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Relationship between Phytoplankton Cell Size and the Rate of Orthophosphate Uptake: *in situ* Observations of an Estuarine Population

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Abstract

Orthophosphate uptake by a natural estuarine phytoplankton population was estimated using two methods: (1) ^{32}P uptake experiments in which filters of different pore sizes were used to separate plankton size-fractions; (2) ^{33}P autoradiography of phytoplankton cells. Results of the first method showed that plankton cells larger than 5 µm were responsible for 2% of the total orthophosphate uptake rate. 98% of the total uptake rate occurred in plankton composed mostly of bacteria, which passed the 5 µm screen and were retained by the 0.45 µm pore-size filter. There was no orthophosphate absorption by particulates in a biologically inhibited control containing iodoacetic acid. Orthophosphate uptake rates of individual phytoplankton species were obtained using ^{33}P autoradiography. The sum of these individual rates was very close to the estimated rate of uptake by particulates larger than 5 µm in the ^{32}P labelling experiment. Generally, smaller cells were found to have a faster uptake rate per μm^3 biomass than larger cells. Although the nannoplankton constituted only about 21% of the total algal biomass, the rate of phosphate uptake by the nannoplankton was 75% of the total phytoplankton uptake rate. Results of the plankton autoradiography showed that the phosphate uptake rate per unit biomass is a power function of the surface:volume ratio of a cell; the relationship is expressed by the equation $Y = 2 \times 10^{-11} X^{1.7}$, where Y is $\mu gP\ \mu m^{-3}\ h^{-1}$ and X is the surface:volume ratio. These results lend support to the hypothesis that smaller cells have a competitive advantage by having faster nutrient uptake rates.

Introduction

The phytoplankton is normally an assemblage of one-celled or multi-celled autotrophs with diverse shapes and sizes. Hutchinson (1961) was puzzled that such diversity could exist in an isotropic environment where competition for resources is supposedly occurring.

Phytoplankton species not only display diversity in shapes and sizes, but they differ in their contribution to the total algal biomass and metabolic activity. Nannoplankton (very small algal cells) which, in the past, were overlooked with net phytoplankton sampling, have recently been recognized as very important autotrophs in the plankton community.

Many studies have documented the dominance of "nannoplankton" in algal standing crop, chlorophyll content, and productivity (Yentsch and Ryther, 1959; Malone, 1971a, b; McCarthy *et al.*, 1974; Berman, 1975). The definition of nannoplankton has varied between cells with a diameter of 65 µm to those less than 3 µm. However, it is clear from these studies and from the work of Watt (1971), who used autoradiography to measure primary production rates of individual phytoplankton species, that the smaller cells provide a large fraction of the total primary production in the euphotic zone. Although nannoplankton were the most important producers in all environments studied by Malone (1971a), the netplankton:nannoplankton productivity and chlorophyll ratios were higher in coastal waters than in oceanic waters. Nannoplankton populations varied within a narrow margin, but generally netplankton standing crop increased in areas of upwelling along the California Current (Malone, 1971b). Yentsch and Ryther (1959) also reported a stable nannoplankton population accompanied by fluctuations in netplankton standing crop.

These observations suggest that perhaps the nannoplankton are the best

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adapted species of the phytoplankton community in many aquatic environments. Possibly they represent the more opportunistic species, since they have faster growth and metabolic rates than larger species (Fogg, 1965). However, their ability to multiply rapidly is dependent upon rapid and efficient utilization of available resources. Do the nannoplankton have a competitive advantage in obtaining the required nutrients?

Munk and Riley (1952), who developed a nutrient absorption model based on fluid dynamics, predicted that small cells have a faster relative nutrient absorption rate than large cells. They studied morphological and size variations of phytoplankton cells and concluded that neither the rate of sinking nor nutrient absorption imposes severe limits upon cells of less than 20 µm in size.

Dugdale (1967) pointed out the possibility for competitive advantage of one phytoplankton species over another based upon Michaelis-Menten nutrient uptake kinetics. Eppley *et al.* (1969) obtained kinetic constants for a number of species taking up ammonia and nitrate. Their results showed several general trends: (1) large species had high K_s values; (2) small-celled species from the ocean showed low K_s values; (3) fast-growing species seemed to have lower K_s values than slow growers. However, the effect of nutrient uptake rates on specific growth rate is modified by efficiency of light utilization of the phytoplankton species. Parsons and Takahashi (1973) have developed a model which predicts that small cells will outgrow larger cells in all environments except those where nutrient levels and light intensities are high.

The work cited above has included either laboratory studies of nutrient uptake kinetics with algal cultures under presumably ideal conditions, or models using kinetic data to predict which cells will have competitive advantage in certain environments. The purpose of the following study was to observe total and individual nutrient absorption rates of a diverse group of phytoplankton cells under natural conditions. This was accomplished by means of ^{32}P uptake experiments employing filters to separate plankton sizes and by ^{33}P autoradiography of plankton organisms. The latter technique enables the researcher to measure the rate of a metabolic function in an individual organism or species. Thus, we can determine whether small cells do have a competitive advantage in nutrient uptake when they are coexisting and perhaps competing with other cells in a natural aquatic environment.

Materials and Methods

This study was carried out on the Rhode River, a subestuary on the western shore of the Chesapeake Bay near Annapolis, Maryland, USA. The Rhode River is a shallow estuary with a mean depth of 2 m. Fresh water enters the river from the Muddy Creek watershed, and saline water enters from the Bay. The experiments were performed in the fall, when water temperatures were dropping, soluble reactive phosphorus levels were declining after a late summer peak, and phytoplankton and bacterial numbers were decreasing.

On October 31, 1974, a 24 l water sample was taken with a peristaltic pump from 1 m depth at a Rhode River station (Latitude 38°53'00" N; Longitude 76°32' 00" W), where the depth is 4 m. The water was placed in glass carboys and transported to the Smithsonian Institution Chesapeake Bay Center dock where the experiments were performed. The water was divided into experimental bottles which were suspended from the dock at 1 m water depth.

The experimental flask for autoradiography consisted of a 12 l spherical flask which had been acid-washed and equipped with a stopper and a wire harness, with float and weight attached. The flask was suspended in the water on the southern side of the dock at 1 m depth. The stopper was pierced by 3 tubes: 1 from a peristaltic pump, 1 from a small air pump, and 1 for the exit for air. For the duration of the experiment, air was bubbled gently through the flask in order to prevent the plankton from settling. 200 µCi of ^{33}P as H_3PO_4 was injected into the flask through a large syringe needle. At timed intervals, 1 l samples were pumped from the flask into bottles containing buffered fixative (final concentration = 0.5% gluteraldehyde and 0.01 M potassium phosphate buffer at pH 7.0). One ml of each sample was also placed in a scintillation vial for counting at the same time. After each sample was taken, the pump was reversed in order to clear the pump lines of water until the next sample was taken. One ml of water was taken at the beginning and at the end of the experiment for counting to check for leaks in the apparatus.

Total phosphorus uptake by the plankton was measured according to the method of Faust and Correll (1976), in three 250 ml bottles: light, dark, and one con-

taining the biological inhibitor, iodoacetic acid. In each experiment, 2 µCi of carrier-free ^{32}P as orthophosphate was added at time zero. The bottles were submerged at 1 m depth and, at timed intervals, the bottles were retrieved and a subsample was removed. Particulates in this subsample were then separated according to size with a 5 µm pore-sized Nitex screen and a 0.45 µm Millipore membrane. The filters and 1 ml of filtrate which had passed the 0.45 µm membrane were placed in scintillation vials for counting.

Autoradiography

Fixed plankton samples were centrifuged in the laboratory for 30 min at 5000 x *g* at 5°C. The pellet was washed three times with 15 ml of 0.002 N HCl at 3000 x *g* and resuspended in 1 ml of distilled water. In order to separate clumped cells, each cell suspension was sonicated for 10 sec (Model W 140 C, Heat System Co., at 60 W output) and filtered via gravity through 8 µm pore-size Nuclepore filters. The cells were rinsed off the filters and resuspended in 2.2 ml of distilled water.

Liquid-emulsion autoradiograms were prepared according to the method of Bogoroch (1972), somewhat modified as described by Correll *et al.* (1975). Each cell suspension was sonicated for 15 sec and 3 drops were spread on a gelatin-coated glass slide. Slides of the 8 µm filtrate were also made. The slides were air-dried for 24 to 48 h.

In a dark room, the photographic NTB-2 emulsion was diluted 1:1 with distilled water and brought to 44°C in a water bath. Two drops of emulsion were placed on each slide and then spread to an even thickness with a stainless steel raclette (Bogoroch, 1972). The slides were allowed to dry for 1 h at room temperature (ca. 23°C), and were then placed in black light-tight, desiccated slide boxes and incubated at 4°C for a 3-day exposure period. The slides were developed in Dektol at 15°C for 2 min, rinsed for 10 sec, and fixed in acid fixer for 5 to 10 min, then rinsed in running tap water for 30 min. The slides were cleared for 1 h in methyl salicylate, and coverslips were mounted with Permount.

Grain counts were made using a Zeiss microscope at 1000 X magnification under phase-contrast optics. The microscope was focused on the top of a cell and the grains in focus were counted. Then the microscope was focused down until the next set of grains was in focus and these were counted. This procedure was repeated until all grains above the cell were out of focus. Since ^{33}P is a moderately high energy emitter which is likely to produce grains at points which do not lie directly over the point of emission, some image-spreading due to "crossfire" is to be expected (Perry, 1964). This was taken into account very roughly by counting grains within 5 µm of the cell margins. Grain counts were made for at least 20 cells of each species on each autoradiogram, i.e., at each time interval of the experiment. Background grains were counted for areas which had no cells, until the standard deviation of grains per unit area was within ± 30% of the mean for each autoradiogram. For each time point, the number of background grains in the area occupied by each type of phytoplankton cell was calculated and subtracted from the mean number of grains per cell.

Analyses for dissolved phosphorus were performed on water samples which had been filtered through a 0.45 µm pore-sized Millipore filter. Inorganic phosphorus was determined colorimetrically on whole and filtered samples by reaction with ammonium molybdate and reduction with stannous chloride (American Public Health Association, 1971). Total phosphorus was analyzed by the same method after digestion with perchloric acid and neutralization (King, 1932). Methods for radioisotope counting were as described by Faust and Correll (1976). Physical parameters were monitored during the experiments with recording instruments at the Chesapeake Bay Center dock.

The population of algal species was estimated in a 1 l sample of water by the direct-count procedure of Campbell (1973). The fixed cells were concentrated to 1 ml by centrifugation at 5000 x *g* for 30 min, sonicated for 10 sec, and 0.03 ml was placed on a microscope slide. A 2.5 cm square coverslip was placed over the liquid and the number of cells of each species in a number of microscope fields was counted under 1000 X magnification. Except for the rarer species, 100 cells of each species were counted in order to estimate cell numbers within ± 20% with a confidence coefficient of 95% (Lund *et al.*, 1958). Volume and surface area of phytoplankton cells were calculated from length and width measurements using standard formulas for either a rod, a sphere, or oblate spheroid. Most of the dinoflagellates were assumed to be oblate spheroid, with an eccentricity of 0.9.

Calculations

The total rate of phosphate uptake by the plankton was calculated from the rate of ^{32}P disappearance from the filtrate. According to the equation for a first-order reaction, the rate constant, k, was obtained from tracer measurements:

$$\log c_0/c = \frac{-kt}{2.303},$$

where c is the dissolved ^{32}P radioactivity, c_0 is the activity at time zero, and t is time. The slope of a linear regression through the data plotted as $\log c$ versus t was used to obtain k. The steady state rate of disappearance of orthophosphate from solution was then obtained as follows:

$$\text{P disappearance rate } (\mu g\ P\ l^{-1}\ h^{-1}) = \frac{k\ c_0}{s},$$

where s is the specific activity of the phosphate ion (CPM $\mu g\ P^{-1}$) at $t = 0$.

Rates of phosphorus uptake by the two particulate sizes were calculated from the slopes of linear regression equations of the first portions of the two appearance curves as follows:

$$\text{P uptake rate } (\mu g\ P\ l^{-1}\ h^{-1}) = \frac{\Delta c/\Delta t}{s},$$

where $\Delta c/\Delta t$ is the slope representing the change in the ^{32}P activity on each of the filters with time.

Obtaining phosphorus uptake rates of individual phytoplankton species from autoradiography requires a known relationship between grain counts and a specific amount of radioactivity. No attempt was made to quantify the grain yield of ^{33}P on Kodak NTB-2 emulsion. Instead, a yield of 1.8 grains per emitted β particle was chosen from literature values for isotopes with similar energy levels and on films of similar sensitivity (Herz, 1959; Perry, 1964; Pelc, 1972; Prescott, 1964). Grain counts were first corrected for radioactive decay to the day of the experiment. The mean number of grains per cell was corrected for the loss of acid-soluble ^{33}P during acid washing. An acid-soluble fraction of 17% of total cell P, as found in *Chlorella pyrenoidosa* by Baker and Schmidt (1964), was used. The (corrected) mean number of grains per cell was then converted to counts per minute (cpm) per liter for each species as follows:

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

Specific activity of dissolved orthophosphate at each time point in the first 2 h was calculated from radioactivity in the filtrate of the ^{32}P uptake experiment and the dissolved orthophosphate concentration. The specific activity of $^{33}PO_4$ during the time course was calculated from specific activity of $^{32}PO_4$ using a ratio of the counts at the beginning of the ^{33}P and ^{32}P experiments. (The dissolved orthophosphate concentration remained constant.)

The rate of P uptake during each time interval was then calculated for each algal species as follows:

$$\frac{\frac{\Delta\ \text{cpm}}{1\Delta t}}{\text{cpm}/\mu g\ \text{P}} = \mu g\ \text{P}/1\Delta t \text{ or } \mu g\ \text{P}\ l^{-1}\ \text{min}^{-1}.$$

The P uptake rates were then averaged over all time intervals for which there was a slope, and a mean rate of phosphorus uptake for each species of phytoplankton was obtained.

Results

Values for physical and chemical parameters during the experiments are presented in Table 1. The plankton labelling experiments were carried out from approximately 10.00 to 16.00 hrs, and the autoradiography experiment was from 09.30 to 15.30 hrs. The largest changes in solar radiation and temperature took place in the later stages of the experiments. The soluble reactive phosphorus concentration was low at 4 $\mu g\ l^{-1}$, and approximately 50% of the total dissolved P was organic. Most phosphorus fractions remained constant throughout the experiments, except for an increase in total reactive phosphorus. Turbidity remained at 18 Jackson units throughout the day.

^{32}P Tracer Experiments

^{32}P uptake was estimated by measuring (1) the rate of ^{32}P disappearance from the dissolved orthophosphate pool in the filtrate, and (2) the rate of ^{32}P appearance in the particulate fraction. The results of these experiments are shown in Figs. 1 and 2. Very little uptake occurred in the bottle containing iodoacetic acid (IAA), where biological uptake of phosphorus was inhibited. Checks of total radioactivity at the beginning and at the end of each bottle experiment confirmed that no ^{32}P was lost to the sides of the bottle or by leakage. $^{32}PO_4$ disappeared rapidly from the water in the dark bottle. Since this uptake was low with IAA present, it was assumed to be due to biological activity. The light bottle was lost during the experiment. However, the uptake in light and dark bottles has rarely differed significantly in experiments of this kind performed

Table 1. Physical and chemical parameters during ^{32}P plankton labelling and ^{33}P autoradiography experiments, October 31, 1974

Time (hrs)	Solar radiation (g-cal cm^{-2} min^{-1})	Water temperature (°C)	Reactive phosphorus (μg P l^{-1})		Total phosphorus (μg P l^{-1})	
			Total	Dissolved[a]	Total	Dissolved
8.00	0.24	15.2				
9.00	0.50	15.2	6	4	49	10
10.00	0.70	15.3				
11.00	0.78	15.5				
12.00	0.80	15.7				
13.00	0.76	16.0				
14.00	0.64	16.4				
15.00	0.40	17.0	16	4	45	7
16.00	0.20	17.2				

[a] Although much has been written about other phosphorus compounds which might react with molybdate if they are present in natural waters, we assume for our purposes that dissolved reactive phosphorus and dissolved orthophosphate are synonymous.

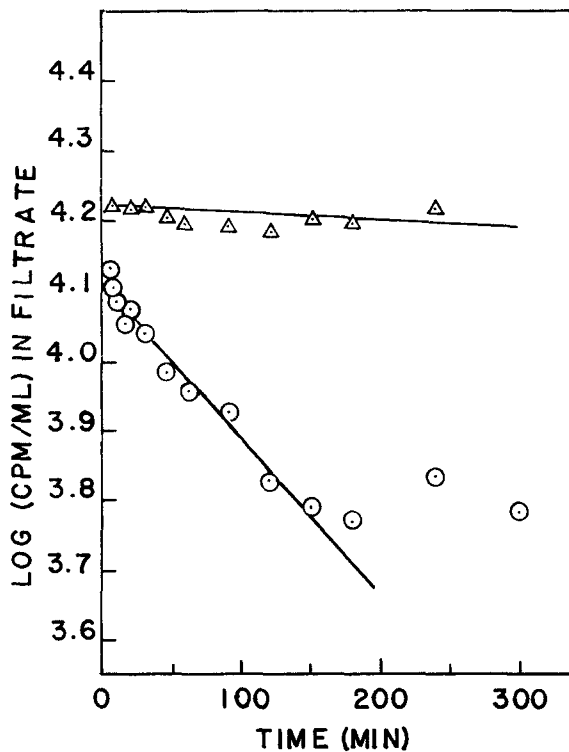

Fig. 1. Disappearance of ^{32}P from the filtrate in dark bottle (circles) and bottle containing the metabolic inhibitor iodoacetic acid, IAA (triangles). The bottles were incubated in the Rhode River at 1 m depth for 4 h

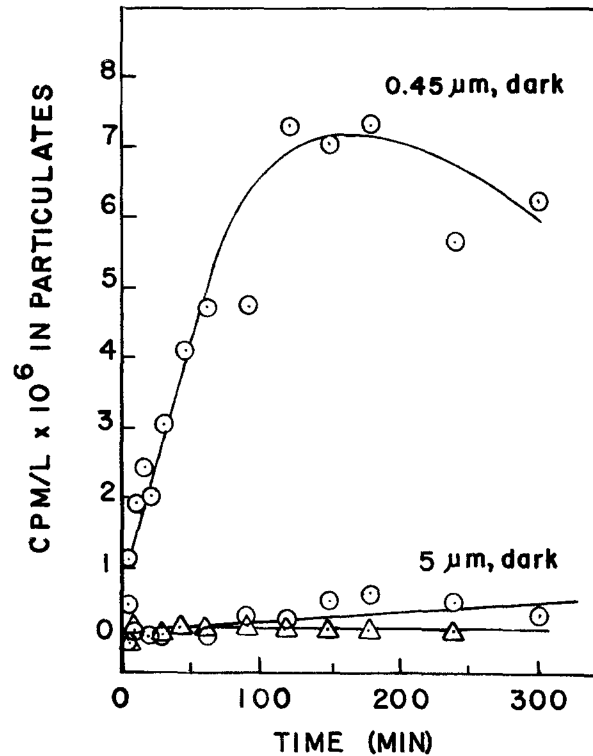

Fig. 2. Appearance of ^{32}P in particulates. Circles: ^{32}P radioactivity on 5 and 0.45 µm pore-sized filters in dark bottle; triangles: ^{32}P radioactivity on 0.45 µm pore-sized filter in IAA bottle

in the Rhode River (Correll *et al.*, 1975; Faust and Correll, 1976).

The appearance of ^{32}P in size fractions of particulates is shown in Fig. 2. In the IAA bottle, there was no P uptake by either size fraction. The majority of dark-bottle uptake was by organisms passing the 5 µm screen and retained on the 0.45 µm membrane.

Disappearance of ^{32}P from the water appeared to follow a pseudo first-order reaction. In Fig. 1, it can be seen that

Table 2. Estimates of population numbers and biomass of phytoplankton species from Rhode River station at 1 m depth on October 31, 1974

Species	Size (μm)	Volume (μm^3)	Cells l^{-1} (x 10^3)	Biomass (mm^3 l^{-1})	% total biomass
Peridineum trochoideum	25 x 28	10300	4.9	0.051	2.5
Gymnodinium sp. 2	25 x 18	5890	140.0	0.825	41.8
Gymnodinium gracilentum	20 x 10	2090	72.3	0.151	7.6
Gymnodinium sp. 1	15 x 11	1300	68.3	0.089	4.5
Gyrodinium mundulum	15 x 10	1180	22.3	0.026	1.3
Gymnodinium galesianum	15 x 15	1170[a]	42.7	0.050	2.5
Gymnodinium subroseum	11 x 8	546	33.4	0.018	0.9
Flagellate	10 x 10	523	50.0	0.050	2.5
Euglena ascus	20 x 5	392	117	0.046	2.3
Katodinium rotundatum	10-15 x 3-10	190[a]	1,300	0.247	12.5
Nannoplankton	<5	34	12,400	0.422	21.4
Total			14,300	1.98	

[a]Values from Campbell (1973).

the log ^{32}P in solution decreases linearly for approximately the first 2 h. It then levels off to an equilibrium value when ^{32}P release into the water by planktonic organisms has begun.

As in any first-order reaction, the appearance of the "product" (i.e., ^{32}P in particulates) is a curved line. Although the rate of phosphate uptake is constant, the rate of appearance of ^{32}P in particulates decreases as the specific activity of orthophosphate decreases. The first portion of the curve is fitted to a straight line.

The total P uptake rate calculated from the disappearance of $^{32}PO_4$ from solution is 1.07 μg P l^{-1} h^{-1}. Uptake rates calculated from the rate of ^{32}P appearance in particulates on the 5 and 0.45 μm filters are 2% (0.024 μg P l^{-1} h^{-1}) and 98% (1.06 μg P l^{-1} h^{-1}), respectively, of the total P uptake rate.

Phytoplankton Population

The phytoplankton in the Rhode River on the day of the experiments was not as abundant as during the warmer months of the year, but the species composition was quite diverse. Ten major species of algae were observed, of which most were dinoflagellates. Phytoplankton population numbers and biomass are given in Table 2. Only one species, *Katodinium rotundatum,* numbered over one million cells per liter. The nannoplankton, which was composed of many small flagellates, chrysophytes, and green algae, was most abundant. Small diatoms were quite numerous, but most of the cells appeared to be senescent. It can be seen from Table 2 that *Gymnodinium* species 2, *K. rotundatum,* and the nannoplankton made up the largest portions of the algal biomass: 41.8, 21.4, and 12.5%, respectively.

^{33}P Autoradiography

Fig. 3 shows the appearance of ^{33}P in fixed, acid-washed cells (concentrated from 1 l of water by centrifugation and later used for autoradiography) with time. The rate of phosphorus incorporation into acid-insoluble compounds inside plankton cells (including bacteria) was calculated from Fig. 3 as 0.768 μg P l^{-1} h^{-1}, which is approximately 75% of the total uptake rate obtained in the ^{32}P uptake experiment.

We observed that autoradiograms of the Rhode River samples contained a greater number of background grains than

was found in plankton preparations by Brock and Brock (1968). This problem was attributed to the large number of bacteria present in estuarine waters. Autoradiograms of plankton at the first time-points were relatively free of background counts, but the number of background grains increased steadily with time during the first 2 h; the number then remained constant during the remainder of the experiment. Therefore, it was necessary to subtract background grains at each time-point as described in "Materials and Methods".

Fig. 4 illustrates the increase in the mean number of grains per cell with time for one dinoflagellate species. Generally, the standard deviation of grain counts for a species at one time point was within ± 30% of the mean. The increase in grains was rapid at first and then leveled off when the specific activity of the orthophosphate pool in the water had decreased. Similar curves were obtained for all 10 species of phytoplankton. All curves had shapes similar to the one shown, with the absolute number of grains and the slope differing for each species.

Rates of phosphorus uptake for all phytoplankton species were computed, as described in the "Materials and Methods" section, from the increase in grain counts per cell over time. These uptake rates are presented in Table 3, with several parameters of cell dimensions. Uptake rate per cell is largest for large cells; however, it is obvious from the data in the fifth column of Table 3 that the phosphorus uptake rate per μm^3 cell volume is highest for smaller cells. The relationship between size and uptake rate is illustrated more clearly in Fig. 5. A plot of log P uptake per cell mass against log surface:volume ratio for the various phytoplankton species is linear. The line represents the best fit (r^2 = 0.93) of the linear regression equation, $\log Y = (-10.7) + (1.7 \log X)$, where Y is uptake in µg P per μm^3 cell volume per hour for a species and X is the surface:volume ratio. Taking the antilogarithm, the equation becomes $Y = (2 \times 10^{-11})\ X^{1.7}$, and we can see that P uptake of these algal cells is a power function of their surface:volume ratio.

To test whether the observed relationship between uptake and surface:volume ratio is true for other Rhode River phytoplankton populations rather than just for the one, we analyzed the ^{33}P autoradiography data of July 9, 1973 from Correll *et al.* (1975) in the same way. Their data shows a similar correlation between grain density per μm^3 cell volume per hour and surface:volume ratio

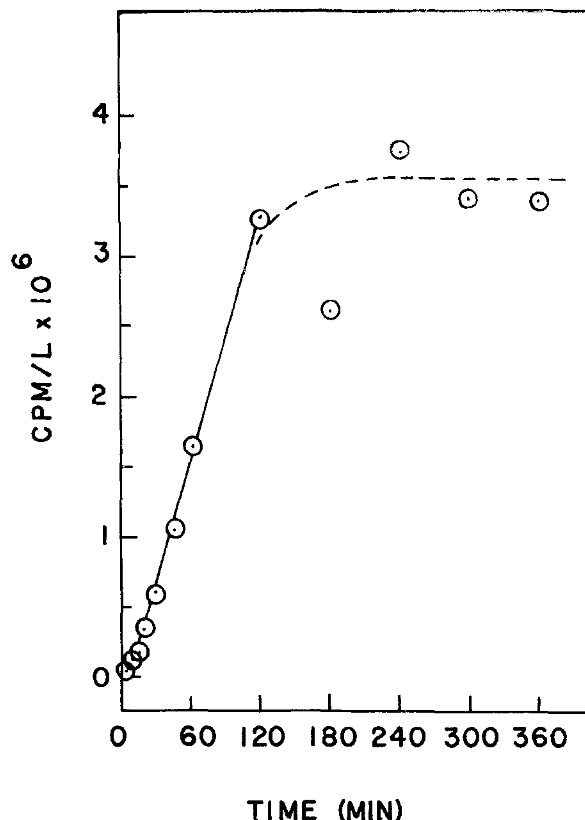

Fig. 3. Autoradiography experiment. Appearance of ^{33}P in acid-washed plankton cells and particulates with time. Rhode River water was incubated with ^{33}P in 12 l bottle in the light at 1 m depth. Each 1 l subsample was fixed, centrifuged, and washed with dilute HCl. Solid line represents a least-squares regression equation giving best fit of first 7 data points; broken line is arbitrarily drawn

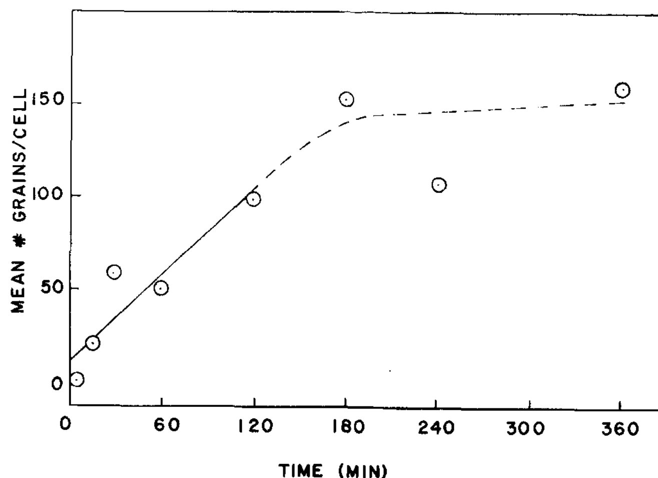

Fig. 4. *Gymnodinium* species 1. Mean number of grains per cell with time. Whole water samples containing this species were incubated with $^{33}PO_4$ in the Rhode River at 1 m depth for 6 h. Solid line gives best-fit linear regression of first 5 data points; broken line is arbitrarily drawn

Table 3. Surface areas, surface:volume ratios, and phosphorus uptake rates for Rhode River phytoplankton species, October 31, 1974. Uptake rates were computed from grain counts of autoradiograms. For phytoplankton cell counts and biomass estimates, see Table 2

Species	Surface area (μm^2 $cell^{-1}$)	Surface: volume ratio	Uptake rate (μg P $cell^{-1}$ h^{-1} x 10^{-9})	Uptake rate (μg P μm^{-3} h^{-1} x 10^{-12})	Uptake rate (μg P l^{-1} h^{-1} x 10^{-3})	% Total phytoplankton uptake rate per liter
Peridineum trochoideum	1927	0.188	11.84	1.15	0.058	0.15
Gymnodinium sp. 2	981	0.167	7.74	1.31	1.084	2.84
Gymnodinium gracilentum	739	0.353	8.52	4.07	0.616	1.62
Gymnodinium sp. 1	490	0.378	3.63	2.80	0.248	0.65
Gyrodinium mundulum	464	0.394	2.60	2.21	0.058	0.15
Gymnodinium galesianum	707	0.604	6.28	5.37	0.268	0.69
Gymnodinium subroseum	261	0.512	4.43	8.76	0.148	0.38
Flagellate	314	0.600	3.48	6.65	0.174	0.45
Euglena ascus	314	0.801	5.92	15.1	0.692	1.81
Katodinium rotundatum	266	1.400	5.02	26.4	6.539	17.14
Nannoplankton	50	1.480	2.30	67.7	28.52	74.75
Total uptake rate					38.41 = 0.038 μg P l^{-1} h^{-1}	

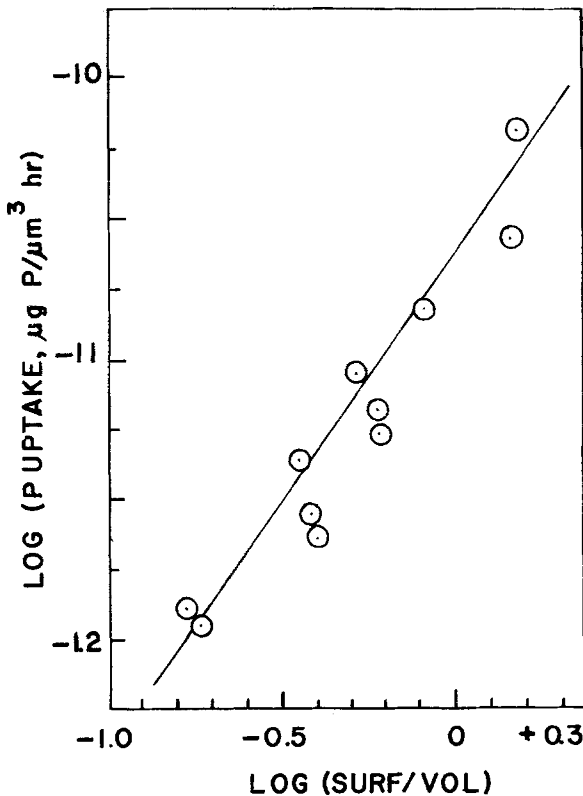

Fig. 5. Relationship between phosphorus uptake per μm^3 cell volume per hour for various phytoplankton species and their surface:volume ratios. Line drawn is linear regression of data: log Y = (-10.7) + 1.7 log X (r^2 = 0.93)

(uptake rates were not available). The regression equation is $Y = 0.17\ X^{2.4}$ ($r^2 = 0.99$). The conversion from grains to uptake in μg P involves several constants which account for the difference between the coefficients of X in the equations for the 1973 and 1974 data. However, the similarity between exponents of X (1.7 for our data, and 2.4 for the 1973 data) is interesting.

The data in the sixth and seventh columns in Table 3 shows that nannoplankton were responsible for the largest part of the phosphorus uptake by the phytoplankton in the Rhode River at this time of year. Other species which showed significant P uptake are *Katodinium rotundatum, Gymnodinium gracilentum, Gymnodinium* species 2, and *Euglena ascus*. These species, which comprise the largest portions of the phytoplankton biomass because of either large numbers of cells or large cell size, are also responsible for the greatest part of the total phosphorus uptake per liter. However, no clear-cut relationship between the total biomass and the rate of phosphate uptake by each species is indicated.

With the correlation in Fig. 5 in mind, an examination of the relationship between total cell surface area for a species and its P uptake per liter is more enlightening. Nannoplankton and *Katodinium rotundatum* had the highest surface: volume ratios (1.48 and 1.4) and highest

cell numbers; these organisms were responsible for 75 and 17% respectively, of the total phytoplankton P uptake rate. In contrast, species with low surface: volume ratios, such as *Gyrodinium mundulum* (0.394) and *Peridineum trochoideum* (0.188) and relatively low cell numbers, had P uptake rates which were 0.15% of the total. Thus, we found that in the water column, the P uptake rate by a species is correlated with its total surface area. The equation $Y = (-1.4 \times 10^{-3}) + (4.15 \times 10^{-5} X)$, where X = surface area in $mm^{-2}\ l^{-1}$ for a species and Y = P uptake in $\mu g\ P\ l^{-1}\ h^{-1}$ for that species, is the linear regression of the data, with $r^2 = 0.90$.

The sum of individual phytoplankton P uptake rates as given in Table 3 is 0.038 $\mu g\ P\ l^{-1}\ h^{-1}$. The rates were calculated assuming that acid-soluble P was 17% of total cell P. If the acid-soluble P fraction were assumed to be 50% as it is in some diatoms, the total uptake rate would be 0.063 $\mu g\ P\ l^{-1}\ h^{-1}$. The sum of the rates of individual species obtained from autoradiography is in very good agreement with the rate of 0.024 $\mu g\ P\ l^{-1}\ h^{-1}$ for plankton cells "larger than 5 µm" obtained in the ^{32}P uptake experiments.

Discussion and Conclusions

The experiments presented here were designed to measure the phosphorus uptake of a natural estuarine phytoplankton community under natural conditions. Environmental variables in these experiments such as solar radiation, water temperature, salinity, and nutrient levels, were those occurring naturally in the Rhode River. Therefore, the nutrient uptake rates observed in this study are likely to be those which occur when a diverse group of algae is present and excess nutrients are not available.

The rate of transfer of phosphorus from the dissolved orthophosphate pool into plankton organisms was measured by (1) tracing the movement of $^{32}PO_4$ into size fractions of particulates, and (2) by following the movement of $^{33}PO_4$ into individual species of phytoplankton. Using the first method, the total phosphorus uptake rate of the plankton was determined to be approximately 1 $\mu g\ P\ l^{-1}\ h^{-1}$. A control experiment with biological inhibitor showed very little adsorption of the radioactive tracer to non-living particles. Plankton cells retained on the 5 µm screen were responsible for about 2% of the uptake (0.024 $\mu g\ P\ l^{-1}\ h^{-1}$), while those passing the 5 µm screen displayed 98% of the uptake. Size fractionation by this method is not perfect by any means, however. Sheldon and Sutcliffe (1969) have studied the accuracy of size separation of plastic particles with different types and pore sizes of filters. Their results show that many particles with a diameter less than the stated pore size of the filter are retained due to clogging of pores and obstruction of particle path through the filters. The type of filter used, as well as its pore size, determined the quality of separation obtained; screens rather than membrane filters gave the best separation. In our experiment, particles smaller than 5 µm (including the nannoplankton) were probably retained by the 5 µm screen. Bacteria are notorious for attaching to surfaces, including suspended sediments and other plankton cells. This phenomenon might lead to overestimation of phosphate uptake by plankton "larger than 5 µm" in size.

Anderson (1965) reported experiments with size fractionation of plankton off the Oregon Coast. He found that large fractions of phytoplankton passed through a 5 µm filter, while very little passed through a 0.8 µm filter. Berman (1975), working in the Gulf of California, found 13 to 83% of total carbon fixation of natural phytoplankton populations was associated with organisms passing a 3 µm Nuclepore filter. The results of these size fractionation experiments depend, of course, upon the size distribution of natural phytoplankton populations. While these two studies were carried out in open marine waters, size fractionation experiments with plankton from a shallow east-coast estuary showed most algal cells to be retained by a 5 µm Nitex screen (Faust and Correll, 1976). Also, autoradiograms of the filtrate of our cell suspensions filtered via gravity through an 8 µm Nuclepore filter contained few phytoplankton cells. Although microscopic examination of the filters was not made in this study, it is assumed that the majority of phytoplankton was retained by the 5 µm screen as shown by previous studies in the same estuary (Faust and Correll, 1976).

Although there are no results from the light bottle, we have found that total P uptake and uptake by different particulate sizes generally do not vary significantly between light and dark bottles in ^{32}P tracer experiments on the Rhode River (Correll *et al.*, 1975; Faust and Correll, 1976). Taft *et al.* (1975) also obtained identical results with light and dark bottles in ^{32}P tracer experiments with plankton of Chesapeake Bay. Thus, it appears that the observed P uptake does not require light and is

not directly driven by photosynthesis; this uptake also is not inhibited noticeably by light.

The majority of uptake is by small organisms which include few algae; light or the absence of light has little effect upon their P uptake. Therefore, most of the phosphate uptake must be attributed to bacteria. Many laboratory studies have established the rapid uptake of inorganic phosphorus by bacteria (Rigler, 1956; Harris, 1957; Johannes, 1964; Rhee, 1972; Beuchler and Dillon, 1974). We have less knowledge of this process occurring in nature. Rigler's experiments (1956) with lake plankton indicated a high phosphate uptake by bacteria. Taft (1974) and Taft *et al.* (1975) argue that bacteria are not important consumers of inorganic phosphate in estuarine waters. They note that particulates absorbing phosphate in their studies had N and P to chlorophyll *a* ratios which are close to those of most algal cells. In contrast, Faust and Correll (1976) have shown a high correlation between phosphate uptake rates by particulates smaller than 5 µm and bacterial biomass in estuarine waters at various times of the year.

Only 2% of the phosphate uptake was by organisms retained on the 5 µm screen, presumably algae. Although the algal absorption rate seems a remarkably small part of the total uptake, it is confirmed by the sum of individual species rates obtained from autoradiography. The phytoplankton rate from the filtration technique is 0.024 µg P l^{-1} h^{-1}, as compared with 0.038 to 0.063 µg P l^{-1} h^{-1} from autoradiography, depending upon the value used for the acid-soluble P content of the phytoplankton cells.

The technique of autoradiography was used to compare the phosphorus uptake rates of individual species of phytoplankton. Knoechel and Kalff (1976) have critically reviewed the use of grain-density autoradiography for comparison of metabolic activity in plankton species. They submit that errors result from several sources: (1) inadequate cell preparation; (2) failure to consider the effect of cell size and geometry on grain density; (3) inadequate methods of determining grain yield.

In this study, the method of cell preparation was not detrimental to the cells, as no acetone, harsh fixatives, heat, or high centrifuge speeds were used. Fixation was complete: Protargol staining of the gluteraldehyde-fixed cells several months later showed excellent fixation and no deterioration of cell organelles.

The second consideration was the effect of cell size and shape upon grain density. For a point source of radioactivity located at the center of a disc, the number of grains resulting from the emitted β particle which are counted over a large disc will be higher than those counted over a small disc. However, this effect is greatly reduced by counting grains at the margins and several micrometers beyond. The data presented by Knoechel and Kalff (1976) shows that the grain counting efficiency over a cell 10 µm in diameter, when counting is extended 5 µm beyond cell margins, is 75%. Thus, since grain counting was extended that far in this study, the efficiency was reduced very little. Also, since our cells were within a narrow size range, any reduction in efficiency would be nearly constant for all the cells. The authors also note that self-absorption of radiation by the cells may cause error. However, self-absorption is greater in large cells; since most of our cells were less than 20 µm in diameter, this effect would reduce actual grain counts by 15% at most, according to estimates by Knoechel and Kalff (1976). For the smallest cells, self-absorption would result in a reduction in grain counts of only a few percent.

Finally, error is introduced in estimating the grain yield of a radioactive isotope on a particular emulsion or film. Ideally, an internal standardization between grains and disintegration should be made using cells with a known radioactivity. As stated before, no attempt at this was made in this study. However, the comparison between algal species is not affected by any error in the grain yield, as it is merely a constant used in calculation of all uptake rates. The sum of individual phosphate uptake rates, i.e., the total phytoplankton uptake rate, might reflect an error in the grain yield, but the grain yield used (1.8) would have to be underestimated by an order of magnitude before a significant difference in the total phytoplankton uptake rate would be observed. The sources of error which Knoechel and Kalff list are significant only if autoradiography is used for studying large phytoplankton cells with a high energy isotope, or if one is comparing the results of several different autoradiography experiments in which there are differences in total radioactivity.

As noted in the results, the autoradiograms contained a number of background grains which increased with time. Autoradiograms of the filtrate obtained by filtering ^{33}P-labelled cell suspensions through an 8 µm pore-size filter

also contained grains without any visible cells under them, and these grains also increased with time. A reasonable source for these grains is bacteria, which are not resolved in autoradiograms at 1000 X magnification without staining. Certainly, adsorption of ^{33}P to sediments or incompleted washing of soluble ^{33}P is ruled out because the number of background grains increased with time. Fuhs and Canelli (1970) observed similar clusters of background grains in ^{33}P autoradiograms. They suspected that clumps of bacteria were the cause, but they could not prove that bacterial cells were present. Faust and Correll (1977) were able to observe grains over bacterial cells in ^{33}P autoradiograms by staining their cell preparations. As explained in "Materials and Methods", a correction for background counts was made for each autoradiogram. However, no correction for the adhering and clumping of bacteria on phytoplankton cells could be made. This could result in overestimation of phosphorus uptake by plankton cells.

Results from autoradiography show that uptake of phosphorus by the nannoplankton (algal cells less than 5 µm in size) accounts for almost 75% of the total uptake by phytoplankton. The importance of nannoplankton in the total primary production of many planktonic communities has been discussed in the "Introduction". Taft (1974) showed that there was no difference in inorganic phosphate uptake rates of plankton passing a 35 µm and a 10 µm filter. He also concluded that smaller plankton cells (which could include bacteria) were responsible for the majority of the phosphorus uptake in Chesapeake Bay.

The fraction of the total rate of uptake by the nannoplankton exceeds their contribution to the total phytoplankton biomass. Watt (1971) found this to be true for nannoplankton productivity in ^{14}C autoradiography experiments. Earlier ^{33}P autoradiography of Rhode River plankton was performed during a bloom of large dinoflagellates (Correll *et al.*, 1975). Although the dinoflagellates made up 90% of the phytoplankton biomass, the rate of phosphorus uptake per µm^3 of cell volume by the nannoplankton was 300 to 500 times the rate of the dominant larger phytoplankton cells. Thus, the total phosphate uptake rate of the nannoplankton was still higher than that of the numerous large dinoflagellates.

Fuhs *et al.* (1972) observed the effect of cell surface:volume ratio on nutrient uptake and maximum growth rate. They found that maximum growth rates of two diatom species occurred at the same phosphate concentration because the lower affinity for phosphate (larger K_s) of the smaller species was offset by its greater surface:volume ratio. Our results of the autoradiography experiment indicate that the algal phosphorus uptake rate per biomass unit is roughly a function of the square of cell surface:volume ratio:

$$U/V \propto K'(S/V)^2.$$

We may ask why this should be so. Is there a simple relationship between cell dimension or geometry and nutrient uptake rate underlying these results?

Let us assume that the P uptake rate per cell is linear with cell radius for spherical cells. (There was no indication from our data that uptake rate was a linear function of cell surface area.)

$$U = Kr$$

then for a sphere,

$$U/V = \frac{Kr}{4/3\pi r^3}$$

and,

$$S/V = \frac{4\pi r^2}{4/3\pi r^3} = 3/r$$

$$r = \frac{3}{(S/V)},$$

therefore,

$$U/V = \frac{K}{4/3\pi\left[\frac{3}{(S/V)}\right]^2} = K'(S/V)^2$$

Taking an average radius for each cell in this study, we examined the assumption of linearity between radius and P uptake per cell. Linear regression of the data gave $U = -0.7 + 0.8\ r$ with $r^2 = 0.76$. The scatter in the data is probably due to the fact that most of these cells are not true spheres. The relationship between axial dimension and volume is, of course, more complex for dinoflagellates than for perfect spheres.

The model of Munk and Riley (1952) predicts that the time required for an algal cell to absorb as many grams of nutrient as are already contained increases with increasing size. According to their calculations, nutrient absorption rate per unit mass is doubled to quadrupled when cell dimension is halved, depending upon cell shape. This is roughly confirmed for spheres by our results. If $U/r \propto K$, and $r/V = \frac{1}{4/3\pi r^2}$, then $U/V \propto \frac{K}{4/3\pi r^2} = \frac{K'}{r^2}$, and it is obvious that when cell dimension is halved, nutrient uptake per cell mass is quadrupled.

The high rate of phosphate uptake by bacterial organisms would be expected from the observed relationship between uptake and surface:volume ratio. Fuhs *et al.* (1972) showed that bacteria having

lower affinity for phosphate than algae (higher K_s and lower V_{max} per unit surface area) were able to outgrow the algae because of their favorable surface: volume ratio. However, Lewis (1976) reports that bacterial $S:V$ ratios can be equaled or exceeded by algae. Unfortunately, no data on bacterial numbers and surface:volume ratios were obtained in our experiments, and the correlation found for algae could not be tested further.

With these results in mind, the "paradox of the plankton" remains. Why, if nannoplankton have such a distinct advantage over large cells in absorbing nutrients, do larger phytoplankton cells exist? One answer is that in some environments where all nutritional resources are plentiful, algal cells may not be competing for nutrients, but this condition is not one that occurs constantly in many environments. Second, if phytoplankton population numbers are low, competition for nutrients may not occur. Hurlburt (1970) followed fluctuations in abundance of marine phytoplankton species and calculated that the maximum nutrient-depleted zones around algal cells could not overlap at the cell densities found in the open oceans; however, overlap, and thus competition for nutrients, occurred at cell densities above 3×10^8 cells l^{-1}, which are sometimes found in estuaries and coastal waters. He found that estuarine phytoplankton forms were significantly smaller, with less diversity and greater numbers.

Another logical answer to the question posed is that other factors as well as nutrient absorption rate act as selective forces on the shapes and sizes of phytoplankton cells. Lewis (1976) showed that there is conservation of $S:V$ ratios for the greatest axial dimension of phytoplankton cells. Certainly a lower limit of the $S:V$ ratio is understandable, but obviously some unknown selective pressures are producing an upper limit on the $S:V$ ratio.

Semina (1972) describes some interesting mutual effects of physical factors on phytoplankton cell size in the oceans. In general, she found increasing cell size with increasing velocity of ascending water, and this effect was amplified by increasing density gradient in the pycnocline and increasing phosphate concentration. Malone (1971b) suggests that increases in netplankton standing crop in areas of upwelling could result from the vertical water movement as well as from increases in nitrate concentration. Thus, certain physical factors can select large cells having low $S:V$ ratios.

Parson's and Takahashi's model (1973) predicts that small cells will be dominant under most light and nutrient regimes. However, the kinetic constants used in their model were obtained with cultures in the laboratory, and are dependent upon many factors, including environmental history and physiological state of the cells. According to observations of Carpenter and Guillard (1971), algal cells are apparently able to adapt nutrient uptake rates to a general range of nutrient conditions encountered in their environment.

Existing data on the relationship between phytoplankton cell size and Michaelis-Menten kinetic constants is confusing. Eppley *et al.* (1969) note a trend of decreasing K_s for nitrogen compounds with decreasing cell size. Malone's (1971b) observation that nannoplankton production increases rapidly at very low nitrate concentrations, while netplankton productivity increases more rapidly at nitrate levels greater than 1.5 µm, lends support to this hypothesis. In contrast, Fuhs *et al.* (1972) found that the larger of two diatom species had a lower K_s for phosphate than the smaller. The response of natural phytoplankton populations to nutrient levels also appears to be ambiguous. MacIsaac and Dugdale (1969) showed that the uptake of nitrogen compounds by natural phytoplankton populations is dependent upon nutrient concentration at the lower concentrations, but that maximum uptake velocity is repressed at higher concentrations by other limiting factors. Taft *et al.* (1975) observed typical Michaelis-Menten kinetics of phosphate uptake by natural phytoplankton of Chesapeake Bay. However, in their long-term study, rate constants for phosphate uptake by natural phytoplankton populations fluctuated randomly throughout the year, regardless of phosphate concentration; the only pattern observed was an inverse relationship between soluble reactive phosphorus concentration and phosphorus uptake rate constants in the winter months.

The relationship between K_s for dissolved nutrients and cell size is thus complex and at this point unclear. Our results do not resolve this problem, but they do show that small cells under natural conditions and at low nutrient levels do have much faster P uptake rates per cell mass than larger cells. It would be interesting to use autoradiography to observe the nutrient uptake of individual species at different (naturally occurring) nutrient levels; however, we would expect the relationship between uptake rate and nutrient concentration to be complicated by other factors, such

as physiological state of the phytoplankton and limiting factors repressing maximum nutrient uptake.

Finally, Gavis (1976) recast the Munk and Riley model in the light of recent knowledge of algal nutrient uptake kinetics and the effect of nutrient diffusion-limited transport (Pasciak and Gavis, 1974). In his mathematical model, the dimensionless parameter P (which includes V_{max} and K_s) increases with decreasing cell size; as a result, the influence of diffusion transport limitation is diminished. He suggests that this is why oceanic regions with low nutrient levels contain cells which are small in size and have low K_s values.

Although the euphotic zone is a somewhat homogenous environment, we can see from the literature that regional differences in light, density gradients, vertical currents, and nutrient levels do exist. However, each local environment usually supports a diverse group of phytoplankton cell shapes and sizes. From our results, the relative nutrient absorption rates of the cells must convey a competitive advantage on smaller cells, but other selective forces must also be at work to balance this metabolic advantage of small cells. Certainly zooplankton grazing, which was not discussed, must play a large role. More research with natural phytoplankton populations in completely natural conditions is needed in order to determine the factors most strongly influencing the types of phytoplankton which successfully coexist.

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Literature Cited

American Public Health Association: Standard methods for the examination of water and waste water, 874 pp. 13th ed. New York: American Public Health Association 1971

Anderson, G.C.: Fractionation of phytoplankton communities off the Washington and Oregon coasts. Limnol. Oceanogr. *10*, 474-480 (1965)

Baker, A.L. and R.R. Schmidt: Further studies on the intracellular distribution of phosphorus during synchronous growth of *Chlorella pyrenoidosa*. Biochim. biophys. Acta *82*, 336-342 (1964)

Berman, T.: Size fractionation of natural aquatic populations associated with autotrophic and heterotrophic carbon uptake. Mar. Biol. *33*, 215-220 (1975)

Beuchler, D.G. and R.D. Dillon: Phosphorus regeneration in fresh water paramecia. J. Protozool. *21*, 339-343 (1974)

Bogoroch, R.: Liquid emulsion autoradiography. *In:* Autoradiography for biologists, pp 65-94. Ed. by P.B. Gahan. New York: Academic Press 1972

Brock, M.L. and T.D. Brock: The application of micro-autoradiographic techniques to ecological studies. Mitt. int. Verein. theor. angew. Limnol. *15*, 1-29 (1968)

Campbell, P.H.: Studies on brackish water phytoplankton, 403 pp. Sea Grant Publication UNC-SG 73-07. Chapel Hill, North Carolina: University of North Carolina 1973

Carpenter, E.J. and R.R.L. Guillard: Intraspecific differences in nitrate half-saturation constants for three species of marine phytoplankton. Ecology *52*, 183-185 (1971)

Correll, D.L., M.A. Faust and D.J. Severn: Phosphorus flux and cycling in estuaries. *In:* Estuarine research: chemistry, biology, and the estuarine system, Vol. I. pp 108-136. Ed. by L.E. Cronin. New York: Academic Press, Inc. 1975

Dugdale, R.C.: Nutrient limitation in the sea: dynamics, identification, and significance. Limnol. Oceanogr. *12*, 685-694 (1967)

Eppley, R.W., J.N. Rogers, and J.J. McCarthy: Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. *14*, 912-920 (1969)

Faust, M.A. and D.L. Correll: Comparison of bacterial and algal utilization of orthophosphate in an estuarine environment. Mar. Biol. *34*, 151-162 (1976)

- - Autoradiographic study to detect metabolically active phytoplankton and bacteria in the Rhode River Estuary. Mar. Biol. *41*, 293-305 (1977)

Fogg, G.E.: Algal cultures and phytoplankton ecology, 175 pp. London: Athlone Press 1965

Fuhs, G.W. and E. Canelli: Phosphorus-33 autoradiography used to measure phosphate uptake by individual algae. Limnol. Oceanogr. *15*, 962-966 (1970)

-, S.D. Demmerle, E. Canelli and M. Chen: Characterization of phosphorus-limited plankton algae. *In:* Nutrients and eutrophication: the limiting nutrient controversy. Special Symposium of the American Society of Limnology and Oceanography, Vol. 1. pp 113-133. Ed. by G.E. Likens. Lawrence, Kans.: Allen Press, Inc. 1972

Gavis, J.: Munk and Riley revisited: nutrient diffusion transport and rates of phytoplankton growth. J. mar. Res. *34*, 161-179 (1976)

Harris, E.: Radiophosphorus metabolism in zooplankton and in microorganisms. Can. J. Zool. *35*, 769-782 (1957)

Herz, R.H.: Methods to improve the performance of stripping emulsions. Lab. Invest. *8*, 71-81 (1959)

Hurlburt, E.M.: Competition for nutrients by marine phytoplankton in oceanic, coastal, and estuarine regions. Ecology *51*, 475-484 (1970)

Hutchinson, G.E.: The paradox of the plankton. Am. Nat. *95*, 137-143 (1961)

Johannes, R.E.: Uptake and release of dissolved organic phosphorus by representatives of a coastal marine ecosystem. Limnol. Oceanogr. *9*, 224-232 (1964)

King, E.J.: The colorimetric determination of phosphorus. Biochem. J. *26*, 292-297 (1932)

Knoechel, R. and J. Kalff: The applicability of grain density autoradiography to the quantitative determination of algal species production: a critique. Limnol. Oceanogr. *21*, 583-590 (1976)

Lewis, W.M.: Surface/volume ratio: implications for phytoplankton morphology. Science, N.Y. *192*, 885-887 (1976)

Lund, J.W.G., C. Kipling and E.D. Le Cren: Inverted microscope method of estimating algal numbers and the statistical basis of estimation by counting. Hydrobiologia *11*, 143-170 (1958)

MacIsaac, J.J. and R.C. Dugdale: The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton. Deep-Sea Res. *16*, 45-57 (1969)

Malone, T.C.: The relative importance of nannoplankton and net plankton as primary producers in tropical oceanic and neritic phytoplankton communities. Limnol. Oceanogr. *16*, 633-639 (1971a)

- The relative importance of nannoplankton and netplankton as primary producers in the California Current System. Fish. Bull. U.S. *69*, 799-820 (1971b)

McCarthy, J.J., W. Rowland Taylor and M.E. Loftus: Significance of nanoplankton in the Chesapeake Bay Estuary and problems associated with the measurement of nanoplankton productivity. Mar. Biol. *24*, 7-16 (1974)

Munk, W.H. and G.A. Riley: Absorption of nutrients by aquatic plants. J. mar. Res. *11*, 215-240 (1952)

Parsons, T.R. and M. Takahashi: Environmental control of phytoplankton cell size. Limnol. Oceanogr. *18*, 511-515 (1973)

Pasciak, W.J. and J. Gavis: Transport limitation of nutrient uptake in phytoplankton. Limnol. Oceanogr. *19*, 881-888 (1974)

Pelc, S.R.: Theory of autoradiography. *In:* Autoradiography for biologists, pp 1-17. Ed. by P.B. Gahan. London: Academic Press 1972

Perry, R.P.: Quantitative autoradiography. *In:* Methods in cell physiology, Vol. 1. pp 305-326. Ed. by D.M. Prescott. New York: Academic Press 1964

Prescott, D.M.: Autoradiography with liquid emulsion. *In:* Methods in cell physiology, Vol. 1. pp 365-370. Ed. by D.M. Prescott. New York: Academic Press 1964

Rhee, G.-Y.: Competition between an alga and an aquatic bacterium for phosphate. Limnol. Oceanogr. *17*, 505-514 (1972)

Rigler, F.H.: A tracer study of the phosphorus cycle in lake water. Ecology *37*, 550-562 (1956)

Semina, H.J.: The size of phytoplankton cells in the Pacific Ocean. Int. Revue ges. Hydrobiol. *57*, 177-205 (1972)

Sheldon, R.W. and W.H. Sutcliffe: Retention of marine particles by screens and filters. Limnol. Oceanogr. *14*, 441-444 (1969)

Taft, J.L., III: Phosphorus cycling in the plankton of the Chesapeake Bay, 193 pp. Ph.D. Dissertation, The Johns Hopkins University, Baltimore, Maryland 1974

-, W.R. Taylor and J.J. McCarthy: Uptake and release of phosphorus by phytoplankton in the Chesapeake Bay Estuary, USA. Mar. Biol. *33*, 21-32 (1975)

Watt, W.D.: Measuring the primary production rates of individual phytoplankton species in natural mixed populations. Deep-Sea Res. *18*, 329-339 (1971)

Yentsch, C.S. and J. Ryther: Relative significance of net plankton and nannoplankton in the waters of Vineyard Sound. J. Cons. perm. int. Explor. Mer *24*, 231-238 (1959)

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