UPTAKE AND RELEASE OF PHOSPHORUS BY PHYTOPLANKTON
IN THE CHESAPEAKE BAY ESTUARY

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ABSTRACT

The phytoplankton uptake and release rates for inorganic phosphate, dissolved organic phosphate and polyphosphate were estimated during five cruises on the Chesapeake Bay over a 9-month period. Phosphorus in all pools turned over in several minutes to 100 hr and each soluble pool appeared to contain fractions which were metabolically useful to the phytoplankton. Maximal uptake rates (V_m) for orthophosphate ranged from 0.02 to 2.95 $\mu\text{g atom P (liter}\cdot\text{hr)}^{-1}$ with half saturation constants (K_s) between 0.09 and 1.72 $\mu\text{g atom P liter}^{-1}$. At low soluble reactive phosphorus concentrations, the uptake rate of trace ^{32}P orthophosphate was initially rapid but declined after 15 to 60 min of incubation. The data suggests that the initial uptake phase was dominated by exchange of $^{32}\text{PO}_4^-$ for $^{31}\text{PO}_4^-$ in the membrane transport systems whereas the subsequent phase represented the net incorporation of orthophosphate into phytoplankton cells.

INTRODUCTION

The cycling of phosphorus between planktonic organisms and the water in which they are suspended is a mechanism which maximizes the biological utilization of the element. There are at least three phosphorus cycles in natural waters. One is an annual cycle, hypothesized in both marine and fresh waters, in which phytoplankton incorporate soluble phosphorus and sink as viable cells, senescent cells or biodebris to deeper layers. Autolysis and bacterial activity remineralize organic phosphorus compounds to orthophosphate which is then returned to the euphotic zone seasonally by vertical mixing. The second cycle, which may occur entirely in the euphotic zone, is that in which zooplankton consume phytoplankton and remineralize orthophosphate with the aid of their intestinal bacteria (Hargrave and Geen, 1968; Butler et al., 1970; Peters and Rigler, 1973). The third cycle is the kinetic steady-state between the phosphorus in either phytoplankton or bacteria and dissolved phosphorus. Hutchinson and Bowen (1947) described the steady-state condition in a small lake, Rice (1953) observed it in laboratory cultures, and Watt and Hayes (1963) and Thayer (1969) further investigated the kinetic steady-state in marine and estuarine waters.

The Chesapeake Bay is a highly productive coastal plain estuary in which orthophosphate concentrations in the euphotic zone seldom exceed $0.2 \mu\text{g atom P liter}^{-1}$ (Carpenter et al., 1969; Taft and Taylor, 1974). Rapid phosphorus cycling, therefore, should be crucial in sustaining primary productivity. The strong two-layered circulation and flushing pattern in Chesapeake Bay diminishes the importance of the first cycle (Taft and Taylor, 1974) so the last two cycles described above are the

most significant over time scales of hours and days. We selected the third cycle, the kinetic steady-state, as the object of this investigation.

We have used a modified form of the steady-state expression of Watt and Hayes (1963) (see also Thayer, 1969), as our model for phosphorus flux. Since the commonly used phosphomolybdate method actually measures soluble reactive phosphorus (SRP) (Rigler, 1968; Strickland and Parsons, 1972), we have used SRP rather than dissolved inorganic phosphorus (DIP) and we have added polyphosphate (Poly P) to the original expression to give:

The principle advantage of the Watt and Hayes model is that it permitted us to estimate the flux of biologically active phosphorus compounds in a living system at ambient nutrient concentrations and, therefore, at near-natural reaction rates. There are constraints on the use of the model, but there is probably more error introduced by experimental manipulation than is inherent in the model, especially over time intervals which are short relative to phytoplankton generation times. The model assumes the biological system is in steady-state with respect to phosphorus flux, a condition that may not prevail during spring in the Chesapeake Bay. The model also assumes that exchange between soluble phosphorus fractions is mediated by live organisms, an assumption which

is not always valid (Lean, 1973). Furthermore, the model, as we applied it, does not distinguish between phosphorus atoms within a given fraction. For example, P atoms in organic molecules such as DNA are not available for rapid exchange with SRP, Poly P, or other DOP so some of the rate constants will be underestimated.

MATERIALS AND METHODS

Cruises on the Chesapeake Bay and adjacent continental shelf (Fig. 1) were undertaken during December 1972, February, April, June and August 1973 to identify the soluble phosphorus fractions, to determine which of the available forms are metabolically useful to Chesapeake Bay phytoplankton, and to compare steady-state phosphorus flux with the rate of supply of soluble phosphorus required to sustain primary productivity in the Bay.

Water was collected from the euphotic zone with Van Dorn bottles, passed through 35 μm Nitex^R mesh to remove most zooplankton, and placed in a polyethylene carboy on deck maintained at surface water temperature. The 35 μm mesh removed only a small fraction of the phytoplankton (McCarthy et al., 1974). All subsequent samples for each station were drawn from the carboy after gentle mixing.

Samples for soluble phosphorus fractions were filtered through pre-rinsed Whatman GF/C filters. Soluble reactive phosphorus (SRP) was determined by the method of Murphy and Riley (1962) as outlined in Strickland and Parsons (1972). Dissolved organic phosphorus (DOP) was determined as SRP following UV irradiation of a filtered water sample (Armstrong et al., 1966). Polyphosphate (Poly P) was determined as SRP following UV irradiation and acid hydrolysis of a filtered sample

(Solórzano and Strickland, 1968). Total phosphorus (Total P) was determined as SRP in an unfiltered sample following UV irradiation (Whaley et al., 1966) and acid hydrolysis (Solórzano and Strickland, 1968). Particulate phosphorus (PP) was taken as the difference between Total P and the sum of the soluble fractions. Reagent blanks and standards were prepared as in Strickland and Parsons (1972). Optical densities were measured in 10 cm cells at 885 nm with a Beckman DU spectrophotometer. Corrections for turbidity were made in the Total P measurements. Corrections for arsenate interference were made on frozen filtered samples by the method of Johnson (1971). To conserve sample all of the above methods were scaled for 5 ml sample volumes.

Primary productivity was measured by the ^{14}C uptake technique described by Strickland and Parsons (1972). Chlorophyll *a* was determined by the method of Loftus and Carpenter (1971) and particulate nitrogen was determined by the method of Holm-Hansen (1968).

Eighteen steady-state experiments were performed at nine different stations along the major axis of Chesapeake Bay and on the adjacent continental shelf (Figure 1). Pyrex bottles were filled with 1 liter aliquots from the carboy and, after an addition of 10 μCi ^{32}P -as carrier free phosphoric acid, they were incubated on deck under natural light attenuated with neutral density filters to the approximate light intensity from which the samples were collected. Near-surface water from the ship's sea water system cooled the samples. Dark experiments were conducted in 1 liter or 150 ml Pyrex bottles covered with black plastic tape. Subsamples were periodically removed from all bottles for chemical analyses of the

phosphorus fractions and ^{32}P extractions. Phosphorus was quantitatively determined in the soluble and particulate fractions as described above. ^{32}P was collected from each soluble fraction by double isobutanol extraction of the phosphomolybdate complex. Particulate material was collected on prerinsed (Rigler, 1964) Millipore^R 0.8 μm filters. The isobutanol extracts or the membrane filters containing ^{32}P were added to 10 ml of a toluene-cellosolve scintillation solution (Parsons and Anderson, 1970) and counted in a Packard model 3320 scintillation spectrometer. Counts were corrected for decay and for counting efficiency by the channels ratio method.

Estimates of precision for both total phosphorus analyses and isobutanol extractions of molybdate complexed ^{32}P were made from a set of six replicates for each: the coefficients of variation (Moroney, 1965) were 2.4% and 8.3% respectively.

Rate constants for phosphorus flux were calculated from the simultaneous solution of a steady-state equation and a rate of change equation

(Watt and Hayes, 1963). For example, the rate constant, α , for SRP movement into the particulate fraction is determined from:

$$\alpha = \frac{\frac{\text{SR}^{32}\text{P}_2 - \text{SR}^{32}\text{P}_1}{\Delta t}}{\frac{\text{SR}^{32}\text{P}_1 + \text{SR}^{32}\text{P}_2}{2} - \left[\frac{\text{SRP}}{\text{PP}} \times \frac{\text{P}^{32}\text{P}_1 + \text{P}^{32}\text{P}_2}{2} \right]}$$

where SR^{32}P_1 and P^{32}P_1 are fractions of total ^{32}P in each respective pool at the beginning of the experimental time interval, SR^{32}P_2 and P^{32}P_2 are fractions of total ^{32}P at the end of the time interval, and SRP and PP are fractions of total P in each pool during the time interval. Most calculations were made from data at time zero and the next sampling time.

Rapid uptake of ^{32}P into the particulate fraction was examined in six steady-state experiments and in an additional experiment at station 834G in February. ^{32}P samples were obtained by filtration at 5- to 20 min intervals. The ordinal intercept on a semilogarithmic plot of ^{32}P against time on the abscissa approximates ^{32}P adsorption onto non-living particles (Correll, personal communication).

Orthophosphate uptake kinetics were examined at 5 stations in June, 1973. In each experiment 100 ml of water from the carboy was dispensed into 6 Pyrex bottles. Sterile KH_2PO_4 solution was added to 5 bottles to yield final concentrations of 0.1 to 9.0 $\mu\text{g atom P liter}^{-1}$. One microcurie ^{32}P was added to each bottle. After 30 minutes of deck board incubation, samples were filtered on prewetted Millipore^R 0.8 μm filters and the radioactivity measured as described above. The maximum uptake rate (V_m), and the half-saturation constant (K_s) and respective standard deviations were calculated by the method of Wilkinson (1961).

RESULTS

Dissolved P, particulate P (PP), particulate N (PN) and chlorophyll *a* concentrations, and various ratios are shown in Table 1. The ratios for station 854G in December (Chl *a* to PN, 1.9; Chl *a* to PP, 27.0; PN to PP, 14.5) provide a good example of the composition of Chesapeake Bay phytoplankton in nutrient-rich water. Compared to the results of Parsons et al., (1961) chlorophyll *a* was usually abundant with respect to both PN and PP during this study. PN to PP atomic ratios ranged from 8 to 55 with a median of 21 and the highest ratios were observed in April.

Rate constants for steady state exchange between P pools are shown in Table 2. Values for α and γ , the flux from SRP and DOP pools respectively into the particulate fraction, were variable and tended to be highest in summer. The only other pattern which could be identified was an inversely proportional relationship in December and February between SRP concentrations and α . Values for κ , the flux from the Poly P pool into particulate material, and values for β_1 , β_2 , and β_3 , the fluxes from the particulate to the soluble pools, have no obvious correlation to other chemical, biological, or physical parameters. The rate constant reciprocal is turnover time, the time required for the mass in a phosphorus pool to pass through that pool. Turnover times for all phosphorus pools ranged from several minutes to 100 hr (Table 3), and in general were shorter for both SRP and PP during warmer seasons.

For a particular pool, the product of the phosphorus mass and the rate constant equals the hourly phosphorus flux. For example, (station 854G in December) $0.18 \mu\text{g atom P liter}^{-1} \times 0.05 (\text{hr})^{-1} = 0.09 \mu\text{g atom P (liter}\cdot\text{hr)}^{-1}$ moving from the SRP to the PP fraction. Since we assume the system to be in steady-state over short time intervals, phosphorus flux from PP to SRP should be identical to the flux from SRP to PP. The data indicates the system was in steady state.

Table 4 compares P flux across the cell membranes, calculated from the rate constants, with the phosphorus required to support carbon assimilation measured at midday, assuming a C:P atomic ratio of 100:1. The phosphorus exchanged across the cell membrane exceeded the phosphorus required in every case. Carbon fixation rates ranged from 1.5 to 100.1 $\mu\text{g C (liter}\cdot\text{hr)}^{-1}$. Highest values were observed in summer and lowest in winter.

Phosphorus uptake as a function of time was calculated for 7 experiments (the data from 4 of these are presented in Figure 2) and the rates were compared to the carbon uptake rates. Carbon to phosphorus uptake ratios should approach the phytoplankton composition ratios if measured over a sufficiently long period. Since long incubations can lead to inaccurate results due to bacterial growth on bottle walls, respiration of fixed ^{14}C , and phytoplankton growth or death, carbon uptake rates from short incubations at midday were normalized to 24 hours by multiplying the midday hourly rate by 7 for February and by 9 for June. These factors were estimated from 2 hr ^{14}C uptake experiments conducted sequentially over a 24 hour period. Phosphorus uptake was equivalent in light and dark (Figure 2A) so daily phosphorus uptake rates were taken as 24 times the hourly rate, ignoring any diel periodicity which is not specifically a function of the quantity of light.

^{32}P uptake into the particulate fraction was initially rapid, as shown in Figure 2, and declined after 15 to 60 minutes. In February (834G) the break in slope occurred when about 50% of the added ^{32}P had been incorporated. In June the incorporation rate slowed when 10% (Ø707V) or 75 to 90% (724R and 834G) had been taken up. Calculations for phosphorus assimilation were made to determine whether the initial rapid uptake rate or the subsequent slow uptake rate could be equated with net uptake. Table 5 shows C to P uptake ratios calculated from the SRP concentration measured in the water and the mean radioactivity remaining in the water during each uptake phase. The C to P ratios during the slow ^{32}P uptake phase (Table 5) are closest to the observed composition ratios for marine phytoplankton (Parsons et al., 1961).

Half saturation constants, K_s , and standard deviations for orthophosphate uptake by the natural phytoplankton assemblages at 5 stations ranged from 0.09 ± 0.06 to 1.72 ± 0.23 $\mu\text{g atom P liter}^{-1}$ (Table 6).

Maximum uptake rates, V_m , and standard deviations ranged from 0.02 ± 0.003 to 2.95 ± 0.23 $\mu\text{g atom P (liter}\cdot\text{hr)}^{-1}$. Figure 3 contains the data from one uptake experiment.

DISCUSSION

Phytoplankton and Bacteria

For the purposes of experimentation such as ours it is virtually impossible to physically separate the nanoplankton of Chesapeake Bay from either the bacterioplankton or the bacteria attached to particle surfaces. The question of whether our phosphorus uptake data represent primarily phytoplankton or bacterial processes can be answered indirectly.

In our study, the chlorophyll a to particulate N and chlorophyll a to particulate P ratios (Table 1) indicate that most particulate nitrogen and phosphorus were associated with chlorophyll a containing organisms (for normal plankton ratios see Parsons et al., 1961). Supporting evidence is found in ATP measurements made throughout this study (Loftus, unpublished data) and the ATP to chlorophyll a ratios which suggest that virtually all of the ATP can be accounted for by the algae.

Functional studies also suggest that the rates we measured were predominantly those of the phytoplankton. Orthophosphate can be utilized by marine bacteria (Watt and Hayes, 1963), and both Rigler (1956) and Correll et al. (1973) have attributed rapid orthophosphate uptake to bacteria in lake and estuarine waters respectively. Rhee (1972) and

Fuhs et al. (1972) have further demonstrated that some bacteria are capable of outcompeting phytoplankters for phosphorus when the orthophosphate concentrations were $0.1-0.5 \mu\text{g atom P liter}^{-1}$ (high values for the Chesapeake Bay), but the outcome was reversed at concentrations less than $0.1 \mu\text{g atom P liter}^{-1}$. Not surprisingly, the K_s values for bacterial orthophosphate uptake ($6.7-12.0 \mu\text{g atom P liter}^{-1}$) measured by Fuhs et al. (1972) exceeded those for phytoplankton ($0.58-1.7$ and $0.01-0.45 \mu\text{g atom P liter}^{-1}$) measured by Fuhs et al. (1972) and Stross and Pemrick (1974) respectively. Our K_s values (Table 6) argue for phytoplankton dominance. The sum of this evidence strongly suggests that both the biomass used in our experiments and the rates which we measured can be attributed almost exclusively to phytoplankton.

Rate Constants

Rice (1953) has shown with cultures of Phaeodactylum that the quantity of intracellular orthophosphate exchanging across the cell membrane was a function of both the extracellular SRP concentration and the organism's recent history. Other investigators (Fitzgerald, 1969; Healey, 1973; Rhee, 1973) also observed that orthophosphate availability influenced the concentration of intracellular orthophosphate, the pool which is potentially exchangeable with the extracellular orthophosphate.

Since the nutrient regime may affect the amount of exchangeable orthophosphate within the phytoplankton, one might expect the rate constants for steady-state exchange (α, β_1) to be related to external orthophosphate concentration and to the state of phytoplankton nutrition. The only such pattern was the inversely proportional relationship between SRP concentra-

tions and α in the winter. Values for α increased in the warmer months independent of SRP concentration. There is no correlation between the rate constants and cell nutrition as measured by the N to P ratio.

DOP is always present in Chesapeake Bay, and its concentration usually exceeds SRP (Carpenter et al., 1969; Taft and Taylor, 1974). Phytoplankton cultures (Kuenzler, 1965, 1970; Kuenzler and Perras, 1965), cell-free extracts of phytoplankton (Antia and Watt, 1965) and natural phytoplankton populations (Berman, 1970; Berman and Moses, 1972; Perry, 1972) hydrolyze phosphomonoesters with alkaline phosphatase when orthophosphate is depleted from the medium. The concentration of phosphomonoester is, however, usually low in ocean water (Strickland and Solórzano, 1966), in Lake Kinneret, Israel (Berman, 1970) and in Chesapeake Bay (Taft, 1974). The bulk (>70%) of the DOP fraction in Chesapeake Bay cannot be hydrolyzed by in vitro preparations of alkaline phosphatase (unpublished data) so our determinations of γ , the rate constants for DOP utilization, are most certainly underestimates and the turnover times for this pool are overestimates. Calculated DOP flux (Table 4) is, however, not affected since a decrease in size of the active DOP pool is balanced by a proportional increase in the calculated rate constant.

The same problem exists for β_1 , β_2 , and β_3 , since the particulate phosphorus fraction is composed of many different inorganic and organic phosphorus molecules. And, likewise, the estimates of P flux through active pools of the particulate fraction are not affected.

Poly P was detectable at about 70% of the stations sampled and then only at low concentrations. For these reasons, errors in the rate constants k and β_3 are probably large. Since Poly P does not accumulate in the open

waters of the Chesapeake Bay, its near-shore waters (Correll, personal communication), in other estuarine waters (Butler and Tibbitts, 1972), or in coastal and open ocean waters (Solórzano and Strickland, 1968) it is presumably utilized rapidly by phytoplankton or bacteria.

The fluxes of phosphorus atoms between the three soluble P pools and the particulate P fraction indicate that at least certain fractions of each soluble pool were utilized by the natural phytoplankton assemblages. The phosphorus species most readily utilized by phytoplankton are the orthophosphate ion, phosphomonoesters and, probably, the linear polyphosphates. The low concentrations and rapid turnover times for these three phosphorus fractions in our experiments indicate that they are the important sources of P for Chesapeake Bay phytoplankton. The low concentrations of these phosphorus species also suggest that phosphorus may limit phytoplankton productivity in Chesapeake Bay during much of the year.

Phosphorus Uptake and Exchange

Harvey (1963) suggested that algae could incorporate merely through exchange relatively large amounts of ^{32}P from medium which contains orthophosphate in low concentration. Rice (1953) was able to measure phosphorus exchange between Phaeodactylum tricornutum (= Nitzschia closterium) cells and the suspending medium. Kuenzler and Ketchum (1962), however, were not able to distinguish phosphorus exchange across the cell membrane from phosphorus uptake into the organism. Since orthophosphate concentrations are very low in the Chesapeake Bay euphotic zone during most of the year, it is necessary for us to attempt to differentiate net uptake from phosphorus exchange characterized by the rate constants.

The common shape of the ^{32}P orthophosphate uptake curves (Fig. 2) suggests that the initial rapid rate of uptake and subsequent reduced rate represent different phenomena. Two possible explanations can be advanced: 1) the entire curve represents net uptake which slows with experiment duration as the level of available orthophosphate approaches the uptake limiting concentrations; and 2) the initial uptake represents exchange across the cell membrane and only the subsequent reduced rate is net uptake.

If a significant portion of the quantity measured as SRP is not orthophosphate, the first explanation above could be correct. In order to test this hypothesis, SRP analyses were disregarded and the orthophosphate concentration was estimated in the following manner. Since most of the ^{32}P orthophosphate was incorporated into the particulate fraction in the time series experiments, we assumed that the concentration of ^{31}P orthophosphate originally present was small compared to the amount of ^{32}P orthophosphate added ($< 2 \times 10^{-4}$ $\mu\text{g atom P liter}^{-1}$). During the rapid uptake phase most of the ^{31}P orthophosphate would have been incorporated along with the ^{32}P . Therefore, little ^{31}P orthophosphate should have remained in solution after the break in slope. The ^{32}P in solution after the break in slope should then nearly equal the quantity of ^{31}P orthophosphate initially present in the water. If this argument is accepted as a best estimate of the natural orthophosphate concentration, then the orthophosphate concentration immediately after ^{32}P addition is approximated by the sum of the mass of ^{32}P orthophosphate added and the mass of ^{32}P orthophosphate remaining in solution after the break in slope. The ^{32}P specific activity used in the calculation of orthophosphate uptake

was determined from the calculated orthophosphate concentration and the mean radioactivity (DPM) in solution during each uptake phase. The C to P atomic uptake ratios were calculated as indicated in the results and compared to the composition ratios in Parsons et al. (1961) which ranged from about 31 to 103 for marine phytoplankton in culture. Only four of our C to P ratios for rapid orthophosphate uptake were near this range and all others were high by factors of 10 to 10^4 , so we rejected the hypothesis that SRP was mostly material other than orthophosphate and also rejected the first explanation for the shape of the ^{32}P uptake curves in Figure 2.

To test the second explanation, orthophosphate uptake was calculated from the measured SRP concentration and the mean radioactivity (DPM) in solution during each uptake phase. The amount of added ^{32}P orthophosphate was considered negligible. The C to P atomic uptake ratios were calculated for each uptake phase under the assumption that only net uptake occurred during each phase. The uptake ratios (Table 5) were low during the rapid initial ^{32}P uptake, but approached expected elemental composition ratios at the reduced uptake rate. This finding suggests that the second explanation offered above is correct.

The two stations (707Ø, Ø707V) with anomalous C to P uptake ratios were different in other respects, so they may not represent a general phenomenon. Carbon to phosphorus uptake ratios should approach expected composition ratios only when P is growth limiting. A low C to P uptake ratio indicates more P is being incorporated than is required for growth so it is not limiting. This appears to be the explanation for the low C to P uptake ratio at the reduced P uptake rate for station Ø707V. Half

saturation constants (K_s) for SRP uptake at 3 of 4 Bay stations in June 1973 were 0.11 to 0.25 $\mu\text{g atom P liter}^{-1}$. The K_s for SRP uptake at 7070 was 1.72 $\mu\text{g atom P liter}^{-1}$ suggesting that the phytoplankton population could not efficiently utilize orthophosphate at the low concentration observed (0.01 $\mu\text{g atom P liter}^{-1}$) in the euphotic zone. The phytoplankton may have utilized stored P to support carbon fixation in which case carbon uptake would be higher than predicted from phosphorus incorporation.

The close agreement between carbon to phosphorus uptake ratios calculated for the reduced phosphorus uptake phase and the expected phytoplankton composition ratios suggests that the semilogarithmic plot of ^{32}P uptake may be used to differentiate orthophosphate exchange across cell membranes from net uptake of orthophosphate by phytoplankton. We have demonstrated that the fraction of ^{32}P incorporated into phytoplankton by exchange alone may be large, and we suggest that experiments designed to measure tracer uptake at low ambient phosphate concentrations should include measurements at several time intervals after the addition of the tracer so that exchange may be distinguished from net uptake. Additional experiments are underway to evaluate the significance of rapidly exchanging phosphorus to phytoplankton productivity.

SUMMARY

1. The rates of short-term phosphorus flux between soluble phosphorus pools and phytoplankton were studied in the Chesapeake Bay estuary and in adjacent continental shelf waters. The three soluble phosphorus pools examined were soluble reactive P, dissolved organic P and polyphosphate.

2. Atomic ratios of N to P in the particulate fraction were 8 to 55 with a median of 21. Chlorophyll *a* was usually abundant with respect to both PN and PP.

3. Turnover times ranged from several minutes to 100 hr for particulate and soluble phosphorus.

4. The mass of phosphorus cycled between the water and the phytoplankton exceeded the phosphorus mass required to sustain primary productivity at a C:P atomic uptake ratio of 100:1.

5. During the summer, maximum orthophosphate uptake rates were 0.02 to 2.95 $\mu\text{g atom P (liter}\cdot\text{hr)}^{-1}$ and half-maximal uptake rates occurred at concentrations between 0.09 and 1.72 $\mu\text{g atom P liter}^{-1}$.

6. At the low SRP concentrations in Chesapeake Bay, the uptake of ^{32}P orthophosphate occurred in two phases: an initial rapid uptake phase of up to 60 min duration followed by a reduced uptake phase. It is suggested that the first phase represents phosphorus exchange between the phytoplankton uptake system and the water, and the second phase represents net incorporation of orthophosphate into phytoplankton cells.

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LITERATURE CITED

- Antia, N. J. and A. Watt: Phosphatase activity in some species of marine phytoplankters. J. Fish Res. Bd. Canada, 22, 793-799 (1965).
- Armstrong, F. A. J., P. M. Williams and J. D. H. Strickland: Photo-oxidation of organic matter in sea water by ultra-violet radiation, analytical and other applications. Nature, 211, 481-483 (1966).
- Berman, T.: Alkaline phosphatases and phosphorus availability in Lake Kinneret. Limnol. Oceanog. 5, 663-674 (1970).
- Berman, T. and G. Moses: Phosphorus availability and alkaline phosphatase activities in two Israeli fishponds. Hydrobiologia 40, 487-498 (1972).
- Butler, E. I., E. D. S. Corner and S. M. Marshall: On the nutrition and metabolism of zooplankton. VII. Seasonal survey of nitrogen and phosphorus excretion by Calanus in the Clyde Sea-area. J. Mar. Biol. Ass. U. K. 50, 525-560 (1970).
- Butler, E. I. and S. Tibbitts: Chemical survey of the Tamar estuary. I. Properties of the waters. J. Mar. Biol. Ass. U. K. 52, 681-699 (1972).
- Carpenter, J. H., D. W. Pritchard and R. C. Whaley: Observation of eutrophication and nutrient cycles in some coastal plain estuaries. In: Eutrophication: causes, consequences, correctives. p. 210-221 Nat. Acad. Sci., Washington (1969).
- Correll, D. L., M. A. Faust and D. J. Severn: Phosphorus flux and cycling in estuaries. Presented at Second Internat. Est. Res. Conf. Myrtle Beach, S. C. (1973).

Fitzgerald, G. P.: Field and laboratory evaluations of bioassays for nitrogen and phosphorus with algae and aquatic weeds. *Limnol. Oceanog.* 14, 206-212 (1969).

Fuhs, G. W., S. D. Demmerle, E. Canelli and M. Chen: Characterization of phosphorus-limited plankton algae (with reflections on the limiting-nutrient concept). *ASLO Special Symposia* 1, 113-133 (1972).

Hargrave, B. T. and G. H. Geen: Phosphorus excretion by zooplankton. *Limnol. Oceanog.* 13, 332-342 (1968).

Harvey, H. W.: The chemistry and fertility of sea waters, 240 pp. Cambridge University Press, London (1963).

Healey, F. P.: Characteristics of phosphorus deficiency in *Anabaena*. *J. Phycol.* 9, 383-394 (1973).

Holm-Hansen, O.: Determination of particulate organic nitrogen. *Limnol. Oceanog.* 13, 175-178 (1968).

Hutchinson, G. E. and V. T. Bowen: A direct demonstration of the phosphorus cycle in a small lake. *Proc. Nat. Acad. Sci.* 33, 148-153 (1947).

Johnson, D. L.: Simultaneous determination of arsenate and phosphate in natural waters. *Environ. soil technol.* 5, 411-414 (1971).

Kuenzler, E. J.: Glucose-6-phosphate utilization by marine algae. *J. phycol.* 1, 156-164 (1965).

Kuenzler, E. J.: Dissolved organic phosphorus excretion by marine phytoplankton. *J. Phycol.* 6, 7-13 (1970).

Kuenzler, E. J. and B. H. Ketchum: Rate of phosphorus uptake by *Phaeodactylum tricornutum*. *Biol. Bull.* 123, 134-145 (1962).

Kuenzler, E. J. and J. P. Perras: Phosphatases of marine algae. *Biol. Bull.* 128, 271-284 (1965)

Lean, D. R. S.: Phosphorus dynamics in lake water. Science 179, 678-680 (1973).

Loftus, M. E. and J. H. Carpenter: A fluorometric method for determining chlorophylls a, b, and c. J. Mar. Res. 29, 319-338 (1971).

McCarthy, J. J., W. R. 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).

Moroney, M. J.: Facts from Figures, 472 pp. Penguin, Baltimore (1965).

Murphy, J. and J. P. Riley: A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27, 31-36 (1962).

Parsons, T. R. and G. C. Anderson: Large scale studies of primary production in the North Pacific Ocean. Deep-sea Research 17, 765-776 (1970).

Parsons, T. R., K. Stephens and J. D. H. Strickland: On the chemical composition of eleven species of marine phytoplankters. J. Fish. Res. Bd. Canada 18, 1001-1016 (1961).

Perry, M. J.: Alkaline phosphatase activity in subtropical central North Pacific waters using a sensitive fluorometric method. Mar. Biol. 15, 113-119 (1972).

Peters, R. H. and F. H. Rigler: Phosphorus release by Daphnia. Limnol. Oceanog. 18, 821-839 (1973).

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

Rhee, G-Y.: A continuous culture study of phosphate uptake, growth rate and polyphosphate in Scenedesmus sp. J. Phycol. 9, 495-506 (1973).

Rice, T. R.: Phosphorus exchange in marine phytoplankton. Fish. Bull. 53, 77-89 (1953).

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

Rigler, F. H.: The phosphorus fractions and the turnover time of inorganic phosphorus in different types of lakes. Limnol. Oceanog. 9, 511-518 (1964).

Rigler, F. H.: Further observations inconsistent with the hypothesis that the molybdenum blue method measures orthophosphate in lake water. Limnol. Oceanog. 13, 7-13 (1968).

Solórzano, L. and J. D. H. Strickland: Polyphosphate in sea water. Limnol. Oceanog. 13, 515-518 (1968).

Strickland, J. D. H. and T. R. Parsons: A practical handbook of sea water analysis, 310 pp. Ottawa: Fisheries Res. Bd. Canada (1972).

Strickland, J. D. H. and L. Solórzano: Determination of monoesterase hydrolysable phosphate and phosphomonoesterase activity in sea water, p. 655-674. In: H. Barnes (ed.), Some contemporary studies in marine science. Allen and Unwin, London (1966).

Stross, R. G. and S. M. Pemrick: Nutrient uptake kinetics in phytoplankton: A basis for niche separation. J. Phycol. 10, 164-169 (1974).

Taft, J. L.: Phosphorus cycling in the plankton of Chesapeake Bay. Ph.D. Thesis, Johns Hopkins University, Baltimore, 193 pp. (1974).

Taft, J. L. and W. R. Taylor: Phosphorus distribution in the Chesapeake Bay. Submitted to Chesapeake Science (1974).

Thayer, G. W.: Phytoplankton production and factors influencing production in the shallow estuaries near Beaufort, North Carolina. Ph.D. Thesis, North Carolina State University, Raleigh. P. 97-123 (1969).

Watt, W. D. and F. R. Hayes: Tracer study of the phosphorus cycle in sea water. Limnol. Oceanog. 8, 276-285 (1963).

Whaley, R. C., J. H. Carpenter and R. L. Baker: Nutrient data summary 1964, 1965, 1966: Upper Chesapeake Bay (Smith Point to Turkey Point) Potomac, South, Severn, Magothy, Back, Chester and Miles Rivers; and Eastern Bay. Ches. Bay Inst. Spec. Report 12, 88pp. (1966).

Wilkinson, G. N.: Statistical estimations in enzyme kinetics. Biochem. J. 80, 324-332 (1961).

Figure 1. Chesapeake Bay (USA) showing location of stations.

Figure 2. Uptake of ^{32}P orthophosphate into the particulate fraction
with time.

Figure 3. Orthophosphate uptake rate (V) vs. concentration in the water (S) and the linear transform $\frac{S}{V}$ vs. S fitted by eye to the data points from station 724R.

Table 1. Soluble reactive phosphorus, dissolved organic phosphorus, polyphosphate, particulate phosphorus, particulate nitrogen and chlorophyll α . Phosphorus and nitrogen in $\mu\text{g atom liter}^{-1}$ and chlorophyll α in $\mu\text{g liter}^{-1}$.

Date	Station	SRP	DOP	Poly P	Part P	Part N	Chl α	Chl α Part P	Part N Part P	Chl α Part N
Dec 72	914S	0.40	0.14	0.09	0.94	7.5	-	-	8.0	-
	854G	0.18	0.18	0.06	0.56	8.1	15.1	27.0	14.5	1.9
	834G	0.01	0.24	0.06	0.44	8.4	15.7	35.7	19.1	1.9
	818P	0.02	0.16	0.00	0.39	8.1	15.0	38.5	20.8	1.9
	804C	0.03	0.27	0.01	0.41	9.6	16.3	39.7	23.4	1.7
	724R	0.00	0.41	0.08	0.24	-	5.0	20.8	-	-
	Ø707V	0.40	0.25	0.02	0.24	-	3.3	13.8	-	-
Feb 73	904N	0.42	0.18	0.05	0.28	9.9	19.2	68.5	35.4	1.9
	854G	0.10	0.23	0.02	0.36	6.9	9.8	27.2	19.2	1.4
	834G	0.04	0.17	0.00	0.53	10.4	20.3	38.3	19.6	2.0
	804C	0.04	0.19	0.06	0.33	8.1	13.1	39.7	24.6	1.6
	744	0.02	0.18	0.08	0.32	8.8	11.6	36.3	27.5	1.3
Apr 73	834G	0.23	0.22	0.03	0.31	13.9	18.1	58.4	44.8	1.3
	818P	0.22	0.20	0.10	0.23	12.6	12.2	53.0	54.8	1.0
	804C	0.11	0.21	0.02	0.32	10.9	9.5	29.7	34.1	0.9
Jun 73	834G	0.04	0.23	0.00	0.69	8.4	12.1	17.5	12.2	1.4
	818P	0.10	0.10	0.00	0.74	9.1	13.7	18.5	12.6	1.5
	744	0.06	0.14	0.00	0.68	9.5	33.5	18.5	22.6	3.5
	724R	0.05	0.16	0.00	0.41	7.1	19.5	47.6	20.9	2.7
	707Ø	0.01	0.20	0.00	0.29	7.2	18.9	65.2	24.8	2.6
	Ø707V	0.06	0.23	0.06	0.16	2.9	3.3	20.6	29.0	1.1
Aug 73	854G	0.04	0.29	0.09	1.42	17.9	24.2	17.0	12.6	1.4
	834G	0.05	0.38	0.05	0.71	11.8	14.7	20.7	16.6	1.2
	818P	0.05	0.47	0.03	0.86	17.3	14.9	17.3	20.1	0.9
	804C	0.09	0.51	0.04	1.21	14.9	10.7	8.8	12.3	0.7

Table 2. Rate constants (hour⁻¹) for phosphorus exchange.

Station	α	β_1	β_2	β_3	γ	κ
Dec 1972						
914S	0.07	0.04	-	-	-	-
854G	0.05	0.01	-	0.06	-	0.57
834G	1.00	0.25	2.00	1.16	3.20	5.60
804C	0.89	0.04	0.16	0.07	0.24	0.40
724R	0.01	0.01	-	-	-	-
Feb 1973						
904N	0.06	0.03	-	-	-	-
854G	0.09	0.02	-	-	-	-
804C	0.70	0.08	0.15	2.12	0.13	11.40
744	0.53	0.03	0.40	2.72	0.72	11.17
Apr 1973						
834G	0.64	0.19	0.08	0.04	0.10	1.18
818P	5.40	7.63	0.07	0.19	0.07	6.67
804C	1.03	0.35	0.17	0.11	0.25	1.02
June 1973						
834G	4.48	0.26	7.19	-	21.01	-
744	10.03	2.00	0.02	-	0.14	-
724R	1.73	0.34	7.30	-	14.59	-
Ø707V	0.27	0.18	0.07	0.36	0.04	0.54
Aug 1973						
854G	15.20	0.43	1.41	0.33	7.09	6.97
804C	2.54	0.13	7.90	-	24.09	-

Table 3. Turnover time (hours) for phosphorus pools.

Station	SRP $1/\alpha$	Particulate P $1/(\beta_1 + \beta_2 + \beta_3)$	DOP $1/\gamma$	Poly P $1/\kappa$
Dec 1972				
914S	14.3	25.0	-	-
854G	20.0	14.3	-	1.8
834G	1.0	0.3	0.3	0.2
804C	1.1	3.7	4.2	2.5
724R	100.0	100.0	-	-
Feb 1973				
904N	16.7	33.3	-	-
854G	11.1	50.0	-	-
804C	1.4	0.4	7.7	0.09
744	1.9	0.3	1.4	0.09
Apr 1973				
834G	1.6	3.2	10.0	0.8
818P	0.2	0.1	14.3	0.1
804C	1.0	1.6	4.0	1.0
June 1973				
834G	0.2	0.1	14.3	-
744	0.1	0.5	7.1	-
724R	0.6	0.1	0.07	-
Ø707V	3.6	1.6	25.0	1.9
Aug 1973				
854G	0.07	0.5	0.1	0.14
804C	0.4	0.1	0.04	-

Table 4. Phosphorus required to support primary productivity at a C to P atomic ratio of 100 to 1 and the calculated flux of P.

Station	C Uptake $\mu\text{g}(\text{liter}\cdot\text{hr})^{-1}$	Required P $\mu\text{g at P}(\text{liter}\cdot\text{hr})^{-1}$	P Exchange Between PP and		
			SRP	DOP	Poly P $\mu\text{g at P}(\text{liter hr})^{-1}$
Dec 1972					
914S	0.3	0.0003	0.028	-	-
854G	6.6	0.0056	0.076	-	0.051
834G	6.7	0.0056	0.100	0.832	0.504
804C	3.0	0.0026	0.018	0.065	0.016
724R	1.5	0.0014	0.002	-	-
Feb 1973					
904N	6.3	0.0054	0.017	-	-
854G	5.8	0.0048	0.005	-	-
804C	5.0	0.0042	0.028	0.025	0.684
744	4.5	0.0038	0.010	0.130	0.893
Apr 1973					
834G	20.0	0.0196	0.051	0.022	0.013
818P	17.2	0.0144	1.188	0.016	0.067
804C	12.1	0.0102	0.113	0.052	0.020
June 1973					
834G	26.8	0.0224	0.179	4.832	-
744	29.3	0.0244	0.091	0.020	-
724R	16.4	0.0138	0.121	2.627	-
Ø707V	3.6	0.0030	0.021	0.010	0.043
Aug 1973					
854G	100.1	0.0834	0.607	2.057	2.328
804C	39.9	0.0334	0.127	7.950	-

Table 5. Carbon to phosphorus atomic ratios for phytoplankton uptake at the initial rapid P uptake rate and the subsequent slow P uptake rate.

		C/P Uptake (atomic) at Rapid P Uptake Rate	C/P Uptake (atomic) at Reduced P Uptake Rate
February 1973	834G	16	40
June 1973	834G	0.16	44
	818P	0.001	28
	724R	0.001	60
	744	0.002	88
	707Ø	0.015	410
	Ø707V	0.45	6

Table 6. Half-saturation constants (K_s) and maximum uptake rates (V_m) for orthophosphate.

Station	K_s $\mu\text{g atom P liter}^{-1}$	V_m $\mu\text{g atom P (liter}\cdot\text{hr)}^{-1}$
854G	0.11 ± 0.03	0.07 ± 0.03
744	0.25 ± 0.08	0.78 ± 0.10
724R	0.13 ± 0.02	0.31 ± 0.01
707Ø	1.72 ± 0.23	2.95 ± 0.23
Ø707V	0.09 ± 0.06	0.02 ± 0.003