-uptake per cell biomass (Figs. 3, 4), the role of algae and bacteria in P-uptake varied with the natural population. Algal and bacterial P-uptake was high in May, June and July when *P. mariae - lebouriae*, nannoplankton and *Gymnodinium* species were dominant. In comparison, *Katodinium* species was dominant in November, when high P-uptake was due to the bacteria.

P-uptake or P levels in the water showed very little correlation with water temperature. For P-uptake into cells larger than 5 μm, the following correlation coefficients were found: total P, 0.048; dissolved ortho-P, 0.029; total

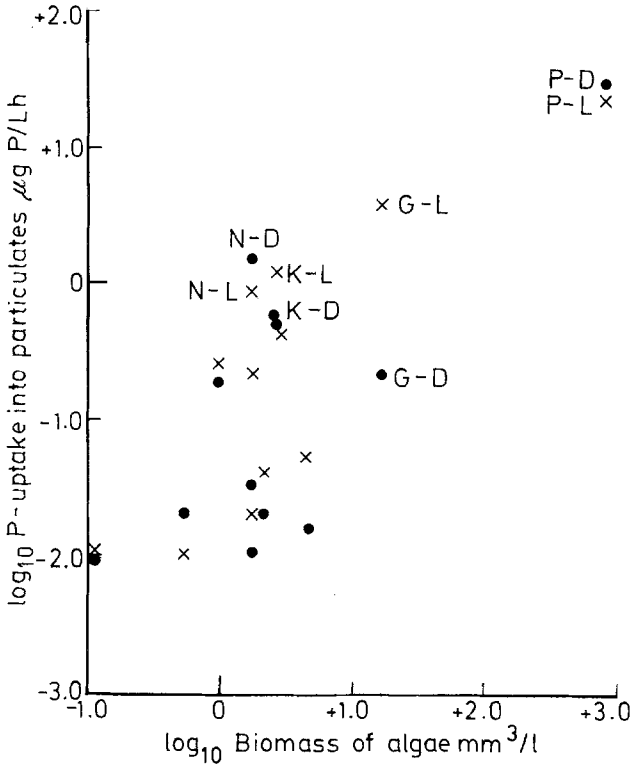

Fig. 3. Correlation between P-uptake into particles >5 µm and algal biomass. Crosses show light- and closed circles dark-bottle experiments. P: May *Prorocentrum mariae - lebouriae* bloom; G: July *Gymnodinium nelsonii* bloom; N: June nannoplankton bloom; K: November *Katodinium rotundatum* bloom. A linear least-squares regression calculation gave for the light (L) data $y = 0.509 + 0.0306\ x$ ($r = 0.984$) and for the dark data $y = 0.184 + 0.0357\ x$ ($r = 0.997$). Logarithms of the values were used for convenience in preparing the figure, but not in the statistical analyses

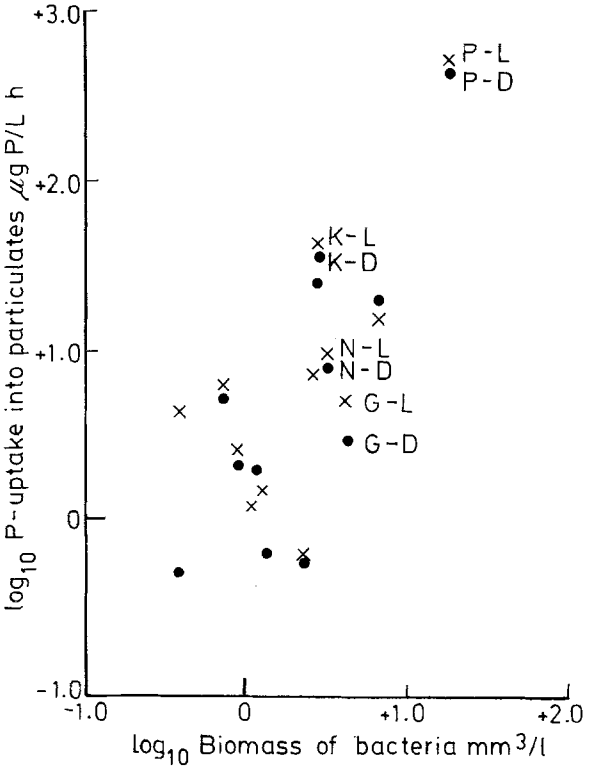

Fig. 4. Correlation between P-uptake into particles <5 µm and bacterial biomass. Symbols as in Fig. 3. A linear least-squares regression calculation gave $Y = 49.1 + 27.22\ X$ ($r = 0.879$). Logarithms of the values were used for convenience in preparing the figure, but not in the statistical analyses

Table 2. Total cell numbers (cells x 10^4/ml) of various bacterial size classes and their seasonal occurrence at sampling station in Rhode River at 1-m depth

Bacterial size classes (µm)	Date (1973-1974) Mar. 28	May 16	June 14	July 9	Aug. 7	Sept. 6	Oct. 3	Oct. 31	Nov. 26	Dec. 18	Jan. 29	Feb. 28
0.6 x 0.6	9.7	0	0	100	0	110	60.7	0	0	0	5.8	8.9
1.0 x 1.0	8.1	980	12.8	710	400	112	21.7	120	51.3	11.3	4.4	2.2
1.7 x 1.7	0.8	0	39.4	10	0	0	13.3	0	0	0	4.5	18.9
0.5 x 1.5	8.5	110	0	0	245	0	29.8	150	98.4	49.1	191	215
0.5 x 5.0	26.7	0	0	0	0	3.6	6.8	0	103	80.6	16.4	11.9
0.5 x 8.5	0	670	0	6	100	15.3	5.3	0	47	19.1	2.6	2.7
1.0 x 5.0	0	0	46	2	26	7.8	7.6	22	10	0	8.7	2.7
1.0 x 100	0	0	0	0	0	1.4	0.2	0	0	0	0	0
2.0 x 4.0	0	0	0	0	5	0	0	0	0	0	0	0
Total cells/ml	53.8	1,760	98.2	828	871	250	145	292	310	160	234	263

Table 3. Total cell numbers (cells/ml) of various algal classes and their seasonal occurrence at sampling station in Rhode River at a 1-m depth. -: None present

Algal classes	Date (1973-1974)											
	Mar. 28	May 16	June 14	July 9	Aug. 7	Sept. 6	Oct. 3	Oct. 31	Nov. 26	Dec. 18	Jan. 29	Feb. 28
Dinophyceae	-	200,000	702	8,061	950	599	262	408	597	682	59	14
Chrysophyceae	-	-	384	-	160	36	238	250	14	50	80	240
Parasinophyceae	-	-	-	-	-	-	82	94	40	640	11	60
Chlorophyceae	250	-	74	-	80	58	468	434	-	174	-	59
Bacillariophyceae	100	-	292	-	82	22	174	1,475	66	186	25	241
Cryptophyceae	60	300	-	230	110	14	96	382	26	226	115	180
Euglenophyceae	110	-	-	1,200	942	18	-	22	-	580	-	-
Haptophyceae	-	-	-	-	92	-	-	252	58	778	-	98
Flagellates	140	75	-	230	41	6	82	4	-	-	8	130
Nannoplankton	800	50	48	404	160	56	69	4	24	384	16	113
Total cells/ml	1,460	200,425	1,500	10,125	2,617	809	1,471	3,325	825	3,700	314	1,135

particulate-P, 0.091; total ortho-P, 0.034; particulate ortho-P, 0.062; temperature, 0.012. For P-uptake into cells smaller than 5 µm, the following correlation coefficients (r) were found: total-P, 0.021; dissolved ortho-P, 0.029; total particulate-P, 0.177; total ortho-P, 0.046; particulate ortho-P, 0.064; temperature, 0.0085. The ratio of P-uptake into cells larger than 5 µm to P-uptake into cells smaller than 5 µm had a low correlation with the level of available dissolved ortho-P (r = 0.0033).

Changes in Algal and Bacterial Population and Biomass During the Year

The number of bacteria, estimated by direct counts, 1 m below the water surface, ranged between 5×10^5 and 10^7 cells/ml throughout the year (Fig. 5a). The highest bacterial numbers were observed during two different algal blooms, a *Prorocentrum mariae - lebouriae* bloom in May and a *Gymnodinium nelsonii* bloom in July. After the July bloom, bacterial numbers declined and remained almost constant from September to February at levels slightly above 10^6 cells/ml. In general, small Gram-negative organisms (1 µm in diameter) were predominant in spring and summer, while rods (0.5 x 1.5 to 8.5 µm in size) including Gram-positive organisms (Table 2) were prominent during the fall and winter. The number of algal cells (determined also by direct counts) was between 10^2 and 10^3 cells/ml, with the exception of two algal blooms which occurred in May and July when the algal count reached 10^5 and 10^4 cells/ml, respectively (Fig. 5b). There was no

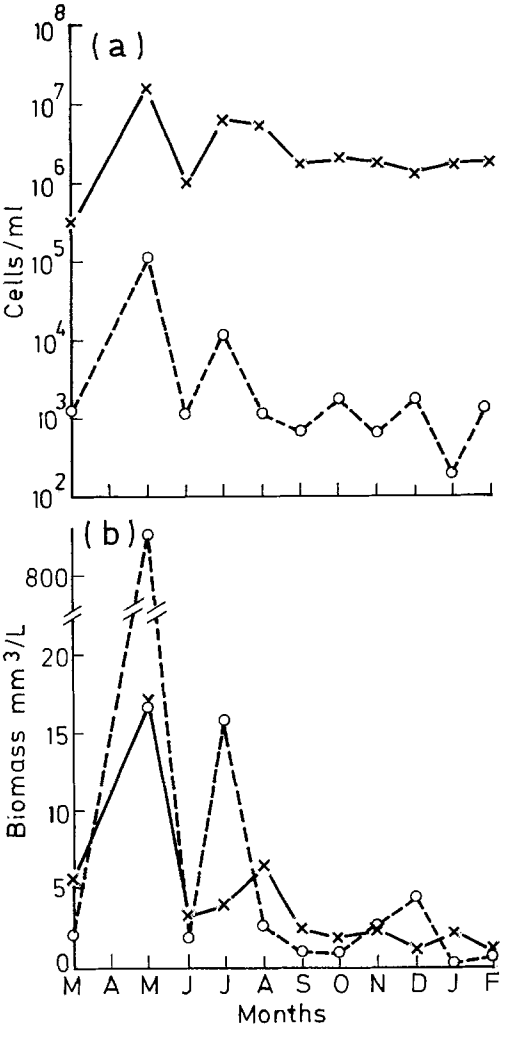

Fig. 5. Seasonal distribution of cell numbers and biomass at Rhode River sampling station at a 1-m depth. Solid lines: bacteria; dashed lines: algae

apparent increase in total number of either bacteria or algae in November, but *Katodinium rotundatum* was the dominant algal species. The total cell numbers per ml of various algal classes and their seasonal occurrence are shown in Table 3.

The highest bacterial biomass was 16.9 mm^3/l during the *Prorocentrum mariae - lebouriae* bloom. Bacterial biomass varied between 1.1 and 6.3 mm^3/l the rest of the year (Fig. 5B). The algal biomass followed algal cell numbers and was 150 times higher in May and 10 times higher in July, during the two algal blooms, than during the rest of the year. Large dinoflagellates were the dominant algal species with respect to biomass during the summer, and nannoplankton of less than 10 µm size were dominant during the rest of the year.

On three occasions, the dominant algal species were *Prorocentrum mariae - lebouriae, Gymnodinium nelsonii* and *Katodinium rotundatum*. It was more difficult to determine the species of bacteria, and they were merely classified according to size (Table 2). The morphology of bacteria varied according to season. The number of cells in certain classifications was high at one sampling and zero at subsequent samplings. A linear least-squares regression calculation gave the result: number of bacterial cells/ml = 23.6 + (0.69)(algal cells/ml), (r = 0.950). The correlation between algal and bacterial biomass was also high, (r = 0.901), indicating that conditions favoring bacterial growth also favored algal growth.

The biomass of phytoplankton had a high correlation (r = 0.803) with the level of dissolved organic-P present in the preceeding 2 weeks and a low correlation (r = 0.0005) with the level of dissolved organic-P present in the succeeding 2 weeks. The same correlations existed between dinoflagellate biomass and dissolved organic-P.

Discussion

We have attempted to distinguish P-uptake by algae from that by bacteria in an estuarine planktonic community by using a differential filtration technique. All experiments were coupled with the estimation of existing biomass throughout a year. This allowed us to calculate P-uptake per biomass and also per volume of water, which gave us not only a measure of the change in biomass with environmental conditions, but also a measure of the metabolic activity of the organisms with regard to P-uptake.

This technique was based on the observation that most of the bacterial population passed through a 5-µm pore-size filter, whereas phytoplankton were retained on it. Even though we separated algae from bacteria as well as our conditions permitted, we recognized the difficulty in separating microorganisms from natural waters into clearly-defined size groups. Some bacterial rods were longer than 5 µm; others adhered to large non-living particulates, or to various algae as reported by other investigators (Bell and Mitchell, 1972; Jones, 1972). In contrast, some broken cells were retained on the smaller pore-size filters in some samples. With all these difficulties, the 5-µm pore-size filter still appears useful in separating estuarine planktonic algae from bacteria.

Another problem was how to discriminate between ^{32}P-orthophosphate bound to non-living particulates and ^{32}P incorporation into the living particulates. Control experiments on ^{32}P-uptake in the presence of IAA, which inhibits biological uptake, gave an estimate of the adsorption of the tracer on surfaces which are largely composed of non-living particulates. Our method of calculation of P-uptake corrects for this nonbiological P-binding. The orthophosphate pool size and its specific activity, as well as frequent data points in the early phase of the experiment, were also needed for the kinetic calculations (Correll *et al.*, 1975). Only the uptake which occurred after the very rapid adsorption of phosphate to the surfaces of particulates could be used in the calculation of biological P-uptake rates. It is possible that we underestimated the relative magnitude of bacterial uptake compared to algal uptake, since we determined this ratio by measuring the slopes of uptake into particulates retained on filters. Since bacterial uptake is very fast compared with algal uptake, there would be a tendency to underestimate bacterial uptake. Since algal uptake was very small, the magnitude of this error could not be very great.

The difficulties in estimating standing crop using total plasma volume or carbon per unit cell volume of a natural sample have been described in detail (Campbell, 1973). In this study we measured standing crop of algae and bacteria by cell counts. This may place a disproportionate emphasis on the smaller-celled specimens, where the total biomass of a number of small cells may ac-

tually be less than a few cells of a larger species. With this method we cannot differentiate between dead or live cells. Smaller cell size provides a greater proportion of surface-to-volume ratio, which can enable more rapid uptake of nutrients and permit a more rapid metabolism of these smaller species. Thus, use of plasma volume in calculating standing crop may overemphasize the importance of larger species (Campbell, 1973). However, we wished to be able to express P-uptake per biomass of cells. To estimate the volume of cells, we needed to know their dimensions, which can be determined only by direct microscopic examination. Jones (1972) has discussed the validity of direct microscopic counts and other methods to establish the degree of association of algae and bacteria in a fresh-water environment. He found a high correlation between algal cell numbers and viable bacterial counts, but not with direct counts of bacteria. However, we obtained a high correlation between algal and bacterial cell numbers using direct counts. It appeared that bacterial numbers follow algal cell numbers. This trend gave a high positive correlation not only with cell numbers but with biomass estimations; r = 0.950 and 0.902, respectively.

The differential filter technique clearly demonstrated that the relative contribution of algae and bacteria to P-uptake varied with season. During the period from August to May, P was assimilated mostly by bacteria, and the algal contribution to P-uptake was less than 6% of total P-uptake. During June and July, P-uptake by algae in the light increased to 9 and 42% of the total P-uptake, respectively. The higher P-uptake throughout the year by bacteria, compared with algae, clearly indicates the importance of bacteria in P assimilation in estuarine environments, as has also been shown for the marine environment (Johannes, 1964). High P-uptake due to algae is possible only in the summer. This is due to higher numbers of algae at that time rather than to higher P assimilating-ability per cell. An increase in population density and a decrease in primary productivity per unit of standing crop in the summer has been reported (Findenegg, 1965). Possible causes are extinction of light, nutrient competition, and increased concentration of excreted algal products. Doubtless, these factors may have occurred in the Rhode River during times when the algal population was high and when the highest supported biomass of algae was 150 times and bacteria 20 times higher than the rest of the year.

The relatively low concentrations of orthophosphate in the water, which prevailed throughout the season with the exception of the summer, did not influence the P assimilating-ability of bacteria and algae, which had high correlations only with biomass. Thus, our results indicate that P-uptake per unit biomass was independent of P concentrations and of water temperature. This differs from the results of Kramer *et al.* (1972), who correlated P levels and total productivity.

Competition between algae and bacteria has been described in laboratory cultures, where growth of algae was severely limited by the presence of bacteria after external P had been exhausted, but growth of bacteria was unaffected by the presence of algae (Rhee, 1972). In our study, no strong competitive advantage in P-uptake by bacteria over algae at low available phosphate levels was indicated. Thus, the ratio of algal to bacterial uptake had a low correlation with dissolved ortho-P (r = 0.0033).

Although algal and bacterial P-uptake, as measured in this study, were both highly correlated with their respective biomasses, high standard deviations from the means were found. For example, the mean algal uptake per biomass in the light was 0.17 ng P/mm^3/h, and the standard deviation was 0.18. One reason for this high deviation was undoubtedly the variation in cell size distribution from experiment to experiment. However, the health or growth rate of the cell population was probably the dominant factor.

Another interesting result of this study was that cells larger than 5 µm (Fig. 3; Table 1) had essentially identical P-uptake rates in both light and darkness. This could be due in part to some bacterial cells contaminating the 5-µm size class, since the average P-uptake by bacteria per biomass was 40 times the average for algae. Since very little bacterial biomass was retained on the 5-µm filter, this is probably not a very major factor in the interpretation of these results. In some experiments (i.e., July 9 and October 3), P-uptake in the dark was much less than in the light. In others (i.e., March 28, October 31, November 26, and December 18), uptake in the dark was about half that in the light. In the rest, it was about the same or even higher. These differ-

ences may reflect differences in the physiology of the dominant species of phytoplankton present. Some species may take up P in the dark, while others cannot (Ketchum, 1939; Kuenzler and Ketchum, 1962).

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