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# Release of Bacterial DNA by Marine Nanoflagellates, an Intermediate Step in Phosphorus Regeneration

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**The concentrations of dissolved DNA and nanoflagellates were found to covary during a study of diel dynamics of the microbial food web in the Adriatic Sea. This observation was further investigated in a continuous seawater culture when nanoflagellates were fed bacteria grown in filtered seawater. Analysis of dissolved organic phosphorus and dissolved DNA showed a sixfold increase of dissolved DNA in the presence of the nanoflagellates (*Ochromonas* sp.). The amount of DNA released suggested that the majority of the consumed bacterial DNA was ejected. Phagotrophic nanoflagellates thus represent an important source of origin for dissolved DNA. The rate of breakdown of dissolved DNA and release of inorganic phosphorus in the pelagic ecosystem is suggested to be dependent on the ambient phosphate pool. In the P-limited northern Adriatic Sea, rapid degradation of the labelled DNA could be demonstrated, whereas the N-limited southern California bight water showed a much lower rate. Phosphorus originating from dissolved DNA was shown to be transferred mainly to organisms in the <3-μm-size fractions. On the basis of the C/P ratios, we suggest that a significant fraction of the phosphorus demand by the autotrophs may be sustained by the released DNA during stratified conditions. Thus, the nucleic acid-rich bacterial biomass grazed by protozoa plays an important role in the biogeochemical cycling of phosphorus in the marine environment.**

Autotrophic growth in oligotrophic waters is primarily limited by the availability of inorganic nutrients, and in most cases, nitrogen is the limiting factor (19, 27). However, inorganic phosphorus ($P_i$) is not abundant in the sea; in fact, $P_i$ limitation has been inferred in several studies (8, 45). Available $P_i$ can sustain microbial growth only for a short time (days), and thus regeneration of this pool must be rapid. The role of zooplankton (including protozoa) in pelagic regeneration of inorganic nutrients, particularly phosphate, has long been known (29). In most aquatic ecosystems, a large fraction, in some cases more than 50% of the primary production, is recycled through a marine protozoan-bacterioplankton predator-prey chain (7, 26, 50). This heterotrophic part of the microbial food web may contain as many as four trophic levels and return nutrients to the autotrophs via release of both inorganic nutrients and dissolved macromolecules (9, 30, 47).

In seawater, nucleic acids are phosphate-rich macromolecules that constitute a reservoir for nutrients and precursors involved in the turnover of phosphorus (35). The amounts of phosphorus in dissolved DNA and in the $P_i$ pool are about the same as that of $P_i$ in the trophogenic layer (17). Studies of temporal and spatial distribution of dissolved DNA in different marine environments have demonstrated numbers typically between 2 and 20 μg of DNA per liter (17, 31, 35, 38). The rate of production of dissolved DNA and the rate of degradation in the sea indicate a rapid turnover time, typically less than a day (37, 38). This clearly indicates that phosphorus in DNA may be recycled in the food web. Ammerman and Azam (1) have shown that degradation of dissolved nucleotides mediated by a bacterial 5′-nucleotidase plays a significant role in the supply of phosphorus to planktonic algae. Thus, in addition to direct release of $P_i$ from phagotrophic organisms, dissolved organic phosphorus (DOP) can be excreted. One such example is the flagellate *Paraphysomonas imperforata*, which has been shown to excrete 10 to 14% of the ingested phosphorus as DOP, presumably when ejecting processed food vacuoles (3, 13).

In coastal waters, phytoplankton and bacterioplankton constitute a significant (70 to 90%) part of the particulate DNA, and it has been suggested that direct release from these organisms could contribute to the pool of dissolved DNA (32, 41). Paul et al. (35) found a good correlation between the seasonal and diel variation of dissolved DNA and the abundance of bacterioplankton and phytoplankton. There are reports of DNA being released from intact bacterial cells, although the physiological rationale for direct release of high-molecular-weight compounds is poorly understood (15, 18). A more simplistic explanation to the mechanism of high-molecular-weight release is perhaps death and cell lysis due to bacteriophages (10, 11, 42). However, this argument assumes that phage-induced cell lysis is a major mortality factor for bacteria. On the contrary, measurements of protozoan grazing on bacteria and the recorded growth of nanoflagellates have clearly demonstrated that the majority of the bacterial biomass is consumed by protozoa (21, 44, 49).

In this study, we observed a clear covariation between flagellate abundance and dissolved DNA in the field and decided to examine the possible production of dissolved DNA by marine nanoflagellates. In addition, we examined the importance of degradation of dissolved DNA in different coastal waters. On the basis of the information from field and laboratory experiments, we can demonstrate a significant source of dissolved nucleotides available for enzymatic activity and thus lend support to the model of phosphorus regeneration by bacterial 5′-nucleotidase activity in the pelagic ecosystem presented by Ammerman and Azam (2).

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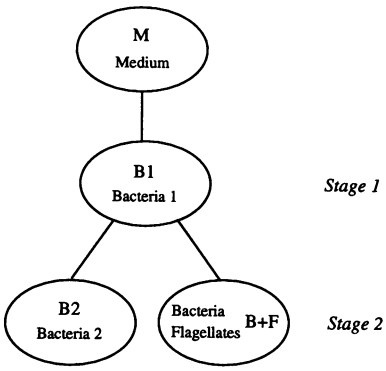


FIG. 1. Scheme for the two-stage chemostat. In the first stage, seawater bacteria were grown in autoclaved seawater (2 liters) (B1). The second stage consisted of two 1-liter bottles, one as a control with bacteria only (B2) and the second bottle with flagellates added (B+F).

## MATERIALS AND METHODS

**Continuous culture.** A two-stage chemostat was set up to examine the relationship between bacteria and nanoflagellates in a steady-state culture. Prefiltered and autoclaved seawater was used in a continuous culture apparatus, similar to that described by Hagström et al. (25). The first stage (B1) was inoculated with Baltic seawater (5 ml/liter), which had been filtered three times through 0.6-μm-pore-size Nuclepore filters and contained mixed strains of heterotrophic bacteria. The chemostat was run at a dilution rate of 0.042/h at 20°C. The overflow from the first stage was fed to parallel bottles in the second stage (Fig. 1). One of these served as a control (B2), while the other was inoculated with a culture of the nanoflagellate *Ochromonas* sp. (B+F) (4).

**Chemical analyses.** In the chemostat experiment, orthophosphate ($P_i$) and total phosphorus (TotP) concentrations were measured in filtered (0.45-μm-pore-size sterile and pyrogen-free filters; FP 030/2; Schleicher & Schuell) as well as in unfiltered samples from the medium and all vessels. Particulate organic phosphorus and DOP were calculated by difference. The instrumentation used was a Technicon TRAACS 800 Autoanalyzer, with a phosphate method based on that of Murphy and Riley (34). The baseline and calibration solutions contained 4.6 g of sodium chloride per liter (close to the chloride concentration in the samples) to avoid baseline shifts. Using a 50-mm flowcell and measuring at 880 nm, the detection limit, defined as twice the noise level, was 0.3 μg/liter. The procedure for the determination of total phosphorus included oxidation of acidified samples with potassium peroxodisulfate in an autoclave at 120°C for 30 min. The accuracy of this procedure was checked by oxidizing solutions containing known amounts of DNA (P/DNA ratio of 0.1) and being 0.46% with respect to sodium chloride. The recovery of phosphorus was found to be 102%, which means that even large molecules are completely oxidized.

TotP and phosphate for field samples were determined in unfiltered samples by standard colorimetric procedures (24).

**Cell counts.** Bacteria and nanoflagellates were counted by using epifluorescence microscopy in formaldehyde-preserved (2%) water samples, after staining with 4′,6-diamidino-2-phenylindole (DAPI) for bacteria (40) and with primuline for nanoflagellates (12).

**Bacterial production.** Bacterial production was measured on subsamples from the chemostat vessels by the incorporation of [$^3$H]thymidine following the protocol described in reference 23. Triplicates of each sample were incubated for 30 min with 250 μCi of [$^3$H]thymidine per liter (80 Ci/mmol). Moles of thymidine incorporated were converted to cells produced by the conversion factor calculated for each stage of the chemostat.

**Dissolved DNA measurements.** Concentrations of dissolved DNA were determined by using the fluorescent dye Hoechst 32258 (Calbiochem-Behring, La Jolla, Calif.), which binds specifically to double-stranded DNA (16). Samples from each vessel (500 ml) were filtered through a 0.2-μm-pore-size Nuclepore filter with a vacuum of ≤150 mm Hg (19.998 kPa). All samples were corrected for DNA loss in processing by the addition of an internal standard. Plasmid pUC18 (46) was prepared from the *Escherichia coli* FL01, linearized with the restriction enzyme *Bgl*II (33), and added to all dissolved DNA measurements as an internal standard.

**[$^{32}$P]DNA uptake into size fractions.** The plasmid (pUC18) was radiolabelled with $^{32}$P, using Amersham's multiple DNA labelling system according to the manufacturer. A 250-ng sample of the plasmid (specific activity, $2.2 \times 10^7$ cpm/μg of DNA) was added to 100 ml of sample water. Subsamples were filtered through 1- and 0.2-μm-pore-size Nuclepore filters at different time intervals (0, 15, 45, 90, 120, 160, 300, 480, and 720 min).

**DNA turnover in situ.** Turnover and transfer of P originating from DNA was determined by using nick-translated $^{32}$P-labelled plasmid DNA (pBR322). [$^{32}$P]DNA was added at tracer levels to seawater (1 liter). The final concentrations were 0.69, 0.16, and 0.55 ng of DNA per ml in the Adriatic, Scripps, and Baltic experiments, with corresponding specific activities of $2.8 \times 10^7$, $1.5 \times 10^7$, and $2.0 \times 10^7$ cpm/μg, respectively. Subsamples (five or more over 24 h) were filtered in series onto 10-, 3-, 1-, and 0.2-μm-pore-size Nuclepore filters to measure uptake of $^{32}P_i$ released from the substrate. A portion of the filtrate was assayed for P added as the substrate and for $P_i$ release from [$^{32}$P]DNA by the method of Ammerman and Azam (1). Samples for microscopic counts were size fractionated with the same equipment as for the fractionation of the incorporated label.

## RESULTS

During a study of diurnal growth dynamics in the microbial food web in the Gulf of Trieste (northern Adriatic), a strong covariation between nanoflagellate abundance and dissolved DNA concentrations was observed. The abundance of nonpigmented nanoflagellates with an average volume of 15.8 μm$^3$ increased from $2 \times 10^6$ to $3.5 \times 10^6$/liter during the night, following a peak in bacterial abundance (Fig. 2a). Periods of high dissolved DNA concentration coincided with the peaks of nanoflagellates, and the concentrations varied from 2 to 7 μg of DNA per liter (Fig. 2b). This led us to believe that DOP released by the nanoflagellates may contain dissolved DNA.

**Continuous seawater culture.** The hypothesis that the nanoflagellates release high-molecular-weight dissolved organic phosphorus was tested in a two-stage continuous seawater culture (Fig. 1). In the first stage (B1), bacteria were grown in particle-free seawater with a dilution rate of 0.042/h. The second stage consisted of two parallel vessels ($D$ = 0.042/h) and was supplied with bacteria from B1. One vessel served as a control with bacteria (B2), and the other contained bacteria and nanoflagellates (B+F). The experiment lasted 1 month, and during the final 10 days of

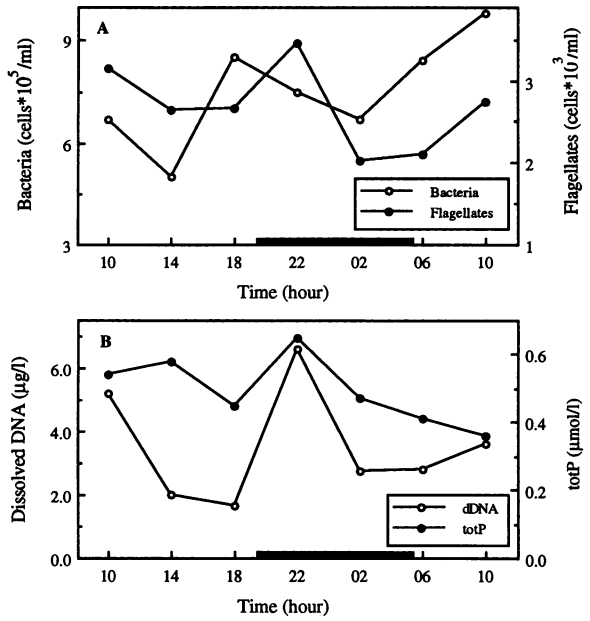


FIG. 2. Abundance of bacteria and nanoflagellates (A), TotP (B), and dissolved DNA (B) during a study of diel dynamics of the microbial food web in the Gulf of Trieste (northern Adriatic). Measurements were made during the period of mixed water column, 28 and 29 October 1987.

sampling, the average bacterial concentration in the vessel B1 was $5.2 \times 10^6$ cells per ml. In the control (B2), bacterial numbers reached an average of $5.8 \times 10^6$ cells per ml. In the parallel culture (B+F) with nanoflagellates (*Ochromonas* sp.), bacterial numbers were reduced compared with the control and declined with increasing flagellate abundance. Average numbers of bacteria and nanoflagellates were $3.6 \times 10^6$ and $1.5 \times 10^3$/ml, respectively (Table 1).

**Partitioning of phosphorus and dissolved DNA.** To determine the level of release of DOP, including dissolved DNA,

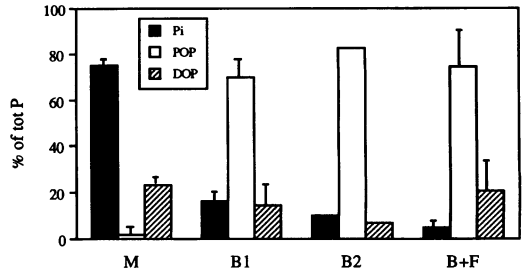


FIG. 3. Partitioning of the phosphorus pool into particulate (POP), dissolved organic (DOP), and inorganic phosphorus ($P_i$) in the different vessels of the chemostat (M, medium; B1, first stage; B2, second stage (bacteria); and B+F, bacteria plus flagellates. Error bars show standard deviation.

and phosphate ($P_i$), we measured the phosphorus content in the total, particulate, and dissolved fractions of the chemostat. Bacteria in B1 used 56% phosphorus in the form of $P_i$ and up to 14% as DOP (Fig. 3). In the second stage, the starving bacteria in B2 used more $P_i$ and DOP, whereas the concentrations of DOP and dissolved DNA increased in B+F culture (Table 1; Fig. 3). The average concentration of dissolved DNA in the outflow of B1 was 3.5 µg/liter, whereas in the control vessel, B2, the concentration of dissolved DNA was below the detection limit. In the parallel culture, B+F, the concentrations ranged between 12 and 22 µg of DNA per liter (Table 1). The dissolved DNA fraction of DOP increased from 4 to 37% in the presence of the nanoflagellates.

**Degradation of DNA and bacterial growth.** The possibility that the released DNA might have been used by bacteria growing in the nanoflagellate culture was tested in a separate experiment. A bacterial plasmid (pUC18) labelled with $^{32}P$ was added to samples from the B+F vessel at a final concentration of 250 ng of DNA per liter. Subsamples were taken at intervals and fractionated with polycarbonate filters. Degradation of the plasmid was linear, with a half-life of 360 min, and only a small amount of $P_i$ was detected. Label

TABLE 1. Release of dissolved DNA by nanoflagellates grazing heterotrophic bacteria[a]

| Culture vessel and flow direction | No. of bacteria ($n$ = 10) ($10^6$ cells/ml) | Bacteria produced ($10^6$ cells/ml) | No. of flagellates ($n$ = 10) ($10^3$ cells/ml) | Bacteria consumed ($10^6$ cells/ml) | [$^3$H]thymidine uptake ($10^{-13}$ mol/ml/h) | Dissolved DNA ($n$ = 3) (µg/liter) |
|---|---|---|---|---|---|---|
| B1 | | | | | | |
| In | 0 | | | | 1.82 | 3.5 ± 0.5 |
| Out | 5.2 ± 0.8 | 5.2 | | | | |
| B2 | | | | | | |
| In | 5.2 ± 0.8 | | | | 0.65 | ≤1[b] |
| Out | 5.8 ± 1.1 | 0.6 | | | | |
| B+F | | | | | | |
| In | 6[c] | | | | 0.81 | 16.3 ± 4.5 |
| Out | 3.6 ± 0.8 | 0.8[d] | 1.5 ± 1.1 | 2.4 | | |

[a] The bacterial standing stock, growth rate, and concentration of dissolved DNA were monitored in a two-stage continuous culture. The overflow from the first stage (B1) was fed to parallel bottles in the second stage, where one served as a control with bacteria only (B2), and the second was inoculated with nanoflagellates (BFF). Samples for cell counts were drawn during 10 consecutive days, and samples for dissolved DNA were drawn every third day.

[b] Detection limit.

[c] Output from B1 ($5.2 \times 10^6$/ml) corrected for growth in B+F ($0.8 \times 10^6$/ml).

[d] Growth calculated from [$^3$H]thymidine uptake assuming the conversion factor to be the same as in B2.

TABLE 2. Turnover of dissolved DNA in relation to the concentrations of inorganic phosphorus in different marine environments[a]

| Sample location | Dissolved DNA | | | Inorganic phosphorus | |
|---|---|---|---|---|---|
| | Ambient (μmol/liter) | Degradation[b] | | Release (μmol/liter/day) | Ambient (μmol/liter) |
| | | %/h | $r^2$ | | |
| Gulf of Trieste | 2–7[c] | 11 | 0.92 | 0.031 | 0.01–0.2[c] |
| Baltic Sea[d] | 17.1 ± 12.7 | 5.6 ± 3.6 | >0.8 | 0.056 ± 0.039 | 0.02–0.1 |
| Off Scripps Pier (m) | | | | | |
| 0 | 2.2 | 1.1 | 0.91 | 0.002 | 0.18–0.4[e] |
| 10 | 1.4 | 0.9 | 0.73 | 0.009 | |
| 50 | 2.2 | 0.6 | 0.97 | 0.001 | |

[a] Labelled DNA was added to seawater samples in tracer levels (<10% of ambient dissolved DNA) and incubated for 24 h (Fig. 4). Degradation of DNA was calculated from the initial linear part of the regression between the amount of labelled DNA and time.
[b] Slope of regression and correlation coefficient.
[c] Range of values during diel measurements ($n$ = 7) 28 to 29 October 1987.
[d] Values from cruise track through the Baltic proper, July 1988, average for seven locations (42a).
[e] Amount of $P_i$ in pier samples measured by Ammerman and Azam (1).

was incorporated both in the >0.2-μm and the >1-μm fractions, indicating that labelled bacteria to some extent were preyed on by the nanoflagellates (data not shown).

The growth of bacteria was measured by the [$^3$H]thymidine incorporation method (23). In B1, the uptake was $1.8 \times 10^{-13}$ mol of thymidine per ml per h. In the same vessel, the production (direct counts in the outflow) of bacteria was $5.2 \times 10^6$ cells per ml per day; hence, the conversion factor for bacteria produced versus uptake of [$^3$H]thymidine was $1.2 \times 10^{18}$ cells per mol of thymidine. In vessel B2, the number of bacteria increased somewhat in abundance because of the residual production of $0.6 \times 10^6$ cells per ml per day, and the uptake of thymidine was $0.65 \times 10^{-13}$ mol of thymidine per ml per h. The conversion factor in this case was $0.38 \times 10^{18}$ cells per mol of thymidine, three times lower than in B1. In B+F, bacterial numbers were reduced because of grazing ($2.4 \times 10^6$ cells per ml per day), and on the basis of the uptake of thymidine (18% higher than in the control B2), we estimated a simultaneous growth of bacteria of $0.8 \times 10^6$ cells per ml per day (Table 1).

**DNA turnover in nature.** Outside the Bay of Piran (Gulf of Trieste), we observed daily changes in the concentration of dissolved DNA (Fig. 2). The importance of this DNA turnover for the supply of $P_i$ to the pelagic food web was investigated by using nick-translated $^{32}$P-labelled plasmid DNA (pBR322). The turnover and transfer of phosphorus from DNA was determined through filtration as the amount of label in different size fractions. In addition, a portion of the seawater was assayed for $^{32}$P decrease and $^{32}P_i$ release by the method of Ammerman and Azam (1). The labelled DNA was rapidly hydrolyzed with a degradation rate of 0.4 μg of DNA per liter per h (Table 2; Fig. 4A). A tightly coupled uptake of labelled $P_i$ was observed during the first 5 h of the incubation, after which the pool of released $P_i$ increased. Uptake of the label was found preferentially in the size fraction from >1 to <3 μm. Only small amounts of tracer (5%) were transferred into the size fractions larger than 3 or 10 μm (Fig. 4B). The distribution of various organisms, determined in the microscope, in the >1- to <3-μm fraction showed bacteria (78%) and cyanobacteria (90%) as well as nanoflagellates (70%) (Table 3).

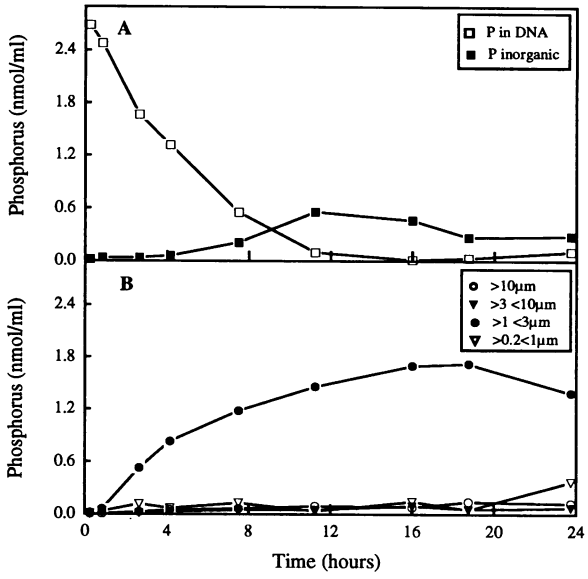


FIG. 4. Degradation and transfer of phosphorus from $^{32}$P-labelled plasmid DNA (pBR322) added to seawater samples from the station in the Gulf of Trieste in October 1987. (A) Amounts of dissolved phosphorus in added substrate and inorganic phosphorus as $P_i$ released from substrate but not taken up by organisms; (B) Amounts of particulate phosphorus as uptake of released $P_i$ in microorganisms in different size fractions.

The results from the P-limited Adriatic Sea indicated a rapid turnover of DNA and a significant uptake of $P_i$ (Table 2; Fig. 4). In contrast, the rate of DNA degradation in the non-P-limited environment of the Southern California Bight was relatively slow (Table 2). In the samples collected from different depths off Scripps Pier, the ambient pool of the dissolved DNA was low (1.4 to 2.2 μg/liter). The degradation rate of $^{32}$P-labelled DNA decreased gradually with depth from 55 to 34 ng of DNA per liter per day, and a low uptake of label and a slow release of $P_i$ were noticed (Table 2). The degradation rates as well as uptake and $P_i$ release in the different sea areas suggests a tight coupling between hydrolysis of dissolved DNA and ambient phosphorus level.

## DISCUSSION

The nucleic acid-rich bacterial biomass potentially plays an important role in the biogeochemical cycling of phosphorus in the marine environment (31, 35). The bacterial biomass turns over rapidly (in the Baltic Sea and the Gulf of Trieste, ~70 to 100 times a year), being controlled mainly by phagotrophic protozoa (5, 22, 47, 48). These protozoa are

TABLE 3. Distribution of microorganisms in different size fractions in seawater samples from the Gulf of Trieste (northern Adriatic, 30 October 1987)

| Organism | No. of cells/ml | Distribution in different size fractions (%) | | | |
|---|---|---|---|---|---|
| | | >10 μm | >3 to <10 μm | >1 to <3 μm | >0.2 to < 1 μm |
| Ciliates | 38 | 59 | 41 | 0 | 0 |
| Flagellates | 1,830 | 0 | 24 | 76 | 0 |
| Cyanobacteria | 4,363 | 0 | 2 | 90 | 7 |
| Heterobacteria | 788,400 | 0 | 3 | 78 | 19 |

known to release a large fraction of the ingested DOP. To determine whether release of dissolved DNA by nanoflagellates contributes significantly to the pool of dissolved phosphorus in the sea, we simulated the flow of phosphorus through a microbial food web using the continuous culture technique. Phytoplankton were not included in the experiment, so as not to confuse our results with the uptake kinetics of the autotrophs. Bacteriophages have been suggested as an important factor for bacterial mortality (10, 11, 28, 42). However, our data do not suggest that phage-induced lysis is important for the production of dissolved DNA. Still, we cannot exclude that part of the DNA released by the flagellates contains phage DNA from bacterial cells carrying phages. This is possible even if bacterial mortality is totally dominated by protozoan grazing, since the bacterial cells can contain phages in different developmental stages and still be subjected to predation.

During the experiment, a mixed culture of seawater bacteria was sustained through a continuous supply of filtered (<0.2-μm pore size) seawater (dissolved organic carbon, 4 mg/liter). In the first stage, inorganic phosphorus and DOP were incorporated into the bacterial biomass (70% of total dissolved phosphorus). The growth rate of the bacteria in B1 was kept at a level of one doubling per day. Bacteria in the control vessel (B2) and in the vessel with nanoflagellates (B+F) were subjected to an additional residence time of 24 h, to allow flagellates to utilize the bacteria grown in B1. A significant amount of DOP was released as the result of nanoflagellates grazing bacteria. The released dissolved organic matter stimulated bacterial growth somewhat, but did not support an extensive growth of bacteria as indicated by measurements of bacterial production by the tritiated thymidine incorporation technique (Table 1). The uptake of thymidine compared with the actual production of bacteria in B1 suggests a conversion factor for moles of thymidine to number of cells of $1.2 \times 10^{18}$ cells per mol of thymidine. This is well within the range of reported values (9, 23, 43). The starving cells in the second stage (B2) apparently incorporated more thymidine per cell since the corresponding conversion factor was $0.38 \times 10^{18}$ cells per mol of thymidine. For the calculation of bacterial growth in the flagellate vessel B+F, we used the same conversion factor as for B2 since the total uptake of thymidine was low (18% above B2), indicating substrate exhaustion.

Our measurements of dissolved DNA showed a fivefold increase of dissolved DNA in the presence of the nanoflagellate *Ochromonas* sp. (Table 1). The rate of production was 16.3 μg of DNA per liter per day. The phosphorus content of this DNA constituted ~37% of the released DOP pool. The dissolved DNA (obtained by ethanol precipitation of filtered [0.2-μm pore size] seawater or chemostat samples) may have included free soluble DNA as well as DNA in virus particles. In the vessels without nanoflagellates (B1 and B2), some release of dissolved organic matter could have been caused by phage-induced cell lysis, since a lesser amount of dissolved DNA was found (3.5 μg of DNA per liter). Paul et al. (39) investigated the fraction of the dissolved DNA in seawater that could be attributed to nucleic acids encapsulated in virus particles. They found that filtration through Nuclepore filters (0.2-μm pore size) reduced the phage counts to one-third of the total numbers. On the basis of published values of phage abundance and DNA content, they concluded that the viral DNA averaged less than 5% of the total dissolved DNA.

Consumption of bacteria by the nanoflagellates was calculated from the difference in bacterial abundance in the inflow and the outflow of the vessel and corrected for resident growth. On average, 67 bacteria were consumed per nanoflagellate. In the first stage, the DNA content of the bacteria of the chemostat was assumed to be 6.0 fg per cell. This is a minimum estimate based on the value of 6.2 fg per cell for exponentially growing marine bacteria in seawater cultures (14) and the range of DNA content in bacteria (5.8 to 14.4 fg per cell) reported from various near-shore locations (36). We assumed that the DNA content of bacteria is the same in all three vessels; thus, the nanoflagellates (*Ochromonas* sp.) consumed bacteria with a total of 14.9 μg of DNA per liter per day. The standing stock of bacteria in B+F was $3.6 \times 10^6$ cells per ml, which corresponds to 22.3 μg of DNA per liter, slightly higher than concentrations of particulate DNA measured in near-shore marine waters (3). The amount of DNA released by the nanoflagellates (16.3 μg of DNA per liter) was almost the same as the amount of ingested bacterial DNA, suggesting that the majority of the bacterial DNA was ejected. If anything, the amount of ejected DNA should be an underestimate, since in the chemostat some of the dissolved DNA was also utilized by bacteria. Although the growth of bacteria in the nanoflagellate culture was limited, as shown by the [$^3$H]thymidine incorporation, the control experiment with added DNA demonstrated a decrease in the labelled DNA with a half-life of about 6 h. Label accumulated in the particulate fractions >0.2 μm and >1 μm; however, the estimated rate of DNA removal from the solution probably depends on both adsorption and uptake capacity (38). In the control experiment, no release of $P_i$ was detected, suggesting a low actual degradation of the dissolved DNA.

To evaluate the release of dissolved DNA in an alternative way, a phosphorus budget can be made. Bacteria in B1 make up all the particulate organic phosphorus (26.2 μg of P per liter), and the average P content of the bacteria (5.04 fg of P per cell) is comparable to results from mixed heterotrophic bacteria in other chemostats (6, 9). The nanoflagellates in the culture consumed 12.2 μg of P in bacteria per liter per day. If we assume a release of 15% of the ingested P in the form of DOP as measured for other cultured protozoa (3), then the release of 1.7 μg of P per liter per day represents 17 μg of DNA per liter per day, which agrees well with our direct measurements of dissolved DNA.

To understand the mechanisms involved in returning nutrients from particulate organic phosphorus to autotrophic organisms, we utilized the chemostat data in field observations. In situ measurements in the Adriatic Sea (Gulf of Trieste) show a strong diel periodicity and relationship between dissolved DNA, bacteria, and protozoa. Based on cell counts of bacteria and bacterial production ($dN/dt$ = PD − BG, where PD is predation on bacteria and BG bacterial growth), nanoflagellates consumed $3.8 \times 10^5$ to $7.2 \times 10^5$ bacteria per ml per day. Assuming the same DNA content per cell as in continuous culture, consumption by flagellates was 2.4 to 4.5 μg of DNA per liter per day. The concentration of dissolved DNA varied between 2 and 7 μg/liter, showing the same diel periodicity as the amount of TotP (Fig. 2). The percentage of phosphorus bound in dissolved DNA represented only a small part of the TotP (3.5%) and DOP (1.5%) normally found at this time of the year (20). However, the rapid turnover of DNA (4.7 h) mediated a release of $P_i$ of 0.03 μmol of P per liter per day. Furthermore, a tight coupling between released $P_i$ and uptake by the nanoplankton and picoplankton size fractions was demonstrated. If we assume a phytoplankton C:P ratio of 110:1, the

release of this amount of $P_i$ can sustain a significant growth of autotrophs (40 μg of C per liter per day).

Degradation of dissolved DNA and uptake of $P_i$ in the Adriatic Sea was found to be similar to the rates observed in the Baltic Sea, while in the non-P-limited waters off Scripps Pier, the rate of DNA degradation and $P_i$ release was much lower (Table 2). The level of $P_i$ is regularly lower in the Adriatic and Baltic seas compared with the southern California bight, suggesting a negative relationship between turnover of DNA and ambient $P_i$ level. The hydrolysis of DNA is dependent on the enzymatic activity of free or cell-associated nucleotidases, and a negative correlation between the uptake of regenerated $P_i$ and the ambient phosphate pool was shown by Ammerman and Azam (2) by measuring the 5′-nucleotidase activity in different $P_i$ environments. This can explain the high rate of degradation of dissolved DNA in P-limited areas compared with the California Bight (Scripps Canyon).

In this study, we demonstrated that excretion by phagotrophic flagellates of bacterial DNA may represent an important source of origin for the dissolved DNA. Furthermore, we demonstrated transfer of phosphorus originating from dissolved DNA to microorganisms. Thus, our results both extend and lend support to the model of $P_i$ regeneration recently presented by Ammerman and Azam (2).

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