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PHOSPHATE TRANSFER BETWEEN MICROBIAL SIZE-FRACTIONS IN VILLEFRANCHE BAY (N. W. MEDITERRANEAN SEA), FRANCE IN AUTUMN 1992

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ABSTRACT

Turnover time of orthophosphate, uptake of phosphate into particulate size-fractions (0.2-1, 1-5, 5-10, > 10 μm), and subsequent release from size-fractions were examined using ^{32}P, in samples from surface waters of Villefranche Bay during the autumnal erosion of the thermocline. Turnover time of orthophosphate increased from 1.6 h in early October to 58 h in December. Throughout the study period, total uptake was dominated (50-68%) by the smallest size-fraction (0.2-1 μm) presumably corresponding to auto- and heterotrophic bacteria as it contained little chlorophyll *a*. Among the size-fractions > 1 μm, representing eucaryotic phytoplankton uptake, the erosion of the thermocline was accompanied by a shift from the total dominance of uptake by the 1-5 μm fraction (98%) toward an increasing contribution from the > 10 μm fraction (~0 to 50%). In cold chase experiments, release of ^{32}P from the size-fractions which dominated uptake (0.2-1 μm & 1-5 μm), was relatively slow (≤ 1% h^{-1}) indicating long turnover times in particulate fractions; transfer to larger size-fractions was undetectable. When the concentration of oligotrich ciliates (predators of organisms 1-6 μm in size) was artifically increased, labeled ^{32}P declined in particulate fractions and increased in the pool of inorganic suggesting a low P retention efficiency.

INTRODUCTION

In pelagic ecosystems, the size-structure and transfer rates of the microbial community govern, to a large extent, the efficiency with which material is passed to higher trophic levels and the export of particulate matter (Ducklow & Fasham 1992). Size-structure and transfer rates of microbial communities can be outlined using carbon, nitrogen or phosphorus as kinds of "common currency" since all are required for the formation of new biomass. However, using phosphorus has certain advantages. In contrast to carbon, no significant gaseous phase is involved and relative to nitrogen, isotopes with high specific activity are readily available. The flows of phosphorus may be of interest in themselves in systems

where phosphorus appears to be in short supply, relative to nitrogen. Recent studies have identified a variety of systems in which phosphorus probably limits (depending on the season) algal or bacterial production: the tropical Atlantic (Raimbault & Pujo-Pay 1993), Chesapeake Bay (Fisher et al. 1992), Florida Bay (Fourqurean et al. 1993), Norwegian fjords (Sakshaug & Olsen 1986; Thingstad et al. 1993), and areas of the Baltic Sea (Lignell et al. 1992; Zweifel et al. 1993).

In Mediterranean systems, phosphorus appears to be the "limiting" nutrient (Berland et al. 1980); both observational and experimental lines of evidence support this conclusion. Observational studies of nutrient concentrations have documented high N:P ratios in subsurface waters of the Western Mediterranean leading to the conclusion that the N. W. Mediterranean is probably P-limited (Raimbault & Coste 1990). Furthermore, the degree of P-limitation, increases from west to east across the Mediterranean basins, based on decreases in P relative to N concentrations in surface waters (Krom et al. 1991). Experimental studies employing nutrient enrichments (bioassays), used to examine the production of either natural phytoplankton communities or selected species, have identified P as limiting primary production in French coastal waters (including Villefranche Bay) during the spring, summer and early autumn (Berland et al. 1973; 1978; Jacques et al. 1973). Similarly, bacterial production in early autumn in Villefranche Bay has been described as P-limited (Zweifel et al. 1993). In addition, global carbon flux in the Mediterranean Sea can be balanced with the inflows and outflows of phosphorus (Bethoux 1989). The mechanisms underlying the apparent P-limitation of the Mediterranean Sea are unclear; proposed hypotheses range from an excess of N input from nitrogen fixation by planktonic and benthic algae (Bethoux et al. 1992) to the removal of P by sedimenting iron-rich particles (Krom et al. 1991).

In a planktonic food web, phosphorus may be incorporated either by osmotrophic uptake of dissolved forms through cell membrane in algae and bacteria or through phagotrophic processes by mixotrophic algae, mixotrophic protists, heterotrophic protists and metazoa. In the first case, dissolved P enters directly into the size class of the osmotrophic organism, in the second case it is transferred through the food chain from one particle to another, usually larger. The size-fraction which dominates osmotrophic uptake should be an index of food web structure, which can range from a short chain (large algae directly to metazoans) to one which involves a microbial complex of bacteria, small phytoplankton, and protists supposedly with a concomitant loss of energy and material by respiration and recycling (Fig. 1). These aspects of the microbial P-cycle are in principle independent of whether the availability of P is limiting population growth rates and/or stocks in the system.

Here we report on the patterns of absorption, transfer, and turnover times of orthophosphate during the autumn in the Bay of Villefranche in the N. W. Mediterranean. For surface waters, early autumn in this system has been charac-

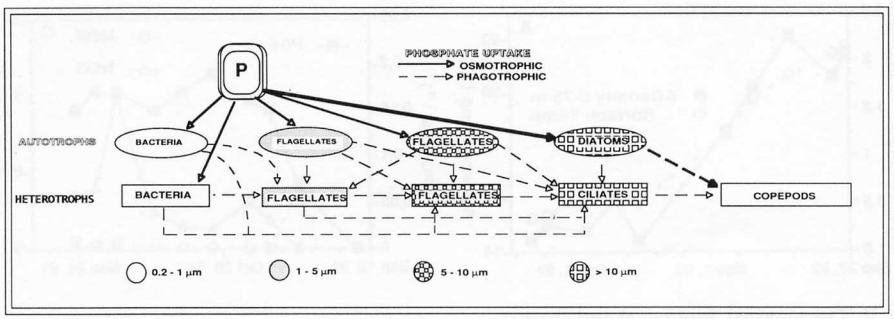

Fig. 1. Phosphorus flow through a simplified planktonic food web. Phosphorus can serve as a "common currency" tracing the formation and transfer of particulate matter. Phosphorus enters a size-fraction through absorption (solid lines) or ingestion (dashed lines). Flow into the dissolved pool, recycling, mixotrophy, etc., are not shown. Transport to the trophic level of typical metazoan grazers, such as copepods, can occur through a short food chain (**bold** solid and **bold** dashed lines) in which P is absorbed by large phytoplankters (e.g., diatoms) which are grazed directly. At the other extreme, heterotrophic bacteria and small picophytoplankton may be responsible for uptake with transfer to higher trophic levels involving passage through 2 or more steps of ingestion within the microbial community. The size-fractions considered in this study are shown at the bottom of the flow diagram.

terized as a period in which cyanobacteria may dominate primary production and heterotrophic bacterial production approaches 50% of primary production (Hagström et al. 1988). Evidence from nutrient enrichment experiments has been used to argue that phytoplankton (Berland et al. 1973) and heterotrophic bacteria (Zweifel et al. 1993) are P-limited in early autumn. The transition from autumn to winter includes declines in the abundances of nanoflagellates and ciliate microzooplankton (Rassoulzadegan et al. 1988), declines in the biomass of picoplankton relative to nanoplankton (Bernard & Rassoulzadegan 1993) and increases in large (> 10 µm) phytoplankters such as diatoms, euglenoids and dinoflagellates (Rassoulzadegan 1979). During this dynamic season we used ^{32}P-labelled orthophosphate to estimate phosphorus turnover rates, changes in the relative importance of various size-fractions (0.2-1, 1-5, 5-10, ≥ 10 µm) in P absorption, and transfer rates between P pools.

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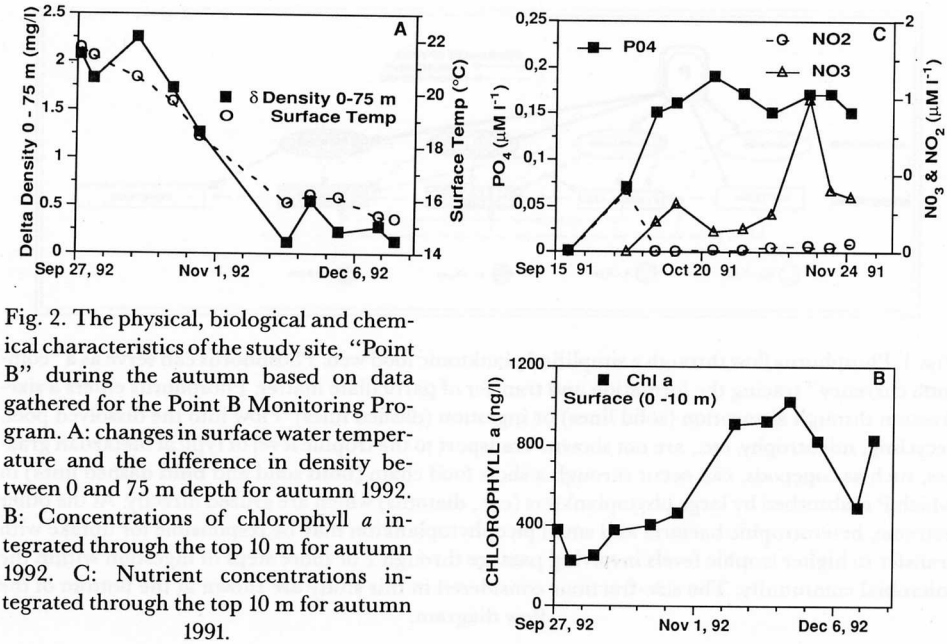

Fig. 2. The physical, biological and chemical characteristics of the study site, "Point B" during the autumn based on data gathered for the Point B Monitoring Program. A: changes in surface water temperature and the difference in density between 0 and 75 m depth for autumn 1992. B: Concentrations of chlorophyll *a* integrated through the top 10 m for autumn 1992. C: Nutrient concentrations integrated through the top 10 m for autumn 1991.

MATERIALS AND METHODS

Study Site

Samples were collected from "Point B" (water column depth ~ 80 m) located at the entrance of Villefranche Bay, France, N. W. Mediterranean (43°41′10″N, 7°19′00″E). The following description is based on data gathered for the "Point B Monitoring Program" (R. Charra, M.-D. Pizay, Station Zoologique, Villefranche). At "Point B", surface waters generally decrease in temperature from a maximum of ~27°C in early September to a minimum of 13°C corresponding to the deep water temperature of the Mediterranean in December. Data gathered during the study period (Sept. 1991-Dec. 1992) showed the erosion of the thermocline in the decreasing difference in density and temperature between the surface and 75 m depth (Fig. 2a); destratification was accompanied by increases in the average concentration of chlorophyll *a* integrated through the top 10 m (Fig. 2b). Seasonal increases in nutrients generally occur with water column mixing in the autumn and can be seen in data from 1991 for the top 10 m (Fig. 2c); reliable data for 1992 are not available due to the freezing of stored samples. Concentrations of microorganisms in samples taken from 5 m at "Point B" during the study period are given in Table 1, based on data gathered by M. Karner (Station

Table 1. Concentrations of selected microorganisms (ml^{-1}) in Villefranche Bay waters during the autumn of 1992, based on samples from 5 m depth at Point B. Data courtesy of Marcus Karner, Station Zoologique, Villefranche.

Date	Heterotrophic Bacteria	Cyanobacteria	Heterotrophic Pico & Nanoflagellates	Ciliates
Oct 1	7.0×10^5	ND	3.9×10^3	ND
Nov 4	10.7×10^5	1.5×10^4	2.7×10^3	0.74
Dec 12	5.9×10^5	1.2×10^4	0.9×10^3	0.3

Zoologique, Villefranche) following protocols outlined in Rassoulzadegan & Sheldon (1986).

Sampling and Incubation

Samples were collected by Niskin bottles from 5 m depth at ~9:00 and immediately brought back to the laboratory in Villefranche where incubations began within 2 h of sampling. Samples of whole water were incubated as either 10 ml subsamples in 20 ml polyethylene scintillation vials (Oct. 1, Nov. 12) or in 500 ml transparent polycarbonate bottles from which 10 ml subsamples were periodically drawn (Oct. 14, 27, Nov. 5, Dec. 15). In previous work, no differences have been noted between large and small incubation volumes (Thingstad et al. 1993). Water samples were incubated in circulating surface water at 30% incident illumination.

^{32}P-Uptake and Release

Carrier-free ^{32}P-orthophosphate (Amersham) was added to give a count of 5×10^5 to 1×10^6 CPM per 10 ml (max of 100 μl fluid per 10 ml yielding pmol l^{-1} ^{32}P). At timed intervals, 10 ml subsamples received a cold chase of orthophosphate (100 μmol l^{-1} final conc.) to halt isotope uptake and were filtered. Samples were filtered in parallel on 25 mm dia. polycarbonate filters of 10, 5, 1, and 0.2 μm pore size supported on GF/F glassfiber filters. Suction was kept low during filtration (>0.2 bar, filter pore size >1 μm, <0.2 bar filter pore size <1 μm). No washing was used and suction was left on (0.2 bar) during filter removal to minimize cell breakage and subsequent loss of incorporated label (Suttle et al. 1990). Inorganic dissolved P was separated from organic by acidification, addition of active charcoal (Ammerman & Azam 1991) and subsequent filtration through GF/F glass fiber filters of the 0.2 μm filtrates.

Cerenkov radiation from ^{32}P was counted in 10 ml of water in polyethylene scintillation vials using a Packard Tri-carb scintillation spectrometer. Added radioactivity was measured in a parallel sample in all experiments. Blanks were

subtracted and all values calculated as % of added activity. Label in each size interval was obtained by subtraction of label on the larger pore size filter from that on the lower. Label as dissolved organic phosphorus was obtained by subtraction as total radioactivity added – (total in particles + inorganic dissolved P).

Following Thingstad et al. (1993), turnover time was assumed to be related to the fraction of radioactivity absorbed following the theoretical expression:

$$r(t) = (1 - e^{-t/T})$$

where r = the fraction of added radioactivity absorbed, t = the incubation time and T = turnover time. The equation is rearranged to allow direct calculation of turnover time T:

$$T = t / -\ln(1-r)$$

Release of label from particulate size-fractions was examined using a cold chase design on October 14th, 27th and November 5th. Samples were incubated with ^{32}P-orthophosphate for 3 h and then uptake of label remaining in solution or recycled dissolved label was prevented by adding a cold chase to yield final concentrations of 100 μmol l^{-1} and 100 μmol l^{-1} AMP (Sigma). Incubation continued for 3.5 to 25 h and subsamples were periodically removed, filtered and processed as described above. Rates of change for a given size-fraction were calculated as the slope of relative concentration (percent added radioactivity) vs. time for the period from the addition of the cold chase until the end of incubation. Linear regressions were used to yield estimates of slopes and associated error.

Ciliate Addition Experiment

The effect of microzooplankton grazing on label redistribution was studied on October 15th by adding ciliates to samples which had been incubated with ^{32}P-orthophosphate. The ciliate species used, the oligotrich *Strombidium sulcatum,* is approximately 30 μm in length and feeds on prey ranging in size from 1 to 6 μm equivalent spherical diameter (Bernard & Rassoulzadegan 1990). Cultures were grown in bacterised batch cultures (Rivier et al. 1985). The basic experimental design was to follow, with time, the distribution of label in samples subjected to 3 levels of microzooplankton grazing pressure: 0, 10, and 50 ciliates ml^{-1} added.

An early stationary-phase culture of ciliates ($\sim$ 500 ciliates and 7×10^5 bacteria ml^{-1}) was used to make 3 solutions of 50 ml to yield final sample concentrations of 0, 10 and 50 ciliates ml^{-1} (but similar concentrations of culture bacteria) when added to 450 ml of pre-incubated sample. The "0 ciliates added" solution consisted of 50 ml of culture reversed filtered through 10 μm mesh Nitex to remove ciliates but not the bacteria. The 10 ciliates ml^{-1} solution consisted of 10 ml of unfiltered culture and 40 ml of 10 μm filtered culture; 50 ml of unaltered ciliate culture were used to yield the 50 ciliates ml^{-1} treatment.

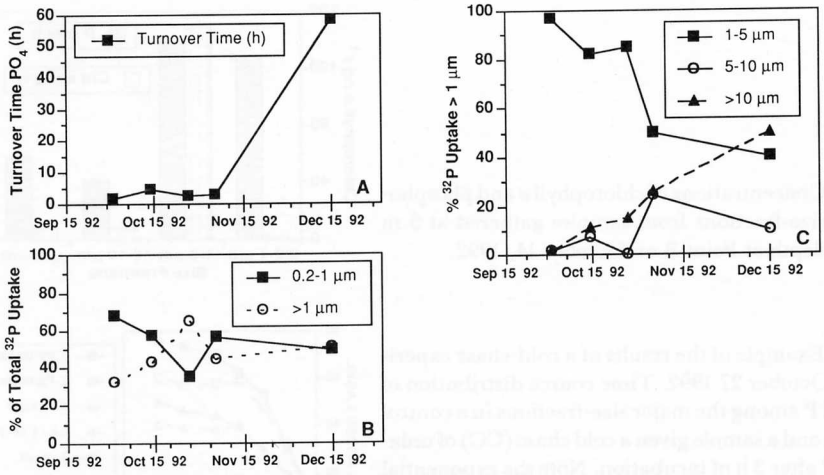

Fig. 3. Phosphorus uptake during autumn 1992. A: turnover times of free orthophosphate. B: distribution of uptake between the size fraction 0.2-1 μm and fractions $\geq$ 1 μm, assumed to represent bacteria (heterotrophic and cyanobacteria) and eucaryotic phytoplankton. C: distribution of uptake among the size-fractions $\geq$ 1 μm, eucaryotic phytoplankton. Lines joining points to emphasise seasonal trends and are not meant to allow interpolation.

After 3 h of incubation with ^{32}P-orthophosphate, 3 samples received a ciliate culture solution and an orthophosphate / AMP cold chase; a control sample received neither a ciliate culture solution nor a cold chase. Subsamples were removed periodically and processed as above for determinations of ^{32}P content in the various fractions. Transfer rates between fractions or pools were determined as described for the simple cold chase experiments.

Distributions of Chlorophyll and Particulate P

On one date, October 15, size distributions of chlorophyll *a* and phosphorus were determined. Material was collected by serial filtration using 47 mm dia. polycarbonate filters of 0.2, 1, 5 and 10 μm pore size. A suction of < 0.2 bar was applied below the 0.2 μm filter. With the filtration "tower" used (Nucleopore) the flow through the 0.2 μm filter controls the flow through the other filters. For chlorophyll, 5 samples of 500 ml were fractionated and the resulting 5 filters for each pore size were pooled to allow spectrophotometric determination of chlorophyll *a* after overnight extraction in 90% acetone. For particulate phosphorus, a 500 ml sample was filtered and particulate P measured as soluble reactive phosphorus after resuspension of filters in 10 ml distilled water and wet oxidation in acid persulphate (Thingstad et al. 1993).

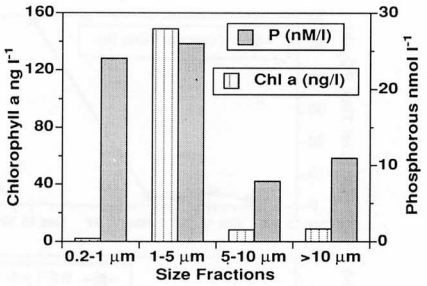

Fig. 4. Concentrations of chlorophyll *a* and phosphorus in size-fractions from samples gathered at 5 m depth at Point B on October 14, 1992.

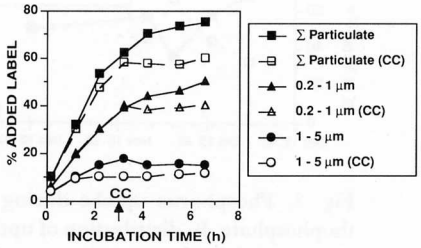

Fig. 5. Example of the results of a cold-chase experiment, October 27 1992. Time cource distribution of labeled P among the major size-fractions in a control sample and a sample given a cold chase (CC) of unlabeled P after 3 h of incubation. Note the exponential uptake in the control. Rate estimates, based on linear regression of points after the cold chase, appear in Table 1.

RESULTS

Uptake of Orthophosphate

Turnover time for orthophosphate varied from 1.6 to ~ 58 h with rapid turnover times estimated from samples in October and November and the maximum value from the mid-December sample (Fig. 3a). The 0.2-1 µm size-fraction generally accounted for most of the uptake, 38 to 68% (avg. 55%) of total absorption (Fig. 3b). Among the fractions above 1 µm, there was a gradual shift from complete dominance of the 1-5 µm fraction in early October towards an increasing importance of the > 10 µm fraction (~ 0 to 50%) from October to December (Fig. 3c).

Distribution of Particulate P and Chlorophyll a

Based on the October 14 samples, chlorophyll *a* was largely restricted (~ 89%) to the 1-5 µm fraction. The 0.2-1 µm fraction accounted for less than 5% of the chlorophyll *a* measured. Relative to chlorophyll, phosphorus was more evenly distributed between size fractions. The 0.2-1 µm and 1-5 µm fractions represented 35 and 38%, respectively, of the particulate phosphorus with the 5-10 µm and > 10 µm fractions accounting for 12 and 16%, respectively (Fig. 4).

Transfer of P: Cold Chase & Ciliate Addition Experiments

In the simple cold chase experiments, addition of unlabeled P stopped absorption

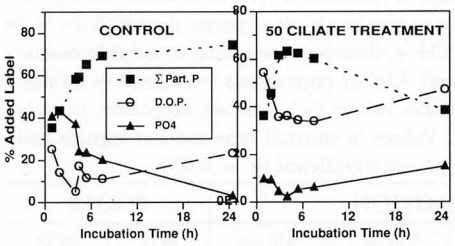

Fig. 6. Time course distribution of labeled phosphorus in the ciliate addition experiment. Distribution of label in the control (without a cold chase or ciliates added) and the sample given a cold chase and 50 ciliates ml^{-1}. Rate estimates, based on linear regression of points after the cold chase, appear in Table 2.

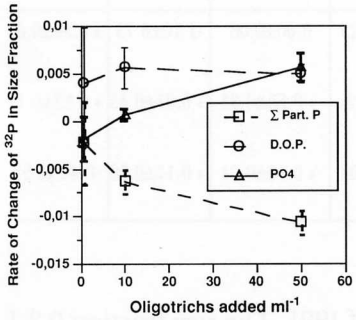

Fig. 7. Phosphorus transport rates from the ciliate addition experiment: transfer between pools as a function of ciliate concentration. Rate estimates for all the pools and size fractions, based on linear regression of points after the cold chase, appear in Table 2.

of the isotopic form (Fig. 5) but most transfer rates of label between size fractions or pools were not significantly different from zero (Table 1) with the precision obtained ($\sim$1% of incorporated label h^{-1}). In only 1 experiment (Oct. 27) was there a significant flux out of the smallest size-fraction. In contrast, in the ciliate addition experiment, significant transfer rates were estimated in samples with increased ciliate concentrations (Fig. 6). Label flux out of the total particulate pool and into the pool of dissolved inorganic P increased with ciliate concentration (Fig. 7). Label loss rates increased with ciliate concentration for the two smallest size-fractions (Table 2).

DISCUSSION

The patterns of P uptake from the early and mid-autumn samples (October and November) indicated a food web apparently dominated by a microbial complex. The smallest size fraction (0.2-1 μm) contained little chlorophyll (based on the mid-October sample: Fig. 4) but generally represented over 50% of absorption (Fig. 3b). These observations suggest that the fraction was composed largely of bacteria (dominated by heterotrophic forms, Table 1) which could compete efficiently with the larger chlorophyll-containing fractions for phosphorus. Together the two smallest size fractions $\leq$ 5 μm accounted for most (78-99%) of P uptake.

Somewhat similar results for the autumn period in Villefranche Bay concerning size-fractionated uptake of ammonium were reported by Selmer et al. (1993)

Table 2. Results of the cold chase experiments. Changes in radioactivity, as % of added isotope h^{-1}, from time course samples (n) following the cold chase in size fractions of particulate P (0.2 - > 10 μm) and pools of P (Σ part. p = total particulate, PO4 = dissolved inorganic or soluble reactive phosphorus, DOP = dissolved inorganic phosphorus). Linear regressions were used to estimate rates as slopes and associated standard errors. Positive values (+) indicate increases, negative values (-) represent decreases in a fraction or pool. Values in normal type are not significantly different from zero, values in bold type are significant (p = 0.05).

Date	Incubation Period (h)	n	SIZE FRACTION				POOLS	
			0.2-1 μm	1-5 μm	5-10 μm	>10 μm	PO4	DOP
Oct 14 1992	3-25	5	+0.14±0.79	+0.4±0.40	+0.01±0.23	0.00±0.06	-1.10±0.75	+0.54±0.61
Oct 27 1992	3-6.5	4	**-1.35±0.28**	+0.12±0.39	-0.18±0.22	+0.68±0.33	+0.07±0.45	+0.67±0.41
Nov 5 1992	3-24	6	-0.06±0.50	+0.11±0.07	+0.08±0.03	+0.10±0.04	+0.17±0.08	-0.40±0.57

for November 9, November 30, and December 4 of 1991. The size fraction 0.2-1 μm accounted for 30-40% of the estimated total ammonium uptake and organisms ≤ 10 μm accounted for 78-90% of total ammonium uptake (Selmer et al. 1993). The difference in the relative importance of the 0.2-1.0 μm fraction in orthophosphate vs. ammonium uptake could reflect differences in the concentrations of cyanobacteria and heterotrophic bacteria between the studies. However, the results of Selmer et al. (1993) were based on uptake of ^{15}N-ammonia supplied in excess of tracer quantities (i.e., isotope additions of 0.2 μM l^{-1} used equaled approximately the in situ ammonia concentrations) which could have altered uptake kinetics in favor of the larger size-classes (e.g., Suttle et al. 1990). Still, for the bay of Villefranche in early autumn, most primary and secondary production appears to occur in small size-classes which would have to pass through 2 or more steps of phagotrophy before being exploited by typical metazoan grazers (see Fig. 1) according to both N and P-based dynamics.

This nutrient-based image of a microbe-dominated food web is in general agreement with a model of carbon flow (Hagström et al. 1988) for Villefranche Bay surface waters based on a carboy experiment carried out in October of 1984; they described a food web in which cyanobacteria accounted for 57% of primary production and production by heterotrophic bacteria was approximately equal to cyanobacterial production. Hagström et al. (1988) reported loss rates for stocks of bacteria and small phytoplankton of 5 and 20% h^{-1}, respectively; the estimates were based on predation experiments conducted separately from production experiments. In contrast, the results of the P cold chase experiments indicated that loss rates from individual fractions were equal to or below 1% h^{-1}. However, the

transfer rates for P reported here are for size-fractions and not specific organisms. Loss rates recorded for a size fraction reflect net loss rates experienced by the entire community of organisms within the size-fraction. P-absorbing organisms in a size-class are a heterogeneous group (e.g. heterotrophic bacteria and cyanobacteria) possibly subjected to very different mortality rates or predation pressures. Furthermore, significant predator-prey relationships may exist within the size-fractions.

For example, among heterotrophic flagellates, small forms (< 5 μm) account for most of the predation pressure on picoplankton (Sherr & Sherr 1991). A certain portion of P ingested by small nanoflagellates would remain in the fraction 1-5 μm. Similarly, some bacterivorous flagellates pass through 1 μm filters (Cynar et al. 1985) and some bacteriophages are retained on 0.2 μm filters (Børsheim 1993) so loss of bacteria to picoflagellate and viral predation could be underestimated in losses from the 0.2-1 μm fraction. Hence, it is not surprising that P loss rate estimates are lower than predation loss estimates for individual groups.

Transfer between pools can be detected as shown in the ciliate addition experiments. Increasing grazing pressure increased transfer of phosphorus between pools, as has been seen previously in laboratory studies (e.g., Andersen et al. 1986, Bloem et al. 1988, Taylor 1986). In our experiment, labelled phosphorus was lost from the particulate pool, including the "ciliate fraction" of ≥ 10 μm, and transfer rates into the inorganic dissolved P pool increased (Fig. 7). These data imply a surprisingly low phosphorus retention, or high regeneration, efficiency for stationary phase *Strombidium sulcatum.* However, regeneration rates of protists are related to their physiological state as well as the nutritional quality of the prey items (Caron & Goldman 1988). For example, Taylor (1986) found that the freshwater ciliate *Colpidium colpoda,* in the exponential growth phase, excreted only 30% of phosphorus ingested when fed P-limited bacteria. In contrast, Andersen et al. (1986) found that regeneration rates (% ingested P excreted) approached 100% for cells in early stationary or transition stages of the growth cycle for cells feeding on P-replete prey in the omnivorous nanoflagellate *Paraphysomonas imperforata.* In a laboratory study of *Strombidium sulcatum* feeding on presumably P-replete prey, excretion of orthophosphate was linearly related to ingestion throughout the different growth phases (Allali et al. 1994).

The nutritional quality of the prey items in our experiment is difficult to judge. Prey could have been P-limited based on the rapid turnover times of P. On the other hand, the addition of phosphorus in the form of the cold chase and the ciliate culture solution may have led to "luxury uptake" (Thingstad et al. 1993), yielding prey items with low N:P ratios, which when ingested, give relatively high P excretion rates.

The phosphorous transfer rates estimates for the early autumn in Villefranche Bay indicate a food web in which little production reaches the trophic level of metazoan herbivores. The rough accuracy of the phosphorus rate measures can

Table 3. Results of the ciliate addition experiment. Changes in radioactivity, as % of added isotope h^{-1}, from time course samples (n) following the addition of ciliates and the cold chase in size fractions of particulate P (0.2 - > 10 μm) and pools of P (Σ part. p = total particulate, PO4 = dissolved inorganic or soluble reactive phosphorus, DOP = dissolved inorganic phosphorus). Linear regressions were used to estimate rates as slopes and associated standard errors. Positive values (+) indicate increases, negative values (-) represent decreases in a fraction or pool. Values in normal type are not significantly different from zero, values in bold type are significant (p = 0.05).

TREATMENT	SIZE FRACTION				POOLS		
Ciliates Added	0.2-1 μm	1-5 μm	5-10 μm	> 10 μm	Σ part. P	PO4	DOP
0	+0.065±0.49	-0.108±0.07	-0.080±.032	-0.101±0.043	-0.225±0.504	-0.172±0.077	+0.397±0.566
10	**-0.197±0.057**	**-0.390±0.083**	-0.016±0.070	-0.016±0.013	**-0.618±0.159**	**+0.064±0.016**	+0.554±0.241
50	**-0.395±0.065**	**-0.643±0.030**	+0.118±0.065	-0.153±0.080	**-1.075±0.097**	**+0.556±0.122**	**+0.519±0.094**

be assessed by checking the balance of pools and flows. Combining the measurement of particulate -P with estimated loss rates yields an estimate of the rate of transfer of P throughout the system; the transfer rate combined with turnover time gives an estimate of the free pool (Thingstad et al. 1993). For the data presented here, assuming all of the 24 $nmol^{-1}$ particulate P in the 0.2-1 μm fraction (Fig. 4) to be bacterial, an upper estimate of loss rate of 1% h^{-1} would correspond to a transfer of 0.24 nmol l^{-1} h^{-1} through the bacterial pool. If all of this is assumed to be taken up from the pool of free orthophosphate, and the bacterial contribution represents ~ 50% of total orthophosphate uptake (Fig. 3b), it will correspond to a total transfer of 0.48 nmol l^{-1} h^{-1} through the pool of free orthophosphate. A turnover time on the order of 2 h (Oct. 1, Fig. 3a) would then indicate a pool of free orthophosphate of about 1 nmol l^{-1}, far below levels measurable with traditional techniques. Employing a turnover time of 58 h (Dec. 15, Fig. 3a) indicates a pool of free (or biologically available) orthophosphate of 29 nmol l^{-1}. These estimates of free orthophosphate agree roughly with nutrient data from autumn 1991 which indicate undetectable levels of orthophosphate in early autumn with mid to late autumn concentrations increasing to 50-150 nmol l^{-1} (Fig. 2c), considering that traditional techniques used to measure soluble reactive phosphorus likely overestimate concentrations of the biologically available forms (Thingstad et al. 1993).

The general seasonal trend of a diminishing contribution to orthophosphate uptake of the small, $\leq$ 5 μm, fractions and the increasing role of the largest, $\geq$ 10 μm, fraction (Fig. 3b & c) agrees well with the seasonal trends in phytoplankton composition documented by Rassoulzadegan (1979). Such changes in phytoplankton community structure fit the general hypothesis that small organisms dominate in stable, stratified systems and larger phytoplankton in seasonal-

ly mixed systems (e.g., Rothhaupt & Güde 1992). One link connecting hydrography to the shift towards larger organisms is presumably nutrient availability. We lack contemporaneous nutrient data, but it is likely that the trend of increased nutrient concentrations with the autumn to winter transition seen in 1991 (Fig. 2c) occurred in 1992 as well. Regardless of the mechanism, phosphorus uptake showed a change in food web structure and such changes are generally considered to have profound influences on the production of higher trophic levels (e.g., Kiørboe 1993).

The data presented here indicate that, at least in early autumn, phosphorus turns over rapidly and is highly conserved within various size fractions. These observations do not prove phosphorus to be the limiting nutrient for phytoplankton or bacterial growth in Villefranche Bay, nor is such a limitation essential for the interpretations suggested. However, it should be noted that Berland et al. (1973) found that phosphorus limited diatom growth in algal bioassays of Villefranche Bay water sampled from spring through summer. Similarly, Zweifel et al. (1993) concluded that heterotrophic bacteria in Villefranche Bay are probably P-limited based on culture enrichment studies and short-term trends (hours) in mesocosms. Thus, the evidence which exists for Villefranche Bay, although based on the relatively low hierarchical levels of bioassays, community cultures and mesocosms (Hecky & Kilham 1988) is highly suggestive. If N (or another element other than P) is limiting the element must be in very near balance with P considering the relatively short turnover times found for orthophosphate in early autumn.

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