

Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients

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ABSTRACT: Seawater cultures were used to study seasonal and diel variations in bacterial growth and nutrient availability. In both the Baltic Sea and the Northeast Mediterranean, the least available component for bacterial growth was phosphorus. In the Baltic Sea, carbon was available in excess for bacterial growth on all sampling occasions. Compared to the controls, additions of nonlimiting concentrations of inorganic nitrogen and phosphorus increased the yield of bacteria compared to the control with 156% and the degradation of dissolved organic carbon (DOC) by 64% (as determined by high temperature catalytic oxidation). Analogous, bacterial growth yield increased along with an accumulation of inorganic nutrients in diel experiments with an intact foodweb (microcosm). The concentration of utilizable carbon (UC) was determined from bacterial consumption of DOC in seawater cultures during non-limiting nutrient conditions. Utilizable phosphorus (UP) and utilizable nitrogen (UN) were calculated by converting the bacterial biomass in the cultures into phosphorus and nitrogen equivalents. In the Baltic the average concentrations of UC, UN and UP were found to be 23 μM C, 0.6 μM N and 0.03 μM P respectively. Heterotrophic bacteria preferentially utilized inorganic nitrogen and phosphorus to support growth on a short time scale (days). Bacterial carbon content decreased as a result of nutrient additions from 51 ± 7 to 32 ± 5 fg C cell⁻¹. Growth efficiencies varied from 11 to 54% in untreated cultures compared to 14 to 58% in cultures supplemented with nitrogen and phosphorus.

INTRODUCTION

Heterotrophic bacteria in the trophic layer of the sea usually encounter a dissolved organic substrate with a surplus of carbon compared to a balanced growth substrate (Williams 1986). Hence, either bacteria must specifically select compounds that are rich in nitrogen and phosphorus or the organic substrate must be supplemented with inorganic N and P (Goldman et al. 1987). Inorganic nutrients as limiting factors for bacterial growth have been observed in the Baltic Sea and experiments by Horrigan et al. showed that seawater off Scripps pier (California, USA) could support an increased bacterial biomass when supplemented with inorganic nitrogen (Horrigan et al. 1988, Heinänen & Kuparinen 1992). Also, Wheeler & Kirchman showed that the net growth rate of heterotrophic bacteria from the Gulf Stream was significantly higher

after the addition of glucose and ammonium compared with an amino acid mixture (Wheeler & Kirchman 1986). Availability of nutrients has traditionally been viewed to be correlated with algal abundance although bacteria, especially in oligotrophic waters, represent a dominant biomass (Fuhrman et al. 1989, Azam & Smith 1991). With high turnover rates, heterotrophic bacteria have the potential to compete successfully with algae for dissolved inorganic nutrients (Currie & Kalff 1984).

Bacterial growth rates generally reach highest levels during summer although measurable substrate levels are low, which means that food transfer through the microbial food web ought to be efficient and tightly coupled. Nevertheless, there are numerous records of diel shifts in most measured parameters, suggesting uncoupling between producers and consumers, and resulting in accumulation of cells

and metabolic products (Mopper & Lindroth 1982, Straskrabova & Fuksa 1982, Hagström & Larsson 1984, Riemann et al. 1984, Fuhrman et al. 1985, Burney 1986, Hama et al. 1988, Paul et al. 1988, Wikner et al. 1990). Rapid turnover and strong diel variations in nutrient supply may therefore be important factors influencing the short-term (hours) growth conditions for heterotrophic bacteria.

In this study, we investigated how different nutrient conditions may affect bacterial growth and utilization of DOC. Nutrient limitations and instantaneous substrate pools were determined in cut-off experiments (no primary producers or predators) using seawater cultures. Pool sizes of utilizable substrates were determined and compared with *in situ* turnover of the bacterial community. Bacterial growth conditions were also studied in 2 non-cut-off experiments, using microcosms. These experiments were performed in the Mediterranean Sea with pronounced dark and light cycles. This allowed us to study the effect on bacterial growth of diel changes in the flow of nutrients through the microbial food web.

MATERIALS AND METHODS

Sampling and seawater cultures. Water was collected at 4 m depth at NB1 (63° 30' N, 19° 48' E), a coastal sampling station in the northern Bothnian Sea. The maximum depth at the station is 24 m and the salinity ranges between 3 and 6‰. On 2 occasions, water was collected at Point B in the bay of Villefranche-sur-Mer (43° 41' N, 7° 19' E) in the Mediterranean Sea. Samples were collected using a polycarbonate water sampler and transferred to acid-rinsed polycarbonate bottles.

Sampled water was filtered through 0.2 µm filters (Gelman Supor) at <200 mm Hg using acid-washed filtration equipment (Nalgene polysulfone unit or Millipore Sterifile unit) (Ammerman et al. 1984). The first 50 ml was discarded and the remainder was transferred to polycarbonate bottles. Predator-free inoculum was prepared by gravity filtration 3 times through 0.6 µm polycarbonate filters (MSI) and added to the cultures to give a 10-fold dilution of the inoculum (final concentration 5×10^4 to 10×10^4 cells ml⁻¹). Cell counts of the inoculum were made under epifluorescent illumination and no predators or autofluorescent primary producers could be detected. DOC does not increase while preparing a 0.6 µm filtrate (checked in separate experiment) and since the same water was used for the inoculate and the experiment the DOC in the seawater culture does not change by addition of the inoculate. All filters used in preparing cultures and inoculum were soaked in 1 M HCl and thereafter

extensively rinsed in ultra-pure water (Millipore Milli-Q) prior to use.

The cultures were incubated in the dark at *in situ* temperature except for cultures prepared in 1991 which were all cultured at 15 °C (the *in situ* temperature ranged between 5 and 15 °C).

Nutrient additions. Cultures were prepared as described above. Bacterial numbers in the cultures were monitored and the water was divided into 4 subsamples when growth rates started to decline. The subsamples were treated as follows: (1) no further treatment, (2) ammonium addition, (3) phosphate addition, (4) phosphate and ammonium addition. The cultures were sampled for bacterial numbers and DOC at regular time intervals. When stationary phase was reached the cultures were sampled for nutrients and POC. Parallel experiments with nutrient additions immediately after inoculation gave identical results.

Nutrients were added to give an enrichment of 0.6 µM PO₄-P (Na₂HPO₄) and 2 µM NH₄-N (NH₄Cl) which approximates inorganic nutrient concentrations at NB1 during unstratified winter conditions. Phosphate and ammonium concentrations were measured at the start and end of the incubations. The remaining amount of the added nutrient (≥ 1.5 µM NH₄ and ≥ 0.5 µM PO₄) confirmed that the added nutrient never became limiting for growth. All solutions used were cross-checked for inorganic nutrient and organic carbon contamination. Carbon additions were only performed in Villefranche-sur-Mer in September 1992. Sucrose was added to increase the DOC concentration by 80 µM which equals approximately a 60% increase in this area.

Microcosm experiment. Microcosm experiments were performed in Villefranche-sur-Mer in September 1984 and 1992. Seawater was prefiltered through a 150 µm net in order to eliminate large zooplankton and thereafter transferred to acid-rinsed and seawater-rinsed bottles (50 l glass 1984, 10 l polycarbonate 1992). The bottles were monitored every 3 h for bacterial and flagellate numbers, ammonium and phosphate concentrations, DOC (1992 only) and yield on 0.2 µm filtered water (Hagström et al. 1988).

Bacterial and flagellate enumeration. Samples for bacterial and flagellate enumeration were preserved with 0.2 µm filtered formaldehyde. 5 to 10 ml of the samples were used for counting bacteria either by DAPI or acridine orange staining and 10 to 20 ml was used for counting flagellates by proflavin staining (Hobbie et al. 1977, Porter & Feig 1980, Haas 1982). Cyanobacteria were counted by autofluorescence. Cells were counted in an epifluorescence microscope (Zeiss Universal). At least 20 fields and 200 objects were counted for bacterial enumeration and 40 fields for flagellates.

Bacterial production. Bacterial production was measured using the [^3H]thymidine incorporation method (Fuhrman & Azam 1982). Samples were incubated for 1 h with 10 nM [^3H]thymidine (final concentration) under *in situ* conditions. A conversion factor of 1.7×10^{18} cells per mole [^3H] thymidine was used to convert moles of incorporated thymidine to number of produced cells.

Dissolved organic carbon (DOC). DOC samples were filtered through 0.2 μm filters (Gelman Supor) using disposable syringes (Terumo) connected to filter holders (Millipore) or by use of polycarbonate filtration units (Nalgene). 7.5 ml of filtered water was transferred to polypropylene test tubes (Falcon, 15 ml), immediately acidified with 100 μl 1.2 M HCl and kept at +4 $^{\circ}\text{C}$ until analysis. All materials in contact with the samples, including the filters and filter units, were carefully acid-rinsed with 1 M HCl and washed with ultra-pure water (Millipore Milli-Q) prior to use. DOC was measured with a high temperature carbon analyzer (Shimadzu TOC 5000) using a 4-point calibration curve with potassium biphthalate as standard. Standard solutions were run at each analysis in order to check for instrumental shifts. Blanks were tested before each analysis by injection of ultra-pure water (Millipore Milli-Q). The total blank (water + system blank) was in all cases less than 0.1 mg l^{-1} . Samples were not corrected for this blank. Triplicate injections showed standard deviations of 0 to 2%. In most cases at least 2 individually filtered samples were analysed and showed standard deviations of 0 to 1% from the mean value of triplicate injections. The detection limit of the system for DOC \approx 100 nM.

Particulate organic carbon (POC). Samples of 200 to 500 ml from the seawater cultures were filtered through precombusted (30 min, 200 $^{\circ}\text{C}$) 0.2 μm silver filters (Poretics) at maximum 200 mm Hg. Blanks were prepared by filtering particle-free media onto precombusted filters. The total blank (filter + nonspecific adhesion of <0.2 μm organic material) made up 3 to 10% of the total sample (filter + nonspecific adhesion of <0.2 μm organic material + sample) and was subtracted. The filters were air-dried and thereafter stored frozen prior to analysis with a CHN analyser (Carlo Erba).

Nutrient measurements. Nutrient determinations in 1984 were performed using standard methods according to Strickland & Parsons (1972) and in 1991 and 1992 with an autoanalyzer (Technicon TRAACS 800). Dissolved organic nitrogen (DON) was calculated from measurements of total nitrogen in filtered samples minus the sum of inorganic nitrogen. Dissolved organic phosphorus (DOP) was calculated accordingly. The detection limit of the system for dissolved inorganic phosphorus (DIP) \approx 10 nM; dissolved inorganic nitrogen (DIN) \approx 30 nM.

RESULTS

Seawater cultures

Bacterial growth was followed in a number of seawater batch cultures during 1991 and 1992. Filtered (<0.2 μm) seawater was inoculated with ambient bacteria from the coastal station NB1. The cultures were used to assay the number of bacteria that can be supported by 0.2 μm filtered seawater and thus the pool of dissolved utilizable substrates. In the text, this assay is referred to as yield of bacteria. The bacteria showed a classical sigmoid growth pattern (Fig. 1). After a lag phase that varied between 10 to 40 h, bacteria grew with a generation time of 12 to 32 h and reached stationary phase within 120 h. The bacterial consumption of dissolved organic carbon varied between 1 and 7% of the total DOC (data not shown). To check if the seawater was contaminated by the filtration procedure, inorganic nutrients and organic carbon were measured before and after filtration. No measurable contaminants could be found within the precision of our methods. In order to further establish the reproducibility of the assay system, we duplicated the cultures on 3 occasions. The results showed only minor deviations in bacterial yield and DOC consumption (Table 1).

Periodic sampling at NB1 showed a seasonal variation in the yield of bacteria (Fig. 2). The number of ambient bacteria, reflecting the carrying capacity of the seawater (as defined in Begon et al. 1986), exceeded the yield of bacteria on all but 2 occasions

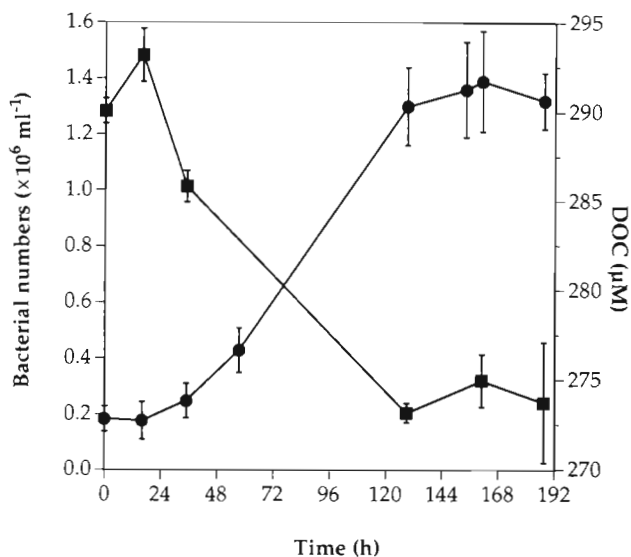


Fig. 1. Growth of a mixed marine bacterial assemblage in particle-free seawater. (●) Bacterial numbers. (■) Concentration of dissolved organic carbon (DOC), mean of duplicated filtrations. Error bars: SD

Table 1. Comparison of bacterial growth and DOC consumption between duplicate predator-free seawater cultures. Cultures (2 l) were incubated at 15 °C for 120 h

Date	Yield of bacteria (cells × 10 ⁶ ml ⁻¹) ^a	Consumption of DOC (μM) ^b
April 9, 1992		
Culture I	0.51 ± 0.05	20 ± 0.2
Culture II	0.53 ± 0.05	20 ± 0.2
April 14, 1992		
Culture I	0.50 ± 0.08	13 ± 0.2
Culture II	0.55 ± 0.06	15 ± 0.1
April 21, 1992		
Culture I	1.19 ± 0.11	19 ± 0.2
Culture II	1.08 ± 0.08	17 ± 0.2

^aMeasured as direct counts of bacteria, mean value of >20 fields of observation ± SD
^bMean value of 3 samples ± SD

(April 1991). Yield was lowest in July and August although the concentration of DOC reached a maximum. The concentration of DOC increased from April to July during both 1991 and 1992 with 60 μM and 90 μM carbon respectively. Since nutrients generally are low during summer, this suggested a demand for a substrate component other than carbon and the assay therefore was expanded to include enrichments with inorganic nutrients in 1992.

Table 2. Growth of mixed heterotrophic bacteria in seawater cultures with the addition of inorganic nutrients. Untreated and enriched seawater cultures from the Baltic station NB1 were compared over the experimental period April to August 1992 (dates as in Fig. 2)

	Treatment			
	Untreated	N-enriched	P-enriched	N&P-enriched
Yield of bacteria ^a (cells × 10 ⁶ ml ⁻¹)	0.9 ± 0.1	0.9 ± 0.1	1.5 ± 0.1	2.3 ± 0.4
Carbon consumption ^b (DOC) (μM)	14 ± 1.5	15 ± 1.9	17 ± 2.5	23 ± 2.8
Particulate carbon ^c (POC) (fg C cell ⁻¹)	51 ± 7	57 ± 12	28 ± 8	32 ± 5
Growth efficiency ^d (range) (%)	27 (11–54)	29 (11–55)	21 (12–40)	27 (14–58)

^aMean values ± standard deviation (n = 10)
^bUtilizable carbon (UC) measured as consumption of DOC in N&P-enriched cultures. Mean values of all experiments ± standard deviation
^cBacteria in seawater cultures collected on 3 occasions on 0.2 μm Ag-filters, mean values ± standard deviation (n = 3)
^dGrowth efficiency calculated as [(Yield × POC)/DOC consumption]

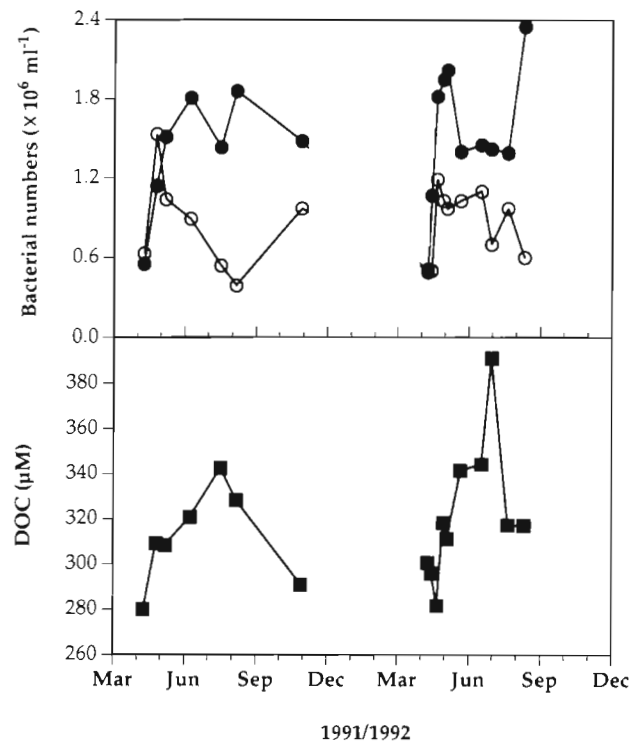


Fig. 2. Seasonal variation in yield of bacteria grown on 0.2 μm filtered seawater, compared to bacterial numbers and dissolved organic carbon (DOC) at the sampling site NB1. (○) Bacterial yield; (●) *in situ* number of bacteria; (■) *in situ* DOC

During the productive season of May to September 1992, the available substrate in the particle-free seawater allowed a significant growth yield of bacteria (Table 2). Since the ambient number of bacteria represents the carrying capacity in the system we related the growth yield in the cultures to the ambient number of bacteria. This ratio is referred to as Growth Capacity [(yield in cultures/ambient number of bacteria) × 100]. Untreated and nitrogen-enriched seawater cultures reached a growth capacity of 56%. Addition of phosphate enhanced the growth capacity to on average 94%, and the addition of both ammonium and phosphate increased the growth capacity of the seawater to 144% suggesting that carbon or an unknown micronutrient became depleted. If carbon is the limiting factor this must be due to poor quality, since DOC was still found in excess in the cultures (average consumption 1 to

Table 3. Composition and amount of available and utilizable dissolved carbon, nitrogen and phosphorus compared to the composition of marine bacteria at the Baltic station NB1. Mean values \pm standard error (n = 10)

	Elemental composition			Ratio of C:N:P
	C (μmol)	N (μmol)	P (μmol)	
Bacteria (cell^{-1}) ^a	1.7×10^{-16}	4×10^{-10}	3.8×10^{-11}	45:10:1
Available organic (l^{-1})	320 ± 9.3	13 ± 3.0	0.13 ± 0.25	2460:100:1
Available inorganic (l^{-1})	–	0.56 ± 0.09	0.035 ± 0.007	– :16:1
Residual inorganic (l^{-1}) ^b	–	0.10 ± 0.03	$<0.009 \pm 0$	
Utilizable (l^{-1})	23 ± 2.8	0.60 ± 0.05^c	0.033 ± 0.003^c	697:18:1

^aCalculated from a C:N:P ratio of 45:10:1 and a mean cell carbon of 20 fg C cell⁻¹ for marine bacteria
^bResidual inorganic nitrogen in phosphate-enriched cultures and residual inorganic phosphorus in ammonia-enriched cultures
^cCalculated from yield of bacteria assuming 100% assimilation of N and P

7%). All in all, the yield of bacteria indicated that the least available component in the seawater was phosphorus followed by nitrogen and carbon.

The consumption of DOC increased from 14 μM C in untreated cultures to 23 μM C when nitrogen and phosphorus were added to the cultures (Table 2). POC varied considerably between limited and nonlimited cells (Table 2). This means that although the carbon consumption per produced cell (DOC consumption/yield of bacteria) was 56% higher for limited cells, the growth efficiency [(yield of bacteria \times POC)/DOC consumption] did not differ much between the cultures (Table 2).

The concentrations of inorganic phosphorus and ammonium were measured at the start and end of all cultures during 1992. The concentrations of available nutrients (start in untreated cultures) and residual concentration of limiting nutrient in enriched cultures (nitrogen in phosphate-enriched cultures and phosphorus in ammonium-enriched cultures) are shown in Table 3.

In order to estimate the pool sizes of utilizable substrates, we converted the yield of bacteria in the cultures into nitrogen and phosphorus equivalents. As inorganic nitrogen (NH_4^+) never stimulated growth of bacteria, the yield in the nitrogen-enriched cultures was regarded as a reflection of the pool of utilizable phosphorus (UP). Furthermore, the yield in phosphorus-enriched cultures was considered as the pool of utilizable nitrogen (UN) since carbon limitations were not reached in these cultures. The nitrogen and phosphorus content of bacteria, used to convert the bacterial yield into phosphorus and nitrogen equivalents (4.0×10^{-16} mol N cell⁻¹ and 3.8×10^{-17} mol P cell⁻¹), were obtained from a bacterial C:N:P ratio of 45:10:1 and a mean cell carbon of 20 fg C cell⁻¹ for marine bacteria (Goldman et al. 1987, Lee & Fuhrman 1987, Neidhardt

et al. 1990). As carbon never became limiting for growth in this study, we have considered the relatively high POC values determined in this paper to reflect a storage of carbon and this should not affect the bacterial content of nitrogen and phosphorus. Furthermore, high POC values were determined in cultures where phosphorus was limiting, hence it does not seem likely that P should be stored in these bacteria. The utilizable pool of carbon (UC) was determined as the consumption of DOC in the cultures with non-limiting N and P conditions. The pools of UC, UN and UP are summarized in Table 3. The available dissolved organic matter (DOC, DON, DOP) at NB1 was found to be highly enriched in carbon compared to the utilizable substrate (Table 3).

Annual growth dynamics

The final yield in the untreated seawater cultures never exceeded the standing stock of bacteria during 1992 (Fig. 2). This result suggested that the ambient bacteria can at most divide once without the substrate pool being replenished. It was therefore of interest to analyze the daily uptake of substrate in relation to the calculated pool size of utilizable nutrients. The *in situ* phosphate concentration ranged between 0.020 and 0.087 μM and the ammonium concentration between 0.21 and 1.16 μM with minimum concentrations in June and July (Fig. 3). Starting from a low level (2.1×10^8 cells l⁻¹ d⁻¹) in April, bacterial production reached the annual maximum at the beginning of June (8.1×10^8 cells l⁻¹ d⁻¹). The average bacterial production during the experimental period corresponded to 4.4×10^8 cells l⁻¹ d⁻¹ and a daily uptake of 0.017 μM phosphorus and 0.18 μM of nitrogen (calculated from the data of bacterial content in Table 3). The ambient number of

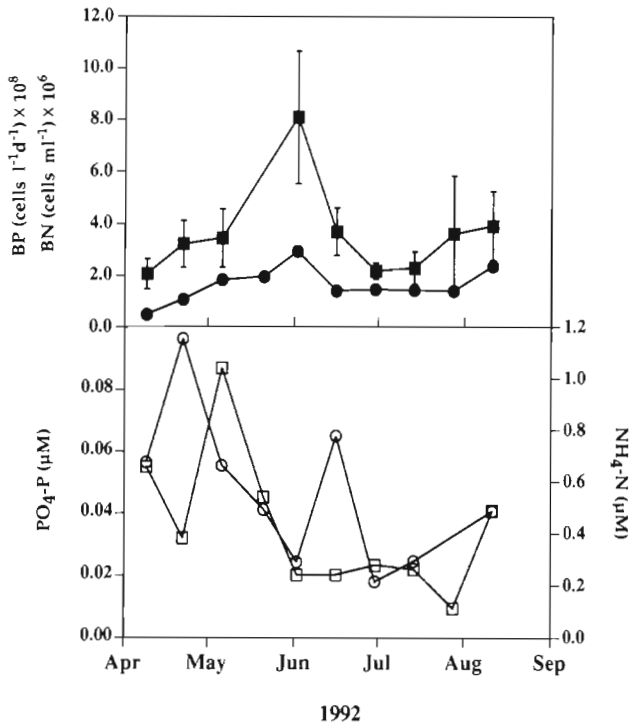


Fig. 3. Bacterial and nutrient dynamics in the surface water (4 m) of the coastal station NB1. (■) Bacterial production using [^3H] thymidine uptake (error bars = SD of triplicate samples); (●) bacterial numbers (same as Fig. 2); (○) ammonium concentration; (□) phosphate concentration

bacteria varied between 0.5×10^9 and 2.9×10^9 cells l^{-1} , with an average of 1.6×10^9 cells l^{-1} thus resulting in a turnover time (calculated from the P/B ratio) of 3.6 d. Consequently 30% of the bacteria must be grazed or lysed each day in order to maintain a constant standing stock.

Diel growth dynamics

Growth and grazing of 30% of the standing stock of bacteria each day suggests a rapid cycling of nutrients. Diel growth dynamics in the microbial food web were therefore studied in microcosm experiments. These experiments were performed in the Northeast Mediterranean in September 1984 and 1992. In this area, the stable weather conditions and pronounced day/night cycle combine to synchronize the growth pattern. Fig. 4 shows the concentration of ammonium and phosphate and bacterial yield in $0.2 \mu\text{m}$ filtered water from the microcosms. Diel variations of phosphate, ammonium and bacterial yield were observed in both years. While the phosphate concentration was 5-fold higher during the 1984 experiment, the same general diel pattern was found. A 1-way ANOVA test

confirmed a systematic variation with time ($p > 0.016$). This was also the case for diel variation of bacterial yield ($p > 0.011$). The ammonium concentration peaked in the afternoon and was lowest during early morning although the comparison between years did not suggest the variation to be systematic ($p > 0.742$). Using a multiple regression test on the 1984 and 1992 experiments, the variation in NH_4 and PO_4 could explain 35% ($p > 0.18$) and 69% ($p > 0.017$) of the change in bacterial yield for the respective year. In the 1992 experiment, DOC was included in the measurements showing a diel variation between 111 and $124 \mu\text{M}$. Extending the multiple regression to include DOC showed that 89% of the variation in bacterial yield could be explained by the variation in phosphate, ammonia and DOC ($p > 0.003$).

In 1984, flagellates and cyanobacteria increased during the night. Flagellate numbers increased from 1.2×10^3 to 3.7×10^3 ml^{-1} , cyanobacteria from 1.1×10^4

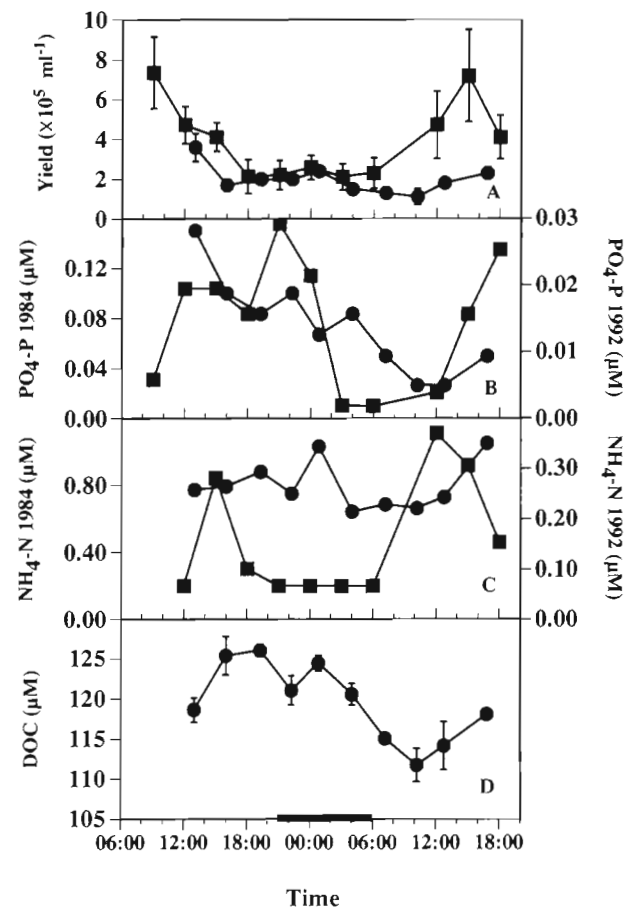


Fig. 4. Diel variations in a Mediterranean microcosm. (A) Bacterial yield on $0.2 \mu\text{m}$ filtered seawater. (B, C) Inorganic nutrients. (D) Dissolved organic carbon. Measurements were performed in 50 l microcosms at ambient light and temperature conditions. (■) 1984; (●) 1992

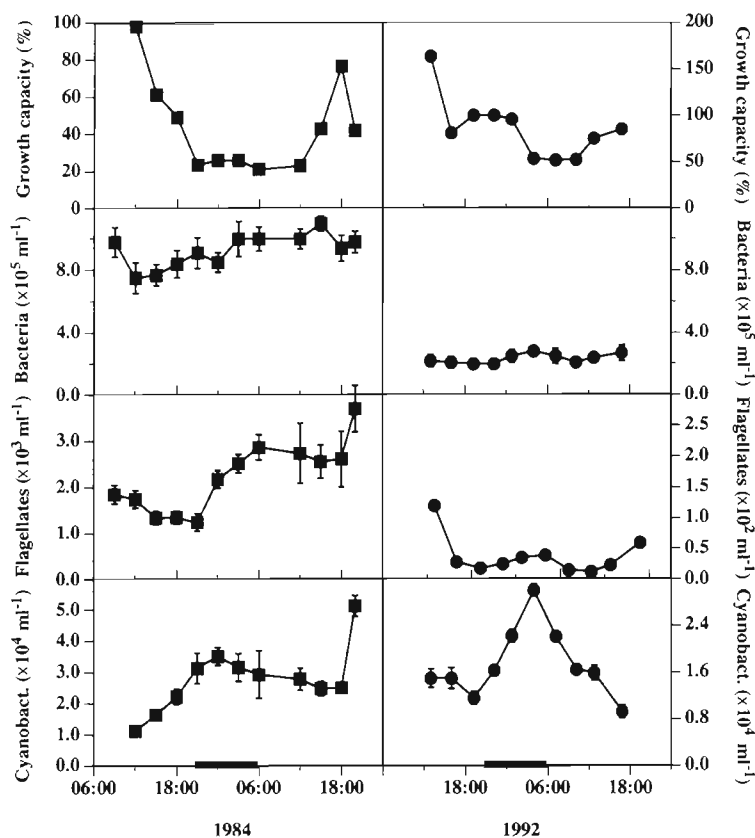


Fig. 5. Diel variation in growth capacity and major organisms in the microbial food web. Measurements were made in the same microcosms as depicted in Fig. 4. Data for 1984 were redrawn from Hagström et al. (1988)

to $5.1 \times 10^4 \text{ ml}^{-1}$ and the numbers of bacteria from 0.21×10^6 to $0.73 \times 10^6 \text{ cells ml}^{-1}$ (Fig. 5). The growth capacity of the seawater decreased markedly during the night to support only 20% of the standing stock.

In the second experiment (1992), the flagellate numbers were low throughout the experiment ranging between 1.2×10^2 and $12 \times 10^2 \text{ ml}^{-1}$, showing the same growth pattern as in 1984. Cyanobacteria counts increased from 0.9×10^4 to $3.0 \times 10^4 \text{ cells ml}^{-1}$ and the numbers of bacteria ranged between 0.20×10^6 and $0.28 \times 10^6 \text{ cells ml}^{-1}$. Both heterotrophic bacteria and cyanobacteria reached maximum numbers during the dark hours. The bacterial growth capacity decreased during the night to 50% of the standing stock.

The least available component for bacterial growth in the Villefranche bay was

determined in seawater cultures. Samples were collected at noon and midnight and enriched with ammonium, phosphate or sucrose (Table 4). Untreated, ammonium-enriched and carbon-enriched samples supported growth of bacteria corresponding to 108% of the standing stock of bacteria at noon and 152% at midnight. Additions of phosphate increased the growth yield to 296% at noon and 291% at midnight. Addition of both ammonium and phosphate did not increase the yield further. Thus, the data suggests that phosphorus was the least available component for bacterial growth in the Villefranche bay, followed by carbon and nitrogen. The DOC consumption during the incubations was determined in both experiments. The amount of consumed carbon per cell showed only minor differences between the treatments except when sucrose was added, in which case an additional 300% of the DOC disappeared in the filtrate.

DISCUSSION

In this study, we used seawater cultures to investigate seasonal and diel variations in bacterial growth and nutrient availability. At the Baltic coastal station NB1, phosphorus was found to be the least available

Table 4. Enrichment studies, Villefranche, 1992. Volume of cultures 200 ml, incubated at *in situ* temperature for 72 h

Date and treatment	PO ₄ (μM)	NH ₄ (μM)	Bacterial yield ± SD ($\times 10^6 \text{ ml}^{-1}$)	Growth capacity (%)
<i>In situ</i> Sep 15, 12:00 h	<0.009	0.26	(0.25 ± 0.08) ^a	–
Enrichment control			0.27 ± 0.05	108
Enrichment N			0.32 ± 0.07	128
Enrichment C			0.30 ± 0.05	120
Enrichment P			0.74 ± 0.12	296
Enrichment N and P			0.74 ± 0.04	296
<i>In situ</i> Sep 15, 24:00 h	0.016	0.25	(0.23 ± 0.05) ^a	–
Enrichment control			0.35 ± 0.04	152
Enrichment N			0.39 ± 0.06	171
Enrichment C			0.33 ± 0.04	143
Enrichment P			0.67 ± 0.11	291
Enrichment N and P			0.68 ± 0.10	296

^a *In situ* numbers of bacteria

component for bacterial growth, followed by nitrogen and carbon. The *in situ* dissolved organic matter (DOM) in the surface water was far from ideal as a bacterial substrate. (Table 3). With a C:N:P ratio of 2460:100:1, it was high in carbon but low in phosphorus when compared to a more typical marine area (Southern California Bight) with a C:N:P ratio of 400:28:1 (Table 3) (Williams 1986). From the seawater cultures the average concentrations of utilizable carbon, nitrogen, and phosphorus were estimated to be 23 μM C, 0.6 μM N, and 0.03 μM P respectively. The ratio of utilizable components (UC:UN:UP), including both inorganic nutrients and dissolved organic matter, was 697:18:1. Relative to carbon, the utilizable substrate was richer in phosphorus but poorer in nitrogen, compared to the *in situ* DOM at NB1.

Our results suggest that over a time scale of days, bacteria preferentially used inorganic sources of nitrogen and phosphorus to support growth. Ammonium and phosphate were measured at the start and end of all enrichment experiments showing that the DIP always dropped below the detection limit ($<0.009 \mu\text{M}$) in the ammonium-enriched cultures while DIN in phosphate-enriched cultures decreased by 82%. Thus, $\geq 79\%$ of the phosphorus found in bacterial biomass could be explained by the decrease in DIP while 77% of the nitrogen in bacterial biomass could be explained by the decrease in DIN (Table 3). Goldman et al. (1987) showed that NH_4^+ -regeneration of organic nitrogen is only found when the C:N ratio of the substrate is $<10:1$, a ratio that is rarely met in nature. Our results also indicate that bacteria are inefficient remineralizers of N and P.

The pool of UN may include peptides and amino acids as well as inorganic nitrogen. Compared to the pool of UN (0.6 μM N), the amount of dissolved free amino acids (DFAAs) in the Baltic has been shown to vary within a range of 30 to 400 nM corresponding to 0.03 to 0.45 μM N (Mopper & Lindroth 1982). This means that 5 to 75% of UN may be supported by DFAAs. In the case of organic phosphorus, phosphate must be enzymatically cleaved from the carbon skeleton before being assimilated by the cell (Ammerman & Azam 1991). DOP is thereby channelled into DIP, and available phosphorus is determined by the concentration of inorganic phosphate. Nucleic acids are found in significant amounts in the DOP and have been shown to be rapidly degraded (Turk et al. 1992). Our results, which indicate a strong coupling between inorganic nutrients and DOC degradation, are therefore puzzling since both the DON and DOP pools are quite variable on a seasonal time scale. However, we believe that seasonal accumulation and concomitant enzymatic degradation of less degradable compounds such as phospholipids, mureic acid and chitin can account

for these changes. In the cultures we cannot expect to detect this degradation during the short incubation period. Thus, while lacking conclusive information on the origin of the degradative enzymes the view of bacteria as poor remineralizers must be regarded with caution.

The pool of UC either exists separately or constitutes the carbon backbone of the organic molecules that are included in the UN and UP pools. The concentration of UC in our cultures was 23 μM C of unknown composition. However, judging from the concentration of free carbohydrates in the oligotrophic Sargasso Sea (10 μM C) it is possible that a major part of the UC was carbohydrates (Burney 1988). The C:N ratio of the utilizable substrate (39:1) was actually higher than the *in situ* dissolved organic substrate (25:1) suggesting that the UC is low in nitrogen. In the cultures, bacteria grew with short generation times (0.5 to 1.7 d) compared to *in situ* growth (3.6 d). This suggested growth on a substrate of fair quality although low in N and P.

When nitrogen and phosphorus were added to the cultures, the consumption of DOC increased by 64% compared to the untreated cultures, and the yield of bacteria increased by 156%. The P-limited cells (untreated and nitrogen-enriched cultures) showed a higher carbon consumption per produced cell (DOC consumption/yield of bacteria). This was first thought to reflect an effort by the cells to free more nutrients through high respiration, which would result in a lower growth efficiency [(yield of bacteria \times POC)/DOC consumption]. However, POC data showed that although P-limited cells were fewer, they had a higher carbon content per cell (ca 54 fg C cell⁻¹) compared with non-limited cells (ca 30 fg C cell⁻¹). Consequently, the growth efficiency showed only minor differences between the cultures (Table 2). We believe that the higher POC values that were reached in limited cultures reflects the capability of bacteria to store carbon such as carbohydrates during N- and/or P-limiting conditions when cell division is no longer possible (Kanopka 1992). This is supported by the fact that when bacteria were grown with excess carbon (sucrose added, Villefranche experiment), during either N or P limitation, the cells increased in size (data not shown). This was possibly caused by an increase in the amount of capsular material and/or grains of particulate matter, as observed in the microscope. Due to the fact that bacterial POC seemed to be a variable component, we do not want to put high emphasis on our determinations of growth efficiencies. Although the numbers correspond quite well with results found by other investigators, we did not measure respiration in our seawater cultures (Bjørnsen 1986). We infer that the decrease of DOC in the filtrate might be a mislead-

ing measure of growth efficiency unless proper measurements of all concerned parameters for bacterial growth (respiration, DOC decrease, POC and bacterial numbers) are available.

In order to study the flow of nutrients in a non-cut-off situation, diel studies in an intact microbial food web were performed in microcosms on 2 occasions. The population dynamics in the microbial food web, as seen in the diel experiments, showed a complex structure with an overall regulation (the sunlight) and a series of random influences that offset the balance between the predators and their prey. During fluctuating nutrient input (changes in remineralisation), the concentration of the limiting substrate may rise if the numbers and/or the activity of the consumers stay too low to assimilate the released nutrients. Uncoupling between bacterial growth and substrate supply may be caused by populations of grazing flagellates lagging behind the bacterial activity. As a result, heterotrophic bacteria and phototrophic organisms will experience pulses of nutrients which can be detected if the sampling interval is frequent enough as shown by Wheeler et al. (1989). Diel variations in phosphate, ammonium and carbon in microcosm experiments performed at Villefranche-sur-Mer suggested that the input of nutrients was pulsed and a high degree of correlation was found between these nutrient pulses and the level of bacterial yield. As a consequence, the level of utilizable phosphate (calculated as P incorporated in bacteria) also showed a sharp diel variation (more than 100%). It is therefore obvious that the instantaneous values of utilizable substrate obtained from the seawater cultures should be regarded as a rough estimate. However, the seawater cultures demonstrated that bacterial growth depended on a high degree of inorganic nutrient utilization. This, and the correlation between nutrient levels and bacterial yield in the diel experiments, suggested that growth of heterotrophic bacteria was controlled by the supply of inorganic nutrients although the precise nature of this control is difficult to outline. Phosphate availability has been found to be important for the control of growth of heterotrophic bacteria in this and other studies (Currie & Kalff 1984, Morris & Lewis 1992). It was therefore interesting to consider the consequence of our results for the *in situ* turnover of nutrients. As calculated above, the bacterial production corresponded to a daily uptake of 0.017 μM phosphorus and 0.18 μM of nitrogen (calculated from the data of bacterial content in Table 3) which in turn corresponds to 52% and 30% of the utilizable pools respectively. Thus, the *in situ* pools of utilizable phosphorus and nitrogen have to be renewed at a rate of every 2 d for phosphorus and every 3 d for nitrogen in order to maintain the *in situ* bacterial production over time.

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