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Indications for low net productivity of pelagic bacterioplankton

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Abstract

In contrast to the current view on the trophic role of bacteria in pelagic environments, the impression of a rather unproductive bacterioplankton arose from an example of the deep mesotrophic Lake Constance. Based on measurements of bacterial DNA, thymidine incorporation rates and grazing rates, turnover times of bacterial biomass exceeding 10 days were estimated during the growth period. Similarly, low productivity of bacterioplankton was indicated by low RNA/DNA ratios (< 1). Additional indications for a rather inactive bacterioplankton were provided by the observation of long lag phases in lake water cultures. Low bacterial productivity has also to be expected from energetic considerations. There is increasing evidence for low bacterial growth efficiencies under natural conditions. The presented material points to the possible need for reconsideration of current estimates of bacterial *in situ* growth.

Introduction

According to the current conception on the role of bacteria in pelagic ecosystems, bacteria convert considerable amounts of carbon produced by algae into secondary production (COLE et al. 1988, AZAM et al. 1990). However, as was emphasized by GÜDE (1990a, b) the methodological and conceptual basis for estimates of bacterial production is possibly still not solid enough to allow such generalizing conclusions. Indeed, there are several lines of argumentation due to which the productivity of bacterioplankton may be regarded as much lower than currently assumed. In the present contribution, an attempt is made to summarize data indicating low bacterial productivity at the example of the prealpine Lake Constance (maximum depth 252 m, surface 540 km²). Because bacterial population dynamics observed in this lake are comparable to other large lakes and to a certain extent also for marine environments (GÜDE 1990b), this example may provide aspects which may have also validity for other pelagic environments.

Material and methods

Water samples were taken at a mid-lake station in the central eastern part of the Lake. Most of the analyses were done with samples from 2 m depth, which are representative for the euphotic water layers. Bacterial numbers were counted by epifluorescence microscopy with DAPI staining. Bacterial production

was estimated by measurements of ^{3}H-methyl-thymidine incorporation (12 nM final concentration). Sampling, counting and production methods were described in detail by GÜDE et al. (1985). The number of heterotrophic flagellates (HNF) was determined by epifluorescence microscopy as described by JÜRGENS and GÜDE (in press). RNA and DNA contents of bacterioplankton were determined in the particulate fraction < 1 µm, which was collected on 0.2 µm cellulose ester filters. Nucleic acids were extracted with 0.1 M ice cold Tris acetate buffer after sonication at 80 W for 30 sec. Nucleic acids contained in the supernatant were determined fluorimetrically using ethidiumbromide and DAPI as fluorochromes of total nucleic acids and DNA, respectively. Further details are described by WALSER and GÜDE (ms. submitted).

Growth of planktonic bacteria was observed in dilution cultures of water samples from 2 m depth which had been made free of eucaryotes by prefiltration through 1 µm membrane filters. These samples were diluted 1 : 10 by lake water which was made free of bacteria by 0.2 µm prefiltration. The cultures were incubated in the dark at *in situ* temperatures. Further details are described by PARTH and GÜDE (ms. submitted).

Results and discussion

The first precondition for considering bacterial productivity is a realistic estimate of bacterial biomass. Because of the given uncertainties of each method we made various independent approaches. On the one hand, bacterial numbers were transformed into carbon units by assuming a carbon content of 12 fg per cell based on mean cell volumes and volume:carbon conversion factors determined by SIMON 1987 and SIMON and AZAM 1989. Alternatively, bacterial carbon (BC) was estimated by determining contents of phosphorus and nucleic acids in bacterioplankton. Bacteria were effectively separated from other organisms by filtration through 1 µm polycarbonate membranes. More than 90 % (except during May with 70-80 %) of bacteria but no eucaryotes passed through these filters (GÜDE 1988). Bacterial carbon was obtained by assuming a mean molar C:P ratio of 60 : 1 (JÜRGENS and GÜDE 1990) and a mean DNA content of 10 % of dry weight and a carbon content of 55 % of dry weight (SIMON and AZAM 1989).

Because these approaches to estimate BC in the euphotic zone resulted in very comparable average values (Table 1), these estimates can be expected to reflect indeed a realistic order of magnitude. The obtained values (70 - 90 µg C l^{-1}) represent about half of the algal carbon (173 ± 128 µg C l^{-1}, n = 25) which was estimated as average for the euphotic zone during the same period. Thus, there can be little doubt that at least with respect to biomass bacteria represent an important compartment of the planktonic community (SIMON and TILZER 1987, CHO and AZAM 1988, AZAM et al. 1990).

Although such high estimates of BC seem to support the view of an important role of bacteria in pelagic food webs, the trophic importance finally depends on the turnover time TB of BC (time required for one renewal of bacterial biomass by net production). Therefore bacterial TB was also estimated by three approaches. One estimate was based on the calculation of DNA synthesis rates obtained from ^{3}H-methyl-thymidine incorporation values, assuming 25 % thymidine content of DNA, and (in favour of low rates) no isotope dilution by de novo synthesis of thymidine, and finally (in favour of high rates) 100 % incorporation of thymidine uptake into DNA. Calculated synthesis rates were divided by actual

Table 1. Bacterial carbon BC, turnover time of bacterial biomass TB, and RNA/DNA ratios of bacterioplankton estimated for the euphotic zone (2 m) of Lake Constance during the period from May to October 1988.

		n	average	range
Bacterial carbon BC ($\mu g\ C\ l^{-1}$)				
	BCm 1)	22	83.6 ± 21.9	41.6 - 134
	BCn 2)	21	73.9 ± 25.4	23.2 - 136
	BCp 3)	22	90.5 ± 38.2	23.1 - 174
Turnover time TB (days)				
	TBn 4)	19	25.7 ± 24.3	5.4 - 115.5
	TBp 5)	20	13.7 ± 17.1	4.3 - 84.2
	TBg 6)	22	12.2 ± 10.5	3.1 - 43.2
RNA/DNA ratios		21	1.05 ± 0.62	0.5 - 2

1) morphometric approach, 2) DNA-content approach, 3) phosphorus content approach, 4) based on DNA concentrations and DNA synthesis rates, 5) based on conversion of thymidine incorporation rates and bacterial numbers, 6) based on estimates of bacterial grazing losses and bacterial numbers. Further explanation in the text.

DNA concentrations observed for the bacterial size fraction. A second estimate was based on the conversion of ^{3}H-methyl-thymidine uptake into bacterial net production assuming a conversion factor of 1×10^{18} cells produced per mole thymidine incorporated which is close to the theoretical values (MORIARTY 1987, RIEMANN et al. 1987). Actual cell numbers were divided by obtained production of cells. A third estimate was based on the calculation of bacterial grazing losses in dependence of the abundance of bacterivorous flagellates. A mean grazing rate of 10 bacteria per flagellate and hour was assumed which was obtained as average from measurements using fluorescent labeled bacteria (JÜRGENS and GÜDE, in press). The actual cell numbers were divided by obtained grazing losses. As is shown in Table 1 all estimates indicated a much higher turnover time of bacterial biomass than anticipated from current production estimates (> 10 days). The highest TB was obtained for the most direct approach, i.e. when relating DNA synthesis rates to actual bacterial DNA-pools. Half as long TB was calculated for the two more indirect approaches based either on the conversion of thymidine uptake into bacterial production or on estimates of bacterial grazing losses. Thus, although differences between the various approaches were obvious, they point uniformly to a rather low bacterial productivity.

Low productivity of bacterioplankton was additionally indicated by low RNA/DNA ratios (mean 1.05, Table 1) observed during summer 1988 (WALSER and GÜDE, ms. submitted). An even lower average of RNA/DNA ratios (0.54) was obtained for the summer 1989 (PARTH and GÜDE, ms. submitted). To our knowledge

such ratios have to date not been reported for natural bacterioplankton. However, ratios in the observed range were predicted from morphometric considerations on cell constituents of very small bacteria as are characteristic for bacterioplankton (SIMON and AZAM 1989). Such low ratios were indeed found for isolates of slow growing minicells from marine habitats. In laboratory cultures, such low values were only observed for non-growing stationary phase bacteria. In contrast, much higher ratios were observed for growing bacteria, irrespective of whether natural bacterial assemblages were grown in lake water or bacterial pure cultures on glucose-mineral media (WALSER and GÜDE, ms. submitted).

A further indication for the existence of a rather inactive bacterioplankton is given by the growth patterns observed in dilution cultures. If bacterioplankton as a whole would indeed permanently grow with a doubling time of about one day, growth should become immediately apparent in such dilution cultures. In contrast, bacterial growth almost regularly initiated only after a more or less pronounced lag phase which could exceed even one day (Fig. 1, PARTH and GÜDE, ms. submitted). Simultaneously, the size distribution of the bacteria shifted from a predominance of very small cells toward much larger cells. Similar observations were made by several authors. Although no unequivocal explanation can be given for these growth patterns they remind strongly to those observed with starving, nongrowing bacterial populations. These also showed growth reactions to additions of nutrients only after a lag phase, the length of which depended on the duration of the previous starvation period (MORITA 1982).

Rather discrepant turnover estimates are to be anticipated according to most current production estimates (COLE et al. 1988, AZAM et al. 1990) with doubling times of approximately one day. As it is made evident by the differences between the turnover estimates made here there is still a high degree of uncertainty which are obviously due to the many assumptions to be made for these approaches. Nevertheless, it is remarkable that all approaches agreed in the order of magnitude. Moreover, a similar magnitude of TB is predicted on the basis of our current knowledge on energetic efficiencies of bacterioplankton (GÜDE 1990b): Because bacterioplankton represents an important part of total planktonic carbon, also a considerable part of the total carbon flux is required for renewal of bacterial biomass. The resulting conversion of this flux into bacterial net production depends on the bacterial growth efficiency. There is increasing evidence (VERSEVELD et al. 1984, BJØRNSEN 1986, TRANVIK and HÖFLE 1987, TRANVIK 1988) that under natural conditions this efficiency is 20 % rather than 50 % as usually assumed (e.g. STRAYER 1988). This means that for one doubling of bacterial biomass a carbon amount equivalent to five times the bacterial biomass would be required. Assuming that allochthonous carbon sources are negligible in large lakes (TILZER 1990) bacterial carbon demands should exclusively rely on primary production. The mean daily carbon supply from primary production estimated for the period from May to October 1988 amounted to 88 ± 63 µg C $l^{-1}d^{-1}$, (n = 19, measured by the 14C-method). This value is of the same magnitude as mean BC estimates (Table 1). It follows that even if all carbon derived from primary production were available exclusively to bacteria, mean TB should be at least five days. In reality, significantly higher TB values should be expected because obviously considerable parts of carbon produced by algae are not available to pelagic bacteria due to irreversible losses by algal respiration, sinking, grazing etc. (TILZER 1984).

Obviously, these conclusions contrast sharply with the currently propagated picture of a very productive bacterioplankton. This discrepancy may be mainly due to methodological and conceptual reasons: 1) It has been proven that physiologi-

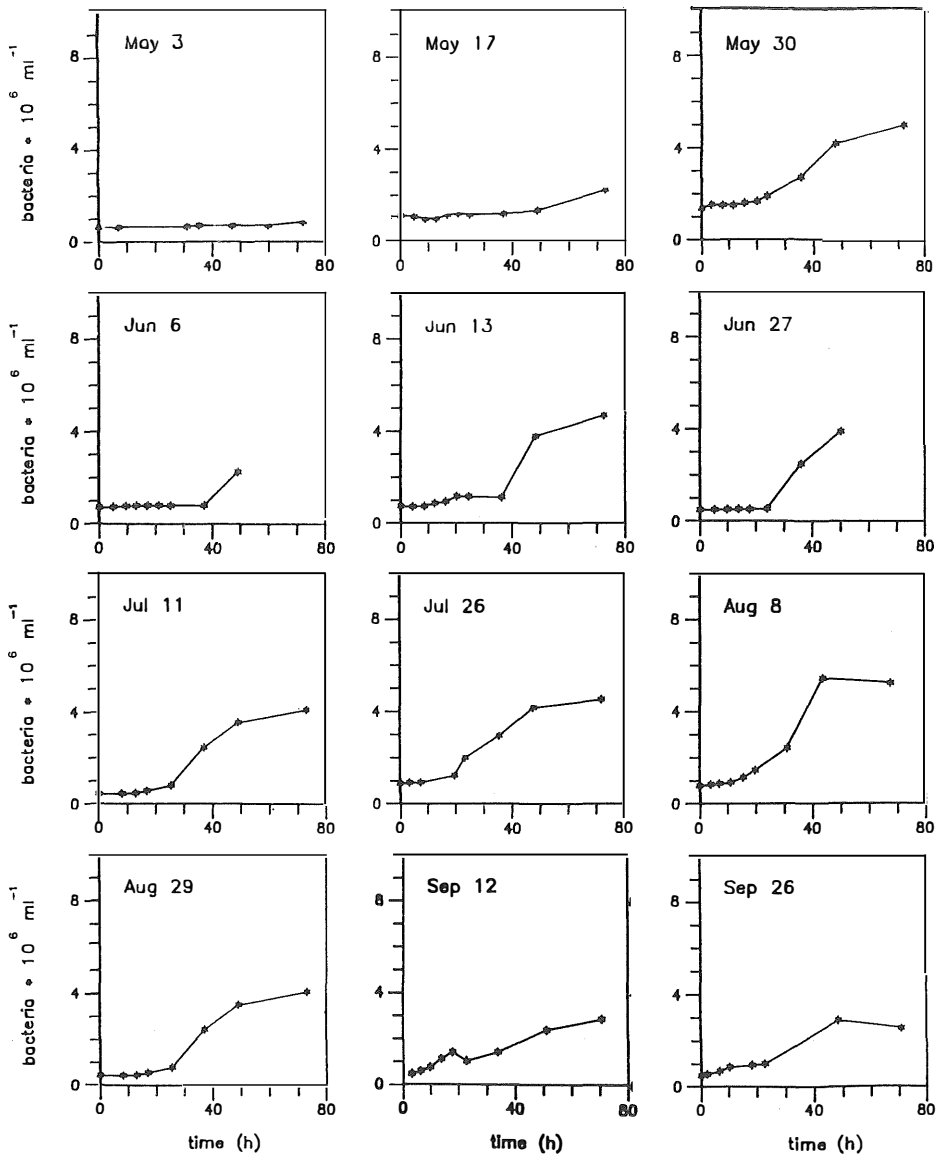

Fig. 1. Patterns of bacterial growth observed in dilution cultures of epilimnetic water samples from 2 m depth during 1989. Bacterial assemblages contained in a 1 µm filtrate of the water sample were diluted 1 : 10 by sterile lake water of the same samples. The cultures were incubated in the dark at *in situ* temperature.

cal and morphological changes of bacterial populations occur almost regularly when natural bacteria are grown in water culture (KIRCHMAN et al. 1982, SIERACKI and SIEBURTH 1985, FERGUSON et al. 1984, WRIGHT and COFFIN 1984, GÜDE 1986, NAGATA 1987). Thus, it is questionable to which extent results obtained in such experiments (e.g. conversion factors) may be valid also for the *in situ* situation. 2) Although growth implies biosynthesis, biosynthesis does not necessarily imply growth. This was clearly demonstrated with nongrowing starving bacteria which synthesized DNA and proteins without any net increase of biomass (MASON et al. 1986, KJELLEBERG et al. 1987).

According to these considerations and indications the role of bacterioplankton seems in some aspects to approach the old paradigm, where bacteria are seen mainly as metabolic catalysts which contribute little to the energy supply of the food chain. We are aware of the very speculative nature of this statement, which leaves many questions open. On the other hand we regard the material and arguments presented here as serious enough to reconsider thoroughly the conceptual and methodological basis for current estimates of *in situ* growth.

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