

Loss rate of an oligotrophic bacterial assemblage as measured by ^3H -thymidine and $^{32}\text{PO}_4$: good agreement and near-balance with production

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ABSTRACT: Growth and loss of planktonic bacteria are thought to be roughly in balance, but are rarely measured together. The loss rate for a bacterial assemblage in surface waters of Villefranche Bay (NW Mediterranean Sea) was estimated using 2 independent techniques. The disappearance rate of ^3H from cold-TCA-insoluble material following a labeling of the natural assemblage with ^3H -thymidine gave a turnover of $2.2\% \text{ h}^{-1}$, while the disappearance of ^{32}P from the bacterial size fraction (0.2 to $1 \mu\text{m}$) following an initial uptake period and a subsequent cold chase with orthophosphate gave a bacterial turnover rate of $2.5\% \text{ h}^{-1}$. The similarity of the 2 estimates suggests that the same loss processes were measured and that processes independent of bacterial population turnover, such as rapid uptake and release of labels, were of minor importance. The mortality estimates were close to thymidine-based production estimates of 2 to $2.3\% \text{ h}^{-1}$. Viral abundance ($\text{ca } 2 \times 10^6 \text{ ml}^{-1}$) was about 3 to 4 times that of bacteria, and relatively constant. Attempts to measure bacterial mortality due to viral infection were complicated by filtration artifacts. Passage of the thymidine-labelled assemblage through a $0.6 \mu\text{m}$ filter in order to separate bacteria and viruses from larger bacterivorous organisms removed 60% of the bacterial label. Label loss rates were undetectable in the filtered assemblage over 96 h incubations, suggesting that viruses were minor loss agents of this (minority) size fraction of bacteria. In the experiments with ^{32}P , most of the label was transferred directly from the bacterial size fraction to dissolved compounds, with relatively minor amounts (10 to 20%) transferred to larger size fractions.

KEY WORDS: Bacterial decay · Phosphate metabolism · Viruses

INTRODUCTION

Population changes reflect the net difference between growth and loss processes. For bacteria, methods aimed at measuring growth include estimating DNA (Fuhrman & Azam 1980, 1982) or protein synthesis (Kirchman et al. 1985, Simon & Azam 1989), or counting the frequency of dividing cells (Hagström et al. 1979). These methods, in principle, attempt to measure the total bacterial growth process. In contrast, most methods for estimating loss rates are specific for individual loss processes such as predation (e.g. McManus & Fuhrman 1988) or viral lysis (Proctor & Fuhrman 1990, Steward et al. 1992). An exception to

this is the approach introduced by Servais et al. (1985, 1989), who labelled a natural bacterial population using ^3H -thymidine and subsequently followed the rate of disappearance of label from the cold-TCA-insoluble fraction. This method presumably measures the total turnover rate of bacterial DNA.

In many systems, phosphate uptake has been found to be dominated by bacteria (e.g. Faust & Correll 1976, Harrison et al. 1977, Lebo 1990), particularly when orthophosphate turnover time is short (Thingstad et al. 1993). When short turnover time and bacterial dominance of the uptake coincide, a rapid and 'hot' labeling of the bacteria is possible. Adding a subsequent cold chase of unlabelled orthophosphate to stop reassimilation of remineralized phosphate allowed Thingstad et al. (1993) to estimate a turnover rate for bacterial P.

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Supposing P leakage from cells to be of minor importance, this should produce results similar to other total bacterial loss estimates.

During summer stratification, the surface waters in Villefranche Bay on the French Mediterranean coast have been shown to have short orthophosphate turnover times and a high percentage of uptake entering the bacterial size fraction (Dolan et al. 1995). In oligotrophic systems such as this one, bacteria are thought to be important reservoirs of C, N, and P (Fuhrman et al. 1989), and regeneration of these nutrients is probably affected significantly by the processes and mechanisms of bacterial loss. It is well known that protists graze bacteria and regenerate nutrients (McManus & Fuhrman 1988). The recent recognition of viruses as active parts of microbial food webs (reviewed by Fuhrman & Suttle 1993, Bratbak et al. 1994) raises the interesting question of how these P-rich agents are involved in such systems. As part of a project to investigate the roles of microorganisms in the P cycling of the Mediterranean, we investigated the turnover of bacterial biomass in this system, with an eye to understanding the contributions of bacteria and viruses to P cycling. Of particular interest were (1) a comparison of P- and thymidine-based loss estimates, (2) comparison of loss and production estimates, and (3) the possible roles of viruses in bacterial turnover.

MATERIALS AND METHODS

Sampling and incubation. Water was collected with a 5 l Niskin bottle from 5 m depth at Point 'B' at the mouth of Villefranche Bay (43° 41' 10" N, 7° 19' 00" E) and pooled in a 20 l carboy. This was brought to the Station Zoologique for distribution into individual bottles for experiments. All incubations were done in a running seawater incubator with a neutral screen removing 70% of natural light.

Size distribution of chlorophyll and particulate P. Samples (500 ml) for chlorophyll and particulate P were filtered through 47 mm polycarbonate filters of 5, 1 and 0.2 μm pore size mounted serially in a tower arrangement with a suction of $\leq 2 \times 10^4$ Pa applied below the 0.2 μm filter at the bottom. Chlorophyll was extracted overnight in 90% acetone and measured fluorometrically (Strickland & Parsons 1972). Particulate P was measured as soluble reactive phosphorus after wet oxidation in acid persulphate (Koroleff 1976) using standard autoanalyzer techniques.

$^{32}\text{PO}_4$ experiments. Two 1 l subsamples were incubated in acid-washed 1 l translucent polypropylene bottles. Carrier-free $^{32}\text{PO}_4$ (Amersham) was added to a radioactive concentration of ca 280×10^3 dpm ml^{-1} . After 3 h of incubation, a cold chase of KH_2PO_4 was

added (0.1 mM final concentration) to one of the bottles. At various intervals during the total incubation period of 24 h, 5 ml aliquots were filtered in parallel through 25 mm polycarbonate filters of 5, 1 and 0.2 μm pore size, supported on Whatman GF/F filters soaked with 10 mM KH_2PO_4 . Suction was regulated by a needle valve that was initially open and was closed successively as the aliquots passed through the filters of larger pore-size, but was not allowed to exceed 10^4 Pa (0.1 bar) before the water had passed through all the filters. The suction was then increased to ca 4×10^4 Pa, and the polycarbonate filters transferred without washing to polyethylene scintillation vials. Three ml of the 0.2 μm filtrate was transferred to a separate vial and distilled water was added to 10 ml final volume for counting of Cerenkov radiation from ^{32}P using a Packard Tri-Carb scintillation spectrometer. Values were checked for unequal quenching based on a quench curve made with Irgalan Black as a color quencher and are reported as % of a triplicate measurement of radioactivity added to each bottle.

Thymidine. Methods for estimating thymidine-based loss were modified from Servais et al. (1985). A 1 l sample was incubated with 0.25 nM tritiated thymidine (Dupont New England Nuclear) in a 1 l polypropylene bottle. At intervals of several hours, duplicate 20 ml subsamples were taken, filtered onto 0.45 μm pore-size Millipore mixed-ester filters and extracted with 5% cold trichloroacetic acid (TCA), then rinsed 7 times with TCA (twice with filter towers removed). The filters were placed into scintillation vials and the nucleic acids were hydrolyzed at 95°C in 0.2 ml of 0.5 N HCl, then cooled and counted with Ready-Safe scintillation fluid (Beckman). After about 24 h of incubation, when radioactivity in the bacteria was already declining, half of the water was filtered gently through a 47 mm diameter, 0.6 μm pore-size Nuclepore polycarbonate filter, and this was subsampled throughout the rest of the experiment and filtered as above.

Thymidine incorporation basically followed Fuhrman & Azam (1982): two 40 ml water samples and a 1% formalin-killed control were incubated with 5 nM tritiated thymidine for 1 h, then filtered through a 0.45 μm pore-size mixed-ester Millipore filter. The organisms on the filter were extracted in cold 5% TCA for 2 min, then rinsed and counted as described above. Radioactivity was converted into a production estimate assuming 2×10^{18} cells produced per mole incorporated (Fuhrman & Azam 1982).

An estimate of the portion of label in nucleic acid and 'protein' fractions was made by comparing hot and cold TCA extractions (Hollibaugh et al. 1980); for the hot extraction, nucleic acids were hydrolyzed in 5% TCA at 98°C for 1 h, then chilled and filtered as above.

Bacteria were enumerated by acridine orange direct counts (Hobbie et al. 1977). Viruses were counted after ultracentrifugation ($200\,000 \times g$, 1.5 h) onto C-stabilized formvar-coated Cu grids, staining them for 20 s with 1% uranyl acetate, blotting excess stain, and viewing on a Jeol 100 CX electron microscope at $27\,000\times$ magnification (modified from Børsheim et al. 1990).

RESULTS

Size distributions of chlorophyll and particulate P.

Total particulate P in samples from 6 and 7 July was 41 and 53 nmol P l^{-1} respectively. Most of the particulate P (avg. = 48%) was in the 0.2–1 μm fraction with the remainder distributed approximately equally between the 1–5 and $>5 \mu\text{m}$ fractions (Fig. 1). Total chlorophyll was 0.52 and 0.23 mg m^{-3} on 5 and 6 July, respectively, with the 1–5 μm size fraction containing an average of 63% of the chlorophyll (Fig. 1). On average, 10% of the chlorophyll passed the 1 μm filter and was collected on the 0.2 μm filter.

Counts of bacteria and viruses. Bacterial abundance averaged ca $5 \times 10^5 \text{ ml}^{-1}$ and viral abundance was nearly invariant at about $2 \times 10^6 \text{ ml}^{-1}$ over the study period (Table 1).

$^{32}\text{PO}_4$ labeling. After 1 h incubation about 4.5% of the added label was found in the $>5 \mu\text{m}$ size fraction, 18% in the 1–5 μm fraction and 40% in the 0.2–1 μm fraction (Fig. 2). A total of about 63% of the label assimilated in 1 h corresponds to an approximate turnover time of $T = -1 \text{ h}/\ln(1 - 0.63) = 1.0 \text{ h}$.

At the time of addition of the cold chase (8 July, 18:00 h), ca 50% of the added label was found in the bacterial size fraction (Fig. 2). In the control, this remained relatively constant throughout the rest of the experiment. In the bottle with cold chase, the label disappeared from this fraction at a rate of $2.5\% \text{ h}^{-1}$ (estimated from a linear regression of log-transformed data for the period from 6 to 24 h after the start of the experiment). In both bottles, label in the 1–5 μm size fraction

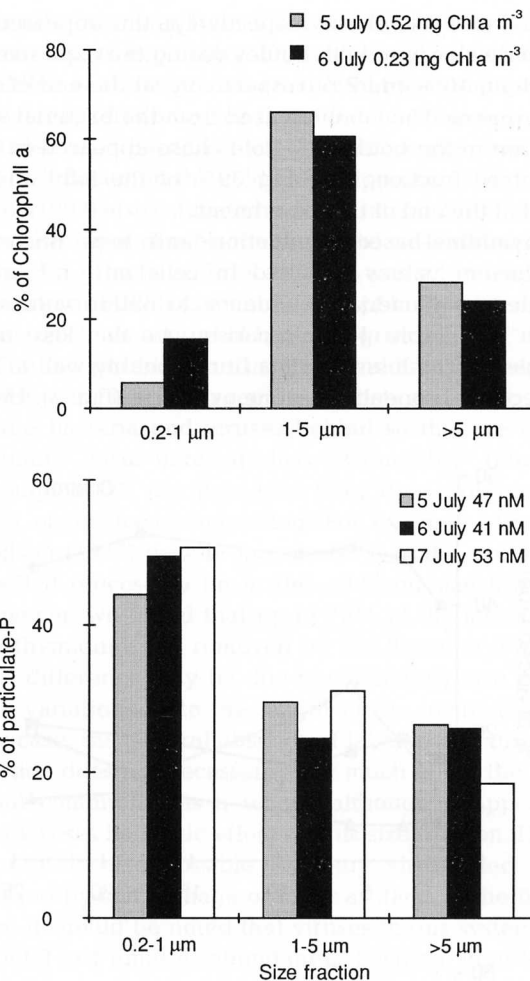


Fig. 1. Size distributions of chlorophyll *a* (upper panel) and particulate P (lower panel). Concentrations of chlorophyll and particulate P for each date are indicated. Note that the size fraction corresponding to bacterial cell sizes, 0.2–1 μm , contained a small portion of the chlorophyll *a*, but a large portion of the particulate P stocks

remained at about 20%. In the period before addition of the cold chase, only a minor fraction of the label was incorporated in the size fraction $>5 \mu\text{m}$ (6% and 3% in

Table 1. Summary of abundance, production and loss data. Values are means \pm range of duplicates or *SE. nd: not determined

Date (1993)	Viruses ($\times 10^6 \text{ ml}^{-1}$)	Bacteria ($\times 10^5 \text{ ml}^{-1}$)	Bacterial production:		Bacterial loss:	
			TdR-based ($\times 10^6 \text{ cells l}^{-1} \text{ h}^{-1}$)	(% h^{-1})	TdR-based (% h^{-1})	^{32}P -based (% h^{-1})
5 Jul	1.8 ± 0.1	4.4	nd	nd	nd	nd
6 Jul	2.0 ± 0.3	nd	8.8 ± 0.4	$\sim 2^a$	$1.7 \pm 0.1^*$	nd
7 Jul	1.7 ± 0.1	nd	nd	nd	nd	nd
8 Jul	2.0 ± 0.3	6.6 ± 0.1	15.0 ± 0.1	2.3	$2.2 \pm 0.1^*$	$2.5 \pm 0.5^*$

^aCalculated with bacterial abundance from previous day

