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Autoradiographic Study to Detect Metabolically Active Phytoplankton and Bacteria in the Rhode River Estuary

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Abstract

The rate of utilization of inorganic carbon (C) and phosphate (P) by phytoplankton and bacteria in the Rhode River estuary has been estimated using liquid-emulsion autoradiography during four times of the year. Metabolic activity of phytoplankton was estimated by calculating silver grains above individual species. From these counts, the relative C and P uptake rates of individual species per unit of biomass and per volume of water were estimated. Examination of the autoradiograms showed that the metabolically most active algae were smaller than 10 µm. Both C and P were taken up by these smaller species at a higher rate than their proportion of the total biomass per volume of water would indicate. Uptake of C and P per unit of cell volume varied within a species and among the various phytoplankton at the different seasons of the year. Metabolic activity of planktonic bacteria in safranin-stained autoradiograms was also estimated by counting bacteria with associated grains and cells without grains. The ratio of ^{33}P-labeled to unlabeled bacteria was highest in November. This high metabolic activity of bacteria in November corresponded with high P uptake rates of the phytoplankton at that time. Throughout this study, only 28 to 42% of the total phytoplankton biomass was metabolically active and 63 to 85% of the bacteria.

Introduction

Size fractionation of natural aquatic plankton populations associated with autotrophic and heterotrophic nutrient uptake has been employed previously for phosphorus (Correll *et al.*, 1975; Faust and Correll, 1976) and for carbon utilization (Derenbach and Le B. Williams, 1974; Berman, 1975). Size fractionation is based upon the assumption that radioactivity collected on various pore-size filters represents the total uptake of nutrients in the photosynthetic (algal; large size) and in the heterotrophic (bacterial; small size) populations. In the above studies, a significant amount of the radioactivity was associated with organisms of less than 5 µm, indicating bacterial involvement, and only a small part of the total radioactivity was associated with algal cells. However, size fractionation does not permit determination of selective uptake by members of plankton; it only measures total nutrient uptake for a specific size class within the plankton community.

Even though a separation of algae from bacteria can be achieved with size fractionation of plankton, certain problems have been recognized which are inherent in the use of this technique (Sheldon and Scutcliffe, 1969). Bacterial uptake cannot be fully determined by filtration, because some bacteria may adhere to algal surfaces (Bell and Mitchell, 1972; Jones, 1972) or to nonliving particulates. Others are large rods and remain in the large size fraction (Faust and Correll, 1976). In contrast, broken algal cell fragments can also pass through smaller pore-size filters and appear in the fraction representing mostly bacteria (Berman, 1975; Faust and Correll, 1976).

The methods widely used in previous studies are inadequate to provide estimations of the nutrient uptake and metabolic activity of individual species of microorganisms in the plankton community. In investigating the role of specific microorganisms in nutrient uptake, autoradiography overcomes some of these difficulties (Brock and Brock, 1966; Fuhs

and Canelli, 1970). This technique enables the investigator to obtain direct information on the function of specific microorganisms, since the presence of a radioactive substance within the microbial cell can be ascertained. It also allows recognition of metabolically active species in a given environment. In this study, we examined the phosphorus and carbon uptake of a natural plankton population by the technique of liquid-emulsion autoradiography. Here we describe: (a) the metabolic activity of associated phytoplankton and bacterial populations; (b) relative nutrient uptake rates of phytoplankton species; (c) contribution of metabolically active bacteria to the nutrient utilization; (d) advantages and difficulties of the autoradiographic and size fractionation methods.

Materials and Methods

Plankton Experiments

The experiments were carried out with natural plankton populations obtained in the main basin of the Rhode River estuary, an arm of the Chesapeake Bay, from March 1973 through February 1974 (Faust and Correll, 1976). A clear glass and a black, epoxy paint-dipped bottle each of 1 l capacity were filled with plankton taken from a depth of 1 m with a peristaltic pump (Correll *et al.*, 1975). Two 4 µCi each of ^{33}P-orthophosphate (carrier-free) or ^{14}C-carbonate were added to each bottle. Bottles were suspended for incubation at a depth of 1 m. At 15 and 30 min after the start of the experiment, 500 ml aliquots from the bottles were removed and fixative was added to give a final concentration of 0.4% gluteraldehyde in 0.01 M sodium phosphate buffer, pH 7.0. Metabolically inhibited controls were run as described before by adding iodoacetic acid (IAA) to control samples (Faust and Correll, 1976). Phytoplankton populations were identified from water samples in the plankton experiments.

Water Chemistry

Water samples for carbon and phosphorus analysis were taken as described under plankton bottle experiments. Inorganic carbon available for uptake was determined by alkalinity titration (American Public Health Association, 1971). Analyses of Rhode River water during this study yielded estimates of inorganic carbon concentrations ranging from 11.6 to 13.3 mg C/l.

Orthophosphate in samples which had been filtered through 0.45 µm pore-size membranes was analyzed colorimetrically by reaction with ammonium molybdate and reduction with stannous chloride (American Public Health Association, 1971). Water was analyzed at the beginning and end of the experiment. Analyses of Rhode River water during this investigation yielded estimates of dissolved orthophosphate concentrations from a high of 82 and 80 µg P/l on June 14 and September 6 to a low of 6.5 and 1.5 µg P/l on November 26 and February 28, respectively.

Autoradiography

Fixed samples were taken to the laboratory, and centrifuged for 30 min at 2000 x *g*. The pellet was washed with 12 ml of 0.002 N HCl to remove any radiotracer which may have been bound to the cell surface. After centrifuging, the pellet was washed two more times with 12 ml distilled water and finally resuspended in 3 ml distilled water. Each sample was then sonicated for 5 to 6 sec to break up any clumps of cells (Model W 140 C, Heat Systems Co., at 60 W output), as described by Maguire and Neill (1971). Acid-washed glass microscope slides were coated with 0.5% aqueous gelatin and air-dried. Slides were prepared from subsamples by placing 5 drops of cell suspension on each of 3 microscope slides. Cells on the slides were quickly frozen on a block of dry ice and dried in a vacuum desiccator for about 6 h.

In a dark room, Kodak NTB-2 emulsion was diluted with an equal volume of distilled water and liquified at 40°C in a water bath. Two drops of emulsion were placed on the end of each slide and the emulsion was spread with a stainless steel "raclette" to obtain a constant thickness throughout the slide (Bogoroch, 1972). The slides were dried in the dark at room temperature (ca. 18°C), and then transferred to a light-proof box and stored in the refrigerator for 3 to 5 days. After exposure, the emulsion was developed in Dektol developer at 15°C for 2 min, followed by a 10 sec distilled water rinse, 5 min in acid fixer and 5 to 10 min washing in running tap water. After drying, the emulsion was cleared for 1 h in methylsalicylate and mounted in Permount with a cover slip (Watt, 1971). Some slides were dipped, after development but before washing, in 0.05% aqueous safranin for 30 sec.

Grain Counting

Silver grain counts were made of phytoplankton on slides prepared both from

light and dark bottles. Whenever possible, enough cells of each species were examined to give 30 net counts per species. Some species were too infrequent, and for these species fewer than 30 counts were enumerated. Grain counts were made under oil immersion, with the microscope focused at the base of the cells and those grains in focus were counted. Then the microscope was focused up until the next set of grains was in focus and these counted. This procedure was repeated until all grains were out of focus. Grains within about 3 µm of the margin of each cell were also counted, since grains activated by radioactivity emitted from the cell may not lie directly over the point of emission.

IAA-treated samples showed little or no silver grains over and around phytoplankton cells. However, dense grain aggregates were occasionally present over detrital particles.

Autoradiograms stained with safranin were used to estimate metabolically active bacteria. Microscopic fields with dispersed bacterial cells were used to estimate the grains over bacteria. Bacteria without grains were also counted and the ratio of labeled to unlabeled cells computed for 40 to 60 microscopic fields. Bacteria were frequently observed in aggregates and attached to detrital particles which were heavily covered with silver grains. Because of the uncertain nature of these aggregates, such fields were not included in the calculations.

Biomass Determination

The estimation of cell numbers of phytoplankton and bacteria by the direct count procedure (Rodina, 1972; Campbell, 1973) has been described by Faust and Correll (1976). Cell biomass of organisms was calculated from the mean diameter, length and depth of individual cells as described by Rodina (1972). The biomass per unit of water volume was determined.

Light and Scanning Electron Microscopy

Microscopic fields from both light- and dark-bottle plankton experiments were examined using the phase contrast or bright-field optics of a Zeiss light microscope at 1000 x magnification. Pictures were recorded on High Contrast Copy or Panchromatic films.

Plankton samples for scanning electron microscopy were prepared as described by Faust (1974).

Uptake Calculations

Carbon (C) and phosphorus (P) uptake rates from autoradiograms were estimated as previously described by Friebele (1975). The initial specific activity of C and P was established from the inorganic C and orthophosphate concentrations of the water after filtration through a 0.45 µm pore size filter and from the cpm/ml of whole water.

The mean number of grains per cell was corrected for ^{33}P decay to the day of experiment. Grain yield for ^{14}C was estimated to be 1.8 to 2.0 on Kodak AR-10 stripping film by Pelc (1972). This value was used in our calculations. Since ^{33}P has a range of maximum energies close to that of ^{14}C, a 1.8 grain yield was also used on Kodak NTB-2 emulsion for ^{33}P, which has a sensitivity similar to that of Kodak AR-10 stripping film (Herz, 1959). After these corrections, the mean number of grains per cell was converted to counts per minute per liter for each species as used by Friebele (1975):

$$\frac{\text{Mean grains/cell} \times \text{no. of cells/l}}{1.8 \text{ grains/count} \times 4320 \text{ min exposure time}} = \text{cpm/l}.$$

The change in cpm/l in a phytoplankton species for a 30-min time interval was divided by the average specific activity for inorganic carbon or orthophosphate. Uptake rates were estimated as µg C/h l^{-1} and µg P/h l^{-1} of individual algal species. Uptake rates were also expressed as µg P/μm^3 and µg C/μm^3 of algal biomass of each species.

Results

Association of Microbial Cells

Bacteria and phytoplankton were observed singly, in aggregates or attached to detritus in the Rhode River, as illustrated in the scanning electron micrographs (Fig. 1). Coccoid bacteria in an aggregate appear to be joined with fibrous material and with small pieces of natural debris in Fig. 1: *1*. Similarly, a filamentous organism forms a firm attachment on *Prorocentrum mariae-lebouriae* cell surface and on debris adjacent to the cell in the lower portion of Fig. 1: *2*. Debris of plankton samples varied in shape and size, and some were closely associated with the microorganisms, while others were not. The above observation makes measurement of *in situ* metabolic activity of algae and bacteria difficult.

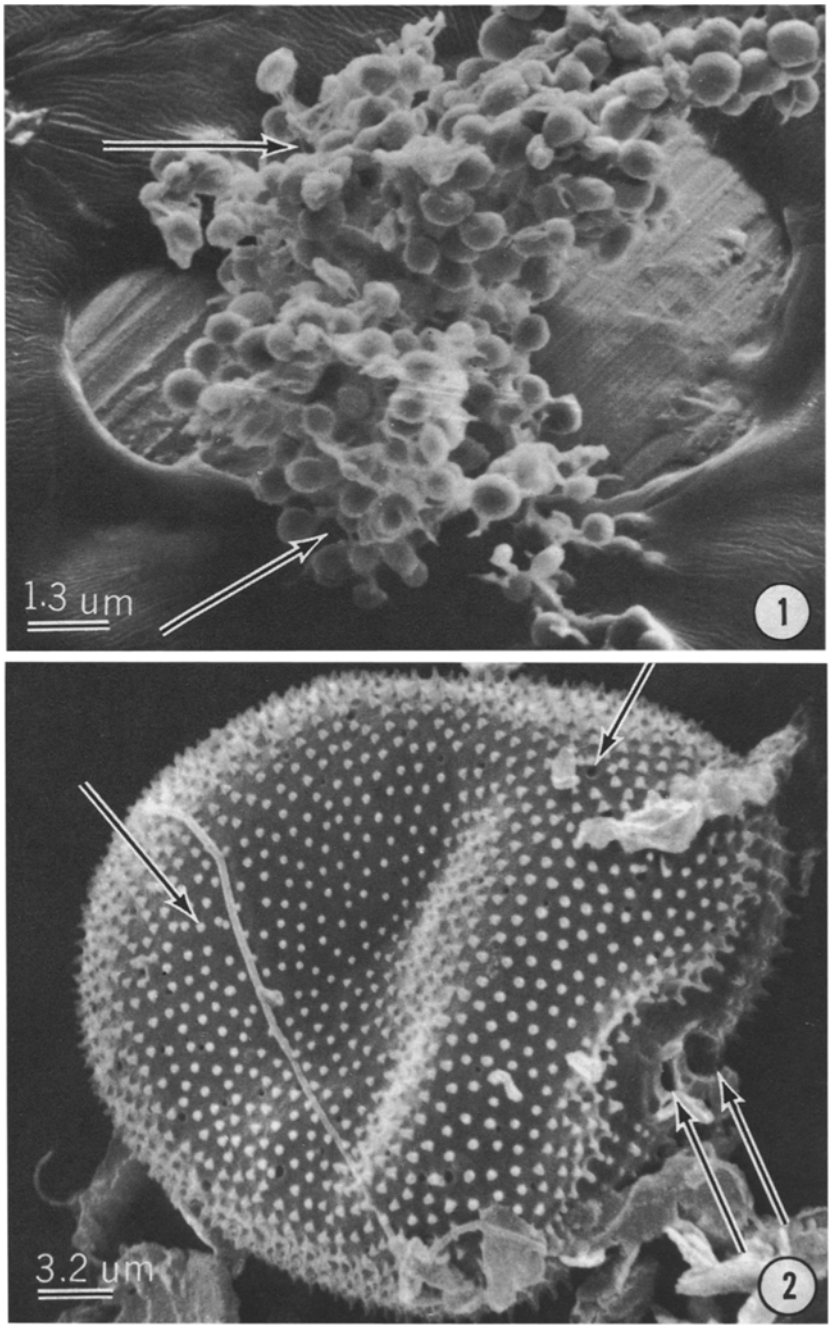

Fig. 1. Scanning electron micrographs (SEM) of association of microorganisms from the Rhode River. *1*: Microorganisms often found in aggregates or attached to surfaces; aggregate of coccoid bacteria is illustrated (x 6300); bacterial cells within aggregate appear to be connected by fibers, indicated by arrows; background delineates surface of SEM stub. *2*: Filamentous organism coherent to strongly compressed and flattened surface of *Prorocentrum mariae-lebouriae* (x 3100); cell surface is covered with tiny spines and trichocyst pores (single arrows); amorphous materials adhering to periphery of cell are debris from natural environment; flagellar pores at anterior end of cell are indicated by double-arrow

Some of the difficulties were overcome, however, by mild sonication of fixed plankton samples before cells were mounted on plates. A few seconds of sonication separated both bacteria and algal cells of small aggregates. Examples of bacteria in an unstained sample after brief sonication, using phase contrast optics is illustrated in Fig. 2. Attempts to separate cells in large aggregates were not successful. A longer time of sonication disrupted the cells; thus, sonication could not be used to break up larger aggregates.

Phytoplankton of fixed plankton samples were also separated after a few seconds of sonication without disrupting their cell morphology (Fig. 2: *6-9*). Phytoplankton that appeared as single cells (Fig. 2: *6,7*), in pairs (Fig. 2: *9*) and in groups of three (Fig. 2: *8*) are illustrated in these light micrographs. Cells dispersed in a microscopic field could be used for grain counts and for taxonomical identification. At the same time, phytoplankton remaining in large aggregates were of little value in this study.

Autoradiography

A few examples of bacteria seen in the autoradiograms of the Rhode River plankton samples are illustrated in Fig. 2: *4,5*. An unstained filamentous organism in an autoradiogram is covered with grains, but the organism cannot be distinguished with the microscope under bright field optics (Fig. 2: *4*). The microscope is focused on the grains. This autoradiogram is an unstained preparation, and the cells are not visible due to the low contrast of the organism under bright-field optics. However, in preparations stained with safranin, bacteria are visible and labeled and unlabeled bacteria can be clearly distinguished (Fig. 2: *5*). This latter autoradiogram is focused on the bacteria, and the grains are somewhat out of focus. Therefore, staining of the autoradiograms was necessary to enhance bacterial morphology under the autoradiographic emulsion. Stained autoradiograms were examined with bright-field optics to observe grain-containing bacteria. In such fields, silver grains appear as black dots and underneath the grains bacteria are visible.

Representative autoradiograms of phytoplankton are illustrated in Fig. 2: *6-9*. The phytoplankton shape and other taxonomical features are recognizable under the emulsion, however the resolution of the cellular features is somewhat lowered.

Autoradiograms of algal cells with silver grains concentrated around and on the cells indicate actively metabolizing organisms. Grains within about 3 µm of the margin of each cell were considered to be due to radioactivity emitted from the cell (Fig. 2: *7,8*). A ^{14}C-autoradiogram of *Gyrodinium dominans* with grains above and around the periphery of the cell is evident in Fig. 2: *7*. Selective uptake of ^{33}P-orthophosphate by phytoplankton species is illustrated in Fig. 2: *8*. Numerous silver grains are above a *Peridinium* species located in the center, none are found above *Prorocentrum mariae-lebouriae*, and only a few grains are above a *Euglenoid* species (arrow). Quite often, phytoplankton incubated in the dark appeared to have numerous grains around the cells at a greater distance than 3 µm. An example of a ^{14}C-autoradiogram of *G. estuariale* incubated in the dark showing numerous grains is shown in Fig. 2: *6*.

A large number of grains were concentrated on and around *Gyrodinium dominans* cells with both tracer ^{14}C and ^{33}P in the light and dark bottles (Fig. 2: *9*). The grains were located within an average diameter of 25 µm around cells, and their appearance was very distinct from the background of the autoradiogram. This organism was the only phytoplankton exhibiting such a grain pattern. The reason for this unusual grain pattern was not readily apparent.

Autoradiograms were also prepared of control plankton samples which had been exposed to the metabolic inhibitor IAA. The number of grains above the microorganisms were greatly reduced when IAA was added. However, grains covering natural debris were often observed in the preparations. Unmetabolized or adsorbed radiotracers were washed out with mild acid and rinsed twice with distilled water from the samples. This produced low background on the radioautograms. It is possible that washing was inadequate to free radiotracers from natural debris. Therefore, grains over clumps of natural debris were not counted.

Estimation of Carbon and Phosphorus Uptake by Phytoplankton

Incorporation of ^{14}C-carbonate and ^{33}P-orthophosphate into phytoplankton was estimated as grains per cell of metabolically active species during the four seasons of the year. Uptake of both tracers computed as grains per cell varied seasonally and from species to species (Table 1). A large number of grains were found only in the dinoflagel-

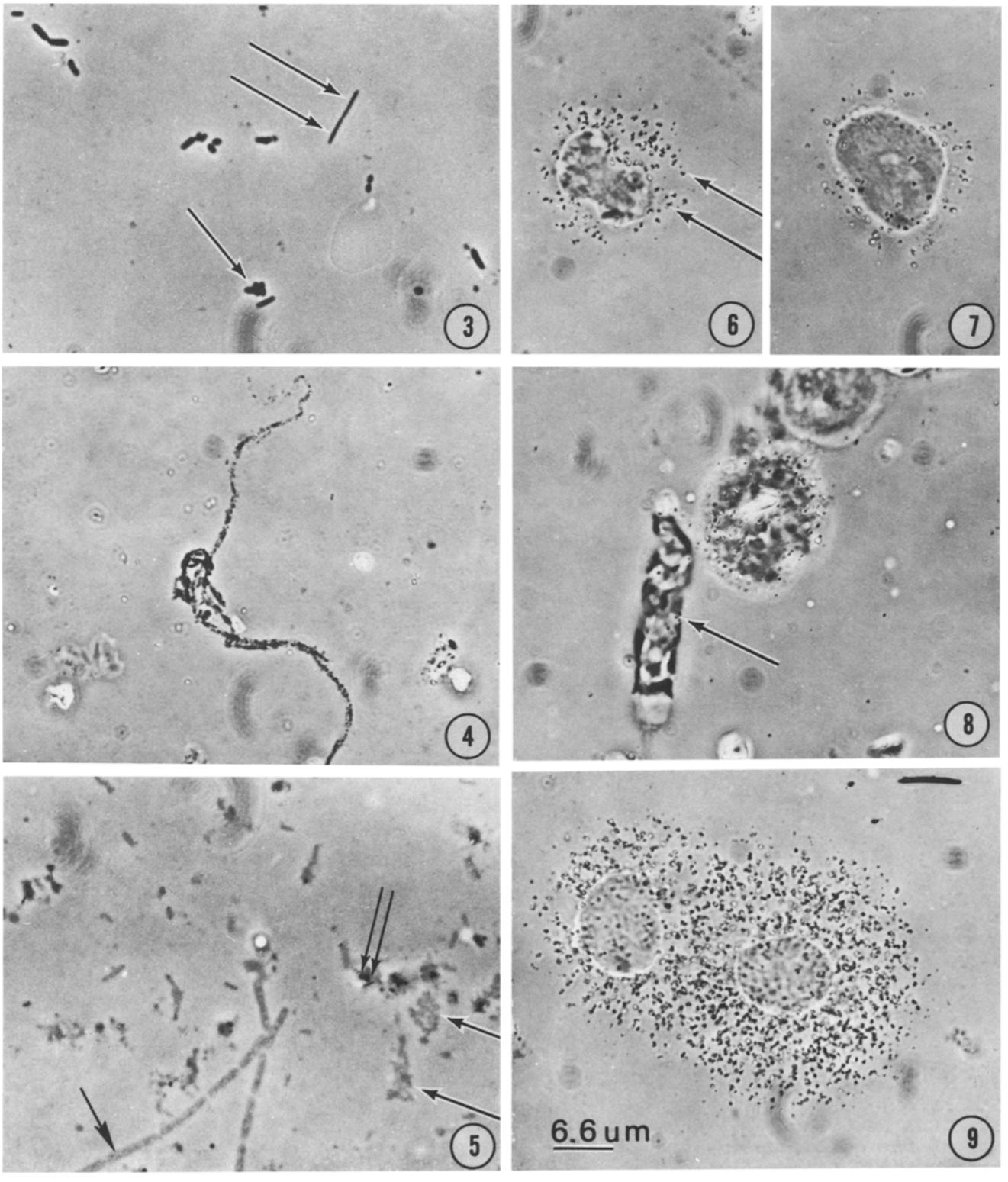
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Table 1. Relative carbon (C) and phosphorus (P) uptake by phytoplankton in Rhode River at depth of 1 m, estimated as grains per cell. -: absent

Organism	Size (μm)	Date (1973-1974)											
		June 14			September 6			November 26			February 28		
		Biomass (mm^3/l)	Grains (no./cell) C	P	Biomass (mm^3/l)	Grains (no./cell) C	P	Biomass (mm^3/l)	Grains (no./cell) C	P	Biomass (mm^3/l)	Grains (no./cell) C	P
Chlamydomonas sp.	13x10	-	-	-	-	-	-	-	-	-	0.01	4.3	1.8
Chlorella sp.	5x5	-	-	-	0.02	11.9	9.3	-	-	-	0.002	-	-
Cryptomonas sp.1	15x12	-	-	-	0.02	--	-	-	-	-	0.04	11.6	3.0
Cryptomonas sp.2	13x10	-	-	-	-	-	-	-	-	-	0.05	7.8	7.8
Diplosalis sp.	15x15	-	-	-	0.04	4.1	3.8	-	-	-	-	-	-
Euglena sp.	5x55	-	-	-	0.02	5.4	6.1	-	-	-	0.05	9.9	2.7
Flagellate	15x12	-	-	-	-	-	-	-	-	-	0.05	9.9	2.7
Gymnodinium aurenticum	13x7	-	-	-	0.01	8.8	4.5	-	-	-	-	-	-
Gymnodinium sp.1	12x8	0.08	9.2	8.2	0.14	14.0	12.5	-	-	-	-	-	-
Gymnodinium sp.2	15x13	-	-	-	-	-	-	-	-	-	0.03	9.6	11.2
Gyrodinium dominans	15x12	0.18	30.6	30.0	-	-	-	-	-	-	-	-	-
G. estuariale	12x10	0.03	-	-	0.09	7.0	6.6	0.10	16.7	15.7	-	-	-
Katodinium rotundatum	10x6	-	-	-	-	-	-	0.05	19.0	17.7	-	-	-
Nannoplankton	10x5	-	-	-	-	-	-	0.02	18.1	16.1	-	-	-
Pavlova gyrans	16x6	-	-	-	-	-	-	0.04	14.5	12.1	-	-	-
Peridinium sp.	17x13	0.02	30.5	18.2	-	-	-	-	-	-	-	-	-
Prorocentrum mariae-lebouriae	16x20	0.35	25.8	21.8	0.08	9.9	4.0	-	-	-	-	-	-
Pseudopedinella pyriforme	5x3	-	-	-	-	-	-	-	-	-	0.05	3.0	2.1
Tetraselmis gracilis	15x12	-	-	-	-	-	-	-	-	-	0.100	13.5	11.7
% biomass of grain-containing cells		37.2			41.9			28.0			36.3		

Fig. 2. Light micrographs of bacteria and phytoplankton from Rhode River examined with phase- and bright-field optics (all x 1500). *3*: Rod-shaped bacteria in pairs (double arrow), coccoid bacteria in small clusters (single arrow), and single cells (phase-contrast optics). *4*: Unstained ^{33}P autoradiogram of a filamentous organism covered with many silver grains (bright-field optics, with the silver grains in focus). *5*: Safranin-stained bacteria are in focus in photomicrograph of a ^{33}P-autoradiogram (bright-field optics); large bacterial filament (single black arrow) and several small bacterial aggregates (single white-edged black arrows) without grains are shown; other bacteria in aggregates (double black arrow) have grains above them, however silver grains are out of focus. Note contrast in detectability of cell morphology between unstained (*4*) and safranin-stained (*5*) preparations. *6*: Example of ^{14}C-autoradiogram of plankton sample incubated in the dark; *Gyrodinium estuariale* cell is shown with numerous grains around the cell (arrows). (In illustrations *6* and *9*, grains are too far from cell and are not considered as algal uptake of the tracer in estimating metabolic activity of phytoplankton.) *7*: *Gyrodinium dominans* with some grains above and around the cell is evident in the ^{14}C-autoradiogram. Grains are within 3 μm of cell periphery and are used in estimating grains per cell. *8*: Selective uptake of ^{33}P-orthophosphate by phytoplankton; numerous silver grains are positioned above a *Peridinium* species in the center, none are found above *Prorocentrum mariae-lebouriae*, and a few grains are above a *Euglenoid* species (arrow). *9*: Two cells of *Gyrodinium dominans* surrounded by many silver grains in ^{33}P-autoradiogram are shown, using phase-contrast optics; large number of grains is accumulated on and around most cells, forming a dense grain halo, indiscriminatedly, in ^{14}C and ^{33}P-autoradiograms incubated in light and dark bottles

late species *Gymnodinium* sp.1, *Gyrodinium dominans*, *Peridinium* sp. and *Prorocentrum mariae-lebouriae* in June. Thus, these species were considered the most metabolically active algae at this time of the year. The biomass of grain-containing algal species was only 37.2% of the total algal biomass in June (Table 1).

Grains per cell of the same algal species varied seasonally. The high number of grains per *Prorocentrum mariae-lebouriae* cell in June declined several fold to 10 grains per cell of C and 4 grains per cell of P in September (Table 1). In contrast, incorporation of the tracers increased from 9 to 14 grains per cell for C and 8 to 12 grains per cell for P in *Gymnodinium* sp.1 from June to September.

Phytoplankton carbon and phosphorus uptake during the summer was due to dinoflagellates and to other species during spring, fall and winter seasons (Table 1). In September, November and February the largest number of grains were found in *Chlorella* sp., *Cryptomonas* sp.1, *Katodinium rotundatum*, nannoplankton, and *Tetraselmis gracilis*. All actively metabolizing cells were less than 20 µm in size and only 28 to 42% of the total algal biomass were metabolically active at any given season (Table 1).

The total biomass of phytoplankton varied from 0.5 to 1.7 mm^3/l during the seasons (Table 2). The biomass of various algal classes was also estimated. Dinoflagellates represented 69 to 80% of the total algal biomass in June, September and November; whereas organisms of the Cryptophyceae, Bacillariophyceae, Crysophyceae, flagellates, and nannoplankton each made up 18 to 24% of the total algal biomass in February. In February the dinoflagellate biomass declined to 5% of total algal biomass.

Rates of carbon and phosphorus uptake for all phytoplankton species are presented in Table 3. The contribution of algal species to the uptake of C and P per volume of water changed seasonally. Uptake of C by *Gymnodinium* sp.1 varied from 8.8 $\mu g/l\ h^{-1} \times 10^{-2}$ in June and 33 $\mu g/l\ h^{-1} \times 10^{-2}$ in September to none in November and February. The P uptake by this alga followed a similar course. In contrast, *Prorocentrum mariae-lebouriae* C and P uptake rates were higher in June and lower in September. The highest observed uptake rates for C and P were exhibited by *Katodinium rotundatum* (145 $\mu gC/l\ h^{-1} \times 10^{-2}$ and 31 $\mu gP/l\ h^{-1} \times 10^{-2}$) and by *Pavlova gyrans* (79.5 $\mu gC/l\ h^{-1} \times 10^{-2}$ and 0.16 $\mu gP/l\ h^{-1} \times 10^{-2}$) in November.

Total C and P uptake per volume of water was also estimated (Table 3). Carbon uptake was comparatively low (41.2 and 51.1 $\mu gC/l\ h^{-1} \times 10^{-2}$) in June and September and high (393.4 and 170.2 $\mu gC/l\ h^{-1} \times 10^{-2}$) in November and February, respectively. Phosphorus uptake rates were 60.0 and 50.9 $\mu gP/l\ h^{-1} \times 10^{-2}$ in June and September and 66.36 and 2.42 $\mu gP/l\ h^{-1} \times 10^{-2}$ in November and February. No persistent relationship was observed between C and P uptake rates per volume of water (Table 3) and total algal biomass (Table 2). It must be noted that only about one-third of the total biomass of algae was active at any given time.

Carbon and phosphorus uptake rates per unit of cell volume were higher for the smaller than for the larger species (Table 4). *Pseudopedinella pyriforme*, the smallest flagellate in the samples, had the highest uptake rates for both C and P per unit of cell volume (817 $\mu gC/\mu m^3\ h^{-1} \times 10^{-10}$ and 5.9 $\mu gP/\mu m^3\ h^{-1} \times 10^{-10}$ in February), followed by a nannoplankton (366.8 $\mu gC/\mu m^3\ h^{-1} \times 10^{-10}$ and 7.5 $\mu gP/\mu m^3\ h^{-1} \times 10^{-10}$ in November); while the third highest rate was by a *Chlorella* sp. (190.7 $\mu gC/\mu m^3\ h^{-1} \times 10^{-10}$ and 15.2 $\mu gP/\mu m^3\ h^{-1} \times 10^{-10}$ in September). Carbon and P uptake per cell volume was much lower for the larger species, e.g. *Prorocentrum mariae-lebouriae* (4.9 to 2.5 $\mu gC/\mu m^3\ h^{-1} \times 10^{-10}$ and 0.2 to 0.15 $\mu gP/\mu m^3\ h^{-1} \times 10^{-10}$) and *Gymnodinium* sp.1 (11.5 to 24.1 $\mu gC/\mu m^3\ h^{-1} \times 10^{-10}$ and 0.18 to 0.22 $\mu gP/\mu m^3\ h^{-1} \times 10^{-10}$) in June and September, respectively. The biomass of *P. mariae-lebouriae* was the largest of any species measured during the experimental period, yet C and P uptake per unit of cell volume was 160 and 30 times less than that estimated for *Pseudopedinella pyriforme*. The uptake may have been governed by the surface:volume ratio in the various plankton size classes.

The relationship C and P uptake per unit of cell volume varied between algal species (Table 4). The ratio of C to P uptake per μm^3 ranged from 10:1 for *Diplosalis* sp., *Gymnodinium aurenticum* and *Peridinium* sp.; and 50:1 for *Katodinium rotundatum*, nannoplankton, and *Pavlova gyrans*, to above 100:1 for *Gymnodinium* sp.1 and *Pseudopedinella pyriforme*.

Autoradiography of Bacteria

Metabolically active bacterial populations from light and dark bottles were detected in stained ^{33}P-autoradiograms. The percentages of labeled and unlabeled bacteria in the autoradiograms were estimated four times a year (Table 5). The distribution of labeled bacteria

Table 2. Biomass of various algal classes and their seasonal occurrence in Rhode River at depth of 1 m. Values as % of total biomass

Algal classes	Date (1973-1974)			
	June 14	September 6	November 26	February 28
Dinophyceae	79.9	72.0	69.1	4.9
Chrysophyceae	2.5	5.7	3.4	10.4
Prasinophyceae	-	-	9.7	1.4
Chlorophyceae	1.1	8.1	-	1.8
Bacillariophyceae	15.9	0.7	4.7	18.3
Cryptophyceae	-	0.9	3.7	23.7
Euglenophyceae	-	1.0	-	-
Haptophyceae	-	-	4.7	3.0
Flagellates	-	4.7	-	18.2
Nannoplankton	0.1	6.9	4.7	18.3
Total biomass (mm^3/l)	1.7	0.9	1.1	0.5

Table 3. Carbon (C) and phosphorus (P) uptake rates ($\mu g\ l^{-1}\ h^{-1} \times 10^{-2}$) of phytoplankton in Rhode River at depth of 1 m

Organism	Size (µm)	Date (1973-1974)							
		June 14		September 6		November 26		February 28	
		C	P	C	P	C	P	C	P
Chlamydomonas sp.	13x10	-	-	-	-	-	-	9.4	0.04
Chlorella sp.	5x5	-	-	7.2	6.0	-	-	-	-
Cryptomonas sp.1	15x12	-	-	-	-	-	-	25.5	0.07
Cryptomonas sp.2	13x10	-	-	-	-	-	-	38.0	0.40
Diplosalis sp.	15x15	-	-	1.0	0.9	-	-	-	-
Euglena sp.	5x55	-	-	0.8	0.9	-	-	-	-
Flagellate	15x12	-	-	-	-	-	-	44.4	0.12
Gymnodinium sp.1	12x8	8.8	14.0	32.9	30.0	-	-	-	-
Gymnodinium sp.2	15x13	-	-	-	-	-	-	12.8	1.50
Gymnodinium aurenticum	13x7	-	-	1.1	0.8	-	-	-	-
Gyrodinium dominans	15x12	8.8	15.0	-	-	-	-	-	-
G. estuariale	12x10	-	-	7.1	6.8	68.4	14.8	-	-
Katodinium rotundatum	10x6	-	-	-	-	145.0	31.21	-	-
Nannoplankton	10x5	-	-	-	-	74.8	15.10	-	-
Pavlova gyrans	10x6	-	-	-	-	79.5	0.16	-	-
Peridinium sp.	17x13	6.4	7.0	-	-	-	-	-	-
Prorocentrum mariae-lebouriae	16x20	17.2	24.0	1.0	4.6	-	-	-	-
Pseudopedinella pyriforme	5x3	-	-	-	-	-	-	40.1	0.29
Tetraselmis gracilis	15x12	-	-	-	-	25.7	5.15	-	-
Total uptake ($\mu g l^{-1}\ h^{-1} \times 10^{-2}$)		41.2	60.0	51.10	50.9	393.4	66.36	170.2	2.42

Table 4. Carbon (C) and phosphorus (P) uptake (μg μm^3 h^{-1} x 10^{-10}) of phytoplankton per unit biomass in Rhode River at depth of 1 m

Organism	Size (μm)	Date (1973-1974) June 14 C	June 14 P	September 6 C	September 6 P	November 26 C	November 26 P	February 28 C	February 28 P
Chlamydomonas sp.	13x10	-	-	-	-	-	-	160.8	6.94
Chlorella sp.	5x5	-	-	190.76	15.20	-	-	-	-
Cryptomonas sp.1	15x12	-	-	-	-	-	-	65.4	0.17
Cryptomonas sp.2	13x10	-	-	-	-	-	-	73.0	0.75
Diplosalis sp.	15x15	-	-	2.42	0.23	-	-	-	-
Euglena sp.	5x55	-	-	5.18	0.05	-	-	-	-
Flagellate	15x12	-	-	-	-	-	-	55.7	1.50
Gymnodinium aurenticum	13x7	-	-	18.32	0.16	-	-	-	-
Gymnodinium sp.1	12x8	11.5	0.18	24.10	0.22	-	-	-	-
Gymnodinium sp.2	15x13	-	-	-	-	-	-	46.1	0.55
Gyrodinium dominans	15x12	4.9	0.84	-	-	-	-	-	-
G. estuariale	12x10	-	-	0.07	0.16	70.4	1.52	-	-
Katodinium rotundatum	10x6	-	-	-	-	164.8	3.53	-	-
Nannoplankton	10x5	-	-	-	-	366.8	7.55	-	-
Pavlova gyrans	16x6	-	-	-	-	127.5	2.42	-	-
Peridinium sp.	17x13	12.7	0.13	-	-	-	-	-	-
Prorocentrum mariae-lebouriae	16x20	4.9	0.20	2.56	0.15	-	-	-	-
Pseudopedinella pyriforme	5x3	-	-	-	-	-	-	817.1	5.91
Tetraselmis gracilis	15x12	-	-	-	-	31.6	0.62	-	-

Table 5. Proportions of ^{33}P-orthophosphate metabolizing bacteria at various seasons in Rhode River at depth of 1 m

Date (1973-1974)	Treatment[a]	No. bacteria/field Labeled	Unlabeled	% labeled ± SD[b]	Labeled: Unlabeled	Cells/ml x 10^6	Biomass (mm^3/l
June 14	L	33.3	20.3	63 ± 3.7	1.64	0.9	4.9
	D	34.1	19.8	63 ± 4.5	1.72		
September 6	L	32.7	13.8	70 ± 3.5	2.36	2.5	3.4
	D	30.1	17.0	63 ± 4.2	1.77		
November 26	L	47.8	7.4	85 ± 5.1	6.45	3.1	5.1
	D	46.1	7.6	84 ± 5.0	6.06		
February 28	L	65.8	14.1	81 ± 4.4	4.66	2.6	1.6
	D	55.4	12.0	82 ± 3.7	4.61		

[a] L: Light bottle; D: dark bottle.

[b] SD: Standard deviation.

within each sample was relatively uniform. In November and February, 81 to 85% of the bacteria were labeled, whereas in June and September 63 to 70% of bacteria had grains. Thus, a greater proportion of planktonic bacteria (Table 5) than phytoplankton (Table 1) had grains above them at the same selected time of the year.

The ratio of ^{33}P-labeled to unlabeled bacteria was the highest (6.4) in November, lower (4.6) in February and the lowest (1.6 to 2.3) in June and September (Table 5). The high metabolic activity of bacteria corresponded with high P uptake rates per volume of water of the phytoplankton in November (Table 3). Similarly the number of bacteria was highest, 3.1 x 10^6 per ml, in November, 2.5 and 2.6 x 10^6 per ml in September and February, and lowest, 0.9 x 10^6 per ml, in June. In contrast, the biomass per volume of water was highest, 4.9 and 5.1 mm^3/l in November and June, 3.4 mm^3/l in September, and lowest 1.6 mm^3/l, in February.

Discussion

Relative grain counts of phytoplankton species as a measure of their metabolic activity have been used previously (Brock and Brock, 1968; Fuhs and Canelli, 1970; Maguire and Neill, 1971; Watt, 1971). However, quantitative measurements from autoradiograms are difficult to obtain. Relative grain densities around organisms depend not only on the geometry and density of the specimen, but also on the energy of the tracer and the thickness and sensitivity of the photographic emulsion (Perry, 1964).

Even though autoradiography is specific, we recognize some of the difficulties in estimating the metabolic activity of algae and bacteria in an estuary. Some phytoplankton and bacteria form mucilaginous capsular material; others adhere to algal cell surfaces and to sediment particles (Bell and Mitchell, 1972; Jones, 1972; Faust and Correll, 1976). Bacteria are also opportunists, utilizing photosynthetic products of phytoplankton efficiently (Watt, 1971). Also, algal heterotrophy may exist in nature (Ketchum, 1939; Faust and Gantt, 1973; Pollinger and Berman, 1976). Despite all these difficulties, autoradiography is still one of the more sensitive techniques for estimation of bacterial and phytoplankton metabolism at the species level.

Grain counts of phytoplankton cells were corrected for ^{33}P-radioactive decay and for background grains occurring within an equivalent area containing no phytoplankton. No correction for the adhering or aggregating bacteria could be made. This is a source of error, and could cause an over-estimation of phosphorus uptake by individual phytoplankton species.

Bacteria are small and difficult to detect under the emulsion of the autoradiograms. Staining enhanced the visibility of these cells. Comparison of counts of labeled and unlabeled bacteria from stained autoradiograms suggested that a large proportion of bacteria were clumped; thus, counts obtained from the autoradiograms might have underestimated unlabeled organisms. The proportion of labeled bacterial cells could also be underestimated in the June experiment when a large number of grains were observed as a dense grain halo around *Gyrodinium dominans*. In this halo, bacteria were not visible and thus this activity was not counted or tabulated as bacterial since activity of bacteria was assessed using grains only above recognizable bacterial cells. Several investigators (Fuhs and Canelli, 1970) experienced similar problems and suspected that clumps of bacteria were the cause of such halos, but were unable to provide proof of the presence of bacterial cells. Nevertheless, the data available suggest that valid counts of labeled bacteria from a mixed population could be obtained from autoradiograms provided the organisms are not clumped and the autoradiograms are stained.

Results from the autoradiograms show that phytoplankton smaller than 10 µm in size were metabolically most active in the Rhode River. McCarthy *et al.* (1974) measured primary production of phytoplankton in the Chesapeake Bay. They reported that 94% of the primary productivity was by "algae" passing through a 10 µm pore-size net. Taft *et al.* (1975) showed also that "ultra-plankton" (which could include bacteria and small algae) were responsible for the majority of the P uptake in the Chesapeake Bay. Our previous results have also indicated that 95% of phosphorus was assimilated by bacteria throughout the year and that P uptake by algae was only a significant percentage of the total during the summer months in the Rhode River (Faust and Correll, 1976).

We have reported earlier (Correll *et al.*, 1975) and in this report that P uptake by nannoplankton was greater than the proportion of their biomass would suggest. Watt (1971) also reported that this was true for primary productivity of nannoplankton in the northwest Atlantic Ocean. Although nutrient uptake by

algal cells may differ, some species may have the advantage in nutrient uptake over others due to higher surface-to-volume ratios. Nannoplankton certainly have a large surface-to-volume ratio (Fuhs *et al.*, 1972). Munk and Riley (1952) calculated nutrient absorption rates from a theoretical expression containing hydrodynamic variables for algal cells and found that absorption increases with decreasing cell dimension. Friebele (1975) and our results with ^{33}P autoradiography of a natural plankton population confirm this theory.

Phosphorus uptake rates per volume of water determined by autoradiography were highest in November (Table 3), when the ratio of labeled and unlabeled bacterial cells was also highest (Table 5). Thus, a close relationship existed between autotrophic and heterotrophic metabolic activity of the plankton population, and bacteria did contribute substantially in this process. This result is similar to reports of bacterial participation in the primary production in the sea (Derenbach and Williams, 1974) and of P uptake in the Rhode River (Faust and Correll, 1976) estimated previously by using size fractionation of the plankton.

The generalized results show summer primary productivity and P uptake in Rhode River phytoplankton to be predominantly attributable to dinoflagellates (70 to 80% from June to November) and to other small species (30 and 95% of total biomass in November and February). The largest dinoflagellate, *Prorocentrum mariae-lebouriae*, contributed significantly to C and P uptake in June, whereas the smaller dinoflagellate *Katodinium rotundatum* and other nannoplankton were the principal primary producers during the rest of the year (Table 3). Thus, the rates of primary production and P uptake per unit of cell volume (Table 4) display a wide range of values. The number of observations are too small to define productivity ranges of the species involved. The metabolic rates encountered within a species such as *P. mariae-lebouriae* in June and September or among the dinoflagellate species *K. rotundatum* and *Gyrodinium estuariale* at the Rhode River estuary and in the ocean (Watt, 1971) are extremely variable.

The metabolic activity per unit of cell volume of small organisms such as *Pseudopedinella pyriforme* (volume = 35 μm^3) was higher than the larger nannoplankton (volume = 196 μm^3) or *Katodinium rotundatum* (volume = 458 μm^3). The C:P uptake ratios followed the previous order (e.g. C:P for *P. pyriforme* was 138:1, nannoplankton 49:1 and *K. rotundatum* 46:1. However, when comparing the uptake rates per volume of water, which reflect the total productivity of the plankton population, *K. rotundatum* was the most productive, followed by the nannoplankton and by *P. pyriforme*. Perhaps there is no universal relationship that exists between a species biomass (volume) and its contribution to the total productivity of the community as suggested by Watt (1971). The reasons for changing patterns of productivity could be such factors as life history, age, size, photosynthetic capacity, nutrient concentrations and diurnal rhythms of phytoplankton species (Maguire and Neill, 1971; Stross and Pemrick, 1974).

In view of the above, we recognize the limitation of this autoradiography study, which only attempted to estimate relative nutrient uptake rates by phytoplankton species and to supply information on the proportion of metabolically active heterotrophic bacterial populations. However, autoradiography and size-fractionation techniques combined may provide a considerable advancement in our understanding of the mechanisms which determine plankton community structure. Estimating short-time *in situ* nutrient uptake by plankton may also be of value to studies of *in situ* algal physiology and the role of algal heterotrophy in the aquatic environment.

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