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ESTIMATES OF CARBON FLOW THROUGH BACTERIOPLANKTON IN THE S. BENGUELA UPWELLING REGION BASED ON ^{3}H-THYMIDINE INCORPORATION AND PREDATOR-FREE INCUBATIONS

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ABSTRACT - Spatial and temporal estimates of bacterial numbers, biomass, activity and production were measured in the S. Benguela upwelling system during the course of a phytoplankton bloom.
Bacterial numbers and biomass were highest in the euphotic zone and correlated closely with particulate carbon in the water column rather than with chlorophyll *a* concentrations. Estimates of bacterial production based on ^{3}H-Thymidine incorporation and growth of bacteria in a predator-reduced environment ($<3\mu$m) showed that results from the latter method agreed well with the lower estimate of production from the ^{3}H-Thymidine incorporation method, giving increased confidence in our estimate of production.
Bacterial carbon production was highest in the euphotic zone at the peak of a phytoplankton bloom and amounted to 15 % of the total fixed carbon. Under these conditions, it was calculated that photosynthetic exudates (*ca.* 30 % of primary production) could entirely meet bacterial carbon demands. As the bloom senesced and decayed, bacterial production exceeded phytoplankton production by an order of magnitude. In such circumstances, photosynthetic exudation was insufficient to meet bacterial carbon demands. However, at this stage, detrital POC dominated the carbon biomass and this source of carbon was utilised by the bacteria. This was also true in the aphotic zone below the pycnocline. Differential utilisation of photosynthetic exudates and detrital POC by bacterioplankton during the course of a bloom might account for the varying significance ascribed to exudates and POC as a source of carbon for bacterial production.

RÉSUMÉ - Des évaluations spatio-temporelles des numérations, biomasse, activité et production bactériennes ont été effectuées durant un développement phytoplanctonique, dans le système d'upwelling au sud Benguela, au large de l'Afrique du Sud. Les plus fortes valeurs en dénombrement et biomasses bactériennes sont observées dans la zone euphotique. Ces paramètres bactériens correspondent plus aux teneurs en carbone particulaire qu'à celles en chlorophylle-*a*. Des évaluations de la production bactérienne basées sur l'incorporation de la ^{3}H-Thymidine et des études de la croissance des bactéries en milieu pauvre en prédateurs (préfiltration sur 3μ) ont été également réalisées. Les résultats obtenus sur la croissance bactérienne correspondent à la plus faible évaluation de la production et confirment donc l'estimation de celle-ci. La production de carbone bactérien est maximale dans la zone euphotique en relation avec le pic de développement phytoplanctonique et correspond à 15 % du carbone total fixé. Dans ces conditions, il a été calculé que les exudats émis lors de la photosynthèse (environ 30 % de la production primaire) sont suffisants pour répondre aux besoins en carbone des bactéries. A la fin du bloom phytoplanctonique, la production bactérienne est supérieure d'un ordre de grandeur à celle du phytoplancton dont les exudats deviennent insuffisants pour la demande bactérienne en carbone. Mais, à cette période, le carbone organique particulaire (COP) est prédominant dans le carbone total et les bactéries peuvent utiliser cette nouvelle source carbonée. Ceci est également vrai pour la zone aphotique en dessous de la pycnocline. L'utilisation différentielle des exudats photosynthétiques ou du carbone organique détritique par le bactério-plancton durant le développement phytoplanctonique pourrait expliquer les résultats variables obtenus sur le rôle respectif des exudats et du POC comme source de carbone pour les bactéries.

INTRODUCTION

Estimates of the transfer of photosynthetically fixed carbon through the bacterioplankton community have recently become widespread (Cole *et al.*, 1982 ; Laake *et al.*, 1983 ; Newell and Linley, 1984 ; Lancelot and Billen, 1984 ; Painting *et al.*, 1985 ; Lochte and Turley, 1985). However, the quantification of carbon transfer and nutrient cycling by bacteria and through the "microbial loop" by microzooplankton bactivory (Azam *et al.*, 1983 ; Wambeke and Bianchi, 1985a&b) are very sensitive to accurate measures of bacterial numbers and biomass, production, net growth yield or carbon conversion efficiency, the activity of the cells both in time and space and to the grazing impact of microzooplankton (Bauerfeind, 1985 and Lucas, 1986).

Recently, attempts have been made (Fallon *et al.*, 1983 ; Riemann *et al.*, 1984 ; Linley and Newell, 1984) to assess the reliability and agreement between the numerous methods employed to measure bacterial productivity. The most notable of these techniques include incorporation of ^{3}H and ^{14}C labelled precursors into DNA (Fuhrman and Azam, 1980, 1982) and RNA (Karl, 1982). In recognition of the numerous assumptions required to convert incorporation of these macromolecules into microbial biomass, more direct estimates of microbial production have also been used, including the FDC technique (Hagström *et al.*, 1979) and direct observations of log phase growth of cells in small volume enclosed predation free incubations (Meyer-Reil, 1977 ; Linley *et al.*, 1983) or *in situ* using dialysis chambers (Lochte and Turley, 1985). However, some investigators have found limited bacterial predation by very small micro-flagellates in the $<0.6\mu m$ fraction (Fuhrman and Mc Manus, 1984) while others have reported inconsistencies between many of the methods outlined (Laake *et al.*, 1983 ; Fallon *et al.*, 1983 ; Riemann *et al.*, 1984 ; Linley and Newell, 1984 and Pollard and Moriarty, 1984).

Estimates of net growth yield, or carbon conversion efficiency required to support estimates of heterotrophic bacterial production also vary widely ; ranging from 10 - 80 % (Williams, 1981 ; Joint and Pomroy, 1982 ; Linley and Newell, 1984 ; Bauerfeind, 1985) depending largely on the nature of the substrate being utilised. Clearly, such a range will significantly affect our estimates of carbon flow through the heterotrophic bacterial community. Erroneous estimates of net growth yield will be seriously compounded if our estimates of bacterial production lack precision.
We have attempted to impose more rigorous constraints on our estimates of bacterial production as measured by ^{3}H-Thymidine incorporation and compared these with direct estimates of bacterial growth under reduced predation pressue in $<3\mu m$ incubations. We have also demonstrated marked changes in bacterial activity associated with a phytoplankton bloom in an enclosed mesocosm.

SAMPLING AND ANALYTICAL METHODS

Sampling

Hydrographic data and biological samples were obtained three times per day using a conductivity, temperature and depth (CTD) rosette sampler for profiles down to 300 m taken from *R S Africana* during the course of a cruise (14.3.83 - 20.3.83) in the S. Benguela upwelling system off the west coast of the Cape Peninsula, South Africa. The cruise track was determined by following a drogue deployed into newly upwelled water. Daily airborne radiation thermometry (ART) flights and CTD profiles confirmed that the drogue remained in the same cell of water. All sampling profiles were taken adjacent to the drogue thus giving a good temporal sequence of data. Further bacterial samples were obtained from a 60L mesocosm containing newly upwelled and nutrient rich water

maintained in a light/dark regime at 12°C and simulating an upwelling event. This allowed us to improve our understanding of small scale temporal processes wich occur during and after upwelling.

Measurements of phytoplankton production and biomass

Primary production was measured according to the ^{14}C-uptake method of Strickland and Parsons (1972). The details are given by Brown (1984). Chlorophyll *a*, as an index of biomass, was measured spectrophotometrically following the procedure of the SCOR/UNESCO Working Group 17 (1966) as described by Brown (1984).

CHN analysis

Water samples filtered onto Whatman GF/F filters were analysed for particulate carbon and nitrogen with a Hereaus (CHN Rapid) Analyser using cyclohexanone (20.14 % N ; 51.79 % C) as a standard.

Bacterial biomass and production

Bacterial numbers and biomass were estimated from the acridine orange direct counting (AODC) fluorescence microscopy method of Hobbie *et al.*, (1977) and S.E.M. estimates of biovolume and biomass as described by Linley *et al.*, (1981). For estimates of bacterial production, the ^{3}H-Thymidine incorporation method of Fuhrman and Azam (1980, 1982) was followed and compared with direct estimates of bacterial growth in small volume (125 ml) predator-free (3μm filtered) incubations in the dark. Details of this method are given by Meyer-Reil (1977) and Linley *et al.*,(1983). Although Fuhrman and McManus (1984) noted that a number of small and apparently bactivorous microflagellates were evident in the <0.6μm fraction, SEM studies of samples from our mesocosm showed no evidence of bacterial predators in the <3μm fraction. We therefore regard this fraction to be essentially predator-free or subject to only insignificant predation pressure on bacterial growth.

Bacterial activity

Bacterial activity was estimated as a function of percentage plateability and the number of colony forming units for samples taken from a 60 L. mesocosm.

RESULTS AND DISCUSSION

Bacterial biomass, activity and production

- Biomass

Profiles for bacterial biomass recorded for three days of the cruise are given in Figure 1.A maximum bacterial biomass (80 m.g.C.m^3) was associated with maxima for chlorophyll *a* concentration and total particulate carbon concentration present in the euphotic zone on 17.03.83. As the chlorophyll *a* concentration and POC concentration declined in subsequent profiles (19th and 20th March), so too did bacterial biomass. Indeed, irrespective of chlorophyll *a* concentration, bacterial biomass was found to closely follow (Fig. 1) and correlate with total particulate carbon (POC) in the water column; $Y = 1.96 + 0.060\,X$, $r = 0.77$ and $n = 85$ where Y = bacterial biomass (mg.C.m^{-3}) and X = POC (mg.C.m^{-3}). Bacterial biomass did not have a simple correlation with chlorophyll *a* concentration which is to be expected since the latter was not well correlated with total POC. The presence of detrital POC may therefore sustain bacterial biomass and production in the absence of living phytoplankton. Bacterial biomass was similarly correlated with detrital POC during the SIBEX I cruise to Antarctica in May 1984, (Painting *et al.*, 1985).

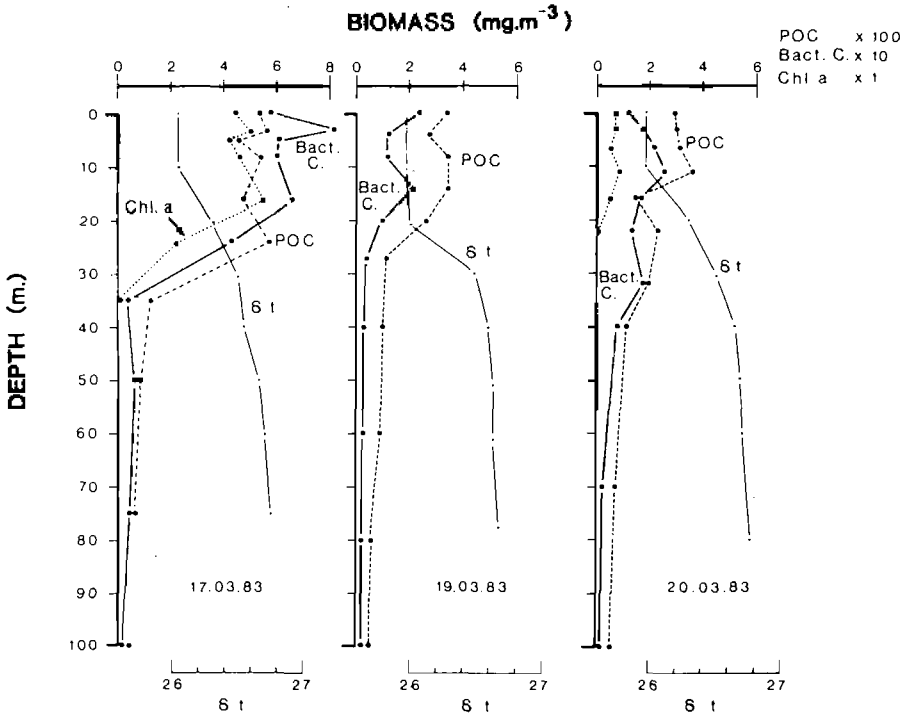

Figure 1 : biomass profiles for three profiles on 17.03.83, 19.03.83 and 20.03.83. Note that Chl. *a* concentration was insignificant (< 0.2 mg.m^{-3}) on 19.03.83 and is not plotted.

- Bacterial activity

In response to good growth conditions, a phytoplankton bloom developed in the mesocosm within 10 days and quickly promoted a three order-of-magnitude increase in the activity of the cells as determined by the percentage plateability and the number of colony forming units (Fig. 2). It is notable that the total bacterial count by AODC remained within one order of magnitude between 10^6 and 10^7 cells ml^{-1}. It is interesting to speculate that the consistent total count but increase in bacterial activity is due to the improved metabolic state of barophilic nutrient-starved cells which originate from S.Atlantic central water off the shelf (200 m) and are advected by upwelling into the nutrient rich euphotic zone adjacent to the coast. This would be in keeping with the findings of Novitsky and Morita (1977) and Morita (1984).

- Bacterial production

Estimates of bacterial production in the euphotic zone in the upper mixed layer (UML), the pycnocline region and the aphotic region below the pycnocline are given for three days in Table 1. The values obtained are based on predation-free and ^{3}H-Thymidine incorporation experiments.

From Table 1, three points emerge clearly. Firstly, the mean values for bacterial production are highest in the euphotic zone and at the pycnocline while production values in the aphotic zone are more than five times lower. The significance of these estimates of bacterial production relative to phytoplankton production and carbon flow will however be discussed later. Secondly, and of more immediate concern, is that bacterial production estimates from the ^{3}H-Thymidine incorporation method of Fuhrman and Azam (1980)

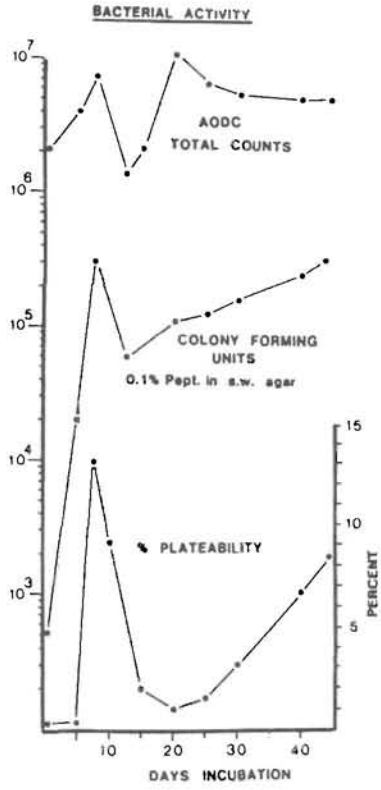

Figure 2 : bacterial activity shown as a function of % plateability and the number of colony forming units relative to the total AODC count. The data were obtained from a 45 day incubation of newly upwelled water in a 60 L. mesocosm which simulated the processes occurring following an upwelling event.

Profile date	Depth (m)	Irradiance (%)	Bacterial production (mg.C.m^{-3} d^{-1}) Predator-free (a)	^{3}H-Thymidine (b) (c)	mean ± S.D. of (a) + (b)	Phytoplankton Production (mg.C.m^{-3} d^{-1})
17.03.83	5	25.0	61.50	58.2-378.4	59.8 ± 2.3	414.4
	24	0.1	138.36	92.7-601.7	115.5 ± 32.3	9.1
	75	-	17.84	0.6- 3.7	17.8 ± -	-
19.03.83	8	25.0	40.73	61.6-401.1	54.0 ± 11.6	54.9
			59.84			
	20	10.0 (14 m)	20.61	40.4-262.6	31.8 ± 10.2	32.1 (14 m)
		1.0 (27 m)	34.56			2.9 (27 m)
	60	-	5.58	12.8- 83.2	12.9 ± 6.6	-
			11.80			
			21.55			
20.03.83	6.5	25.0	69.36	117.7-726.9	90.5 ± 29.9	155.6
	22	1.0	28.92	26.4-171.3	27.6 ± 1.8	7.2
	75	-	5.59	4.7- 30.4	5.1 ± 0.7	-

Table 1 : estimates of bacterial and phytoplankton production. The 25 % irradiance depth corresponds to the euphotic zone. Irradiance depths between 0.1 and 1 % correspond to the pycnocline region. Depths below that are in the aphotic zone. The mean estimate of bacterial production is obtained from the predator-free ($< 3\,\mu$m) values and the lower estimate of production from the ^{3}H-Thymidine incorporation method. Daily phytoplankton production values were calculated for a 12 h daylight period and corrected for 10 % night respiration.

give a wide range (x 6,5) for each calculated value. This is due to the number of conversion factors and assumptions required to convert ^{3}H-Thymidine incorporation into bacterial biomass. Fuhrman and Azam (1980) estimated that 2.0×10^{17} to 1.3×10^{18} cells are produced per mole of ^{3}H-Thymidine incorporated into DNA. Subsequently, Fuhrman and Azam (1982) proposed that conversion factors of 1.7×10^{18} and 2.4×10^{18} should be used for nearshore and offshore waters respectively. This range in production values reflects, in part, maximum and minimum estimates of chromosomal DNA corresponding to actively growing and dormant cells. (Fuhrman and Azam, 1980, 1982). The accuracy of the ^{3}H-Thymidine incorporation method is also subject to variable isotopic dilution due to internal synthesis of thymine and in consequence, this method is always likely to produce an underestimate of production. Furthermore, we have recent evidence to show that some bacteria (Flavobacteriacea) do not take up ^{3}H-Thymidine (Lucas, 1986). It may be that Flavobacteria do not have an appropriate uptake mechanism (see also Pollard and Moriarty, 1984).

Thirdly, it appears that the lower estimate of bacterial production by ^{3}H-Thymidine incorporation is in good agreement with estimates of bacterial production based on predator-free incubations ; although it should be pointed out that the latter method may give an underestimate of bacterial growth if small microflagellates ($<3\mu m$) are found to be present in the incubation media (Fuhrman and McManus, 1984). Here, this agreement can however be expressed as a linear regression of the lower estimate of ^{3}H-Thymidine production against production values based on predator-free incubations (Fig. 3). Two regression slopes are given ; A and B. Regression slope B is fitted to all data points for the three profiles and includes data from 5 m down to 75 m. Although there is a significant relationship between the results of the two methods ($r = 0.82$; $P<0.02$), a better correlation is obtained from slope A ($r = 0.95$; $P<0.0005$) in which the two highest estimates of production associated with surface waters are omitted. Note that regression A has a slope of 1.04 and a Y intercept close to zero (1.44) relative to the intercept of slope B (15.4) indicating that regression A describes an almost one to one agreement between production estimates based on predator-free incubations and the lower estimate of the ^{3}H- Thymidine incorporation method of Fuhrman and Azam (1980).

Bacterial and phytoplankton relationships

The more rigorous constraints that we have been able to impose on our estimates of bacterial biomass and production now allow us to more confidently estimate the quantitative significance of the transfer of photosynthetically fixed carbon through the bacterioplankton community.

Bacterial consumption of carbon (Cc) can be estimated from the net growth yield of bacteria (Williams, 1981) and expressed as a % of the carbon substrate utilised. Estimates of bacterial net growth yield have however been shown to vary widely (between 10-90 %) depending on the complexity of the substrate being utilised and also on the extent of nitrogen available to the bacteria. (Newell *et al.*, 1981 ; Williams, 1981 ; Joint and Morris, 1982 ; Linley and Newell, 1984). Soluble molecules such as amino-acids and glucose are efficiently utilised (60-90 %) relative to refractory detrital components (10-15 %).

To estimate carbon flow through bacterioplankton, based on primary production, it is necessary to estimate bacterial carbon consumption on the basis of the differing net growth yields recorded for the particulate and photosynthetic exudate (PDOC) fractions of primary production. Photosynthate exudates as DOC (PDOC) have been shown to account for as much as 30 % of the total fixed carbon (Williams, 1981) and have been considered as the major source of carbon for bacteria in the euphotic zone (Joiris *et al.*,

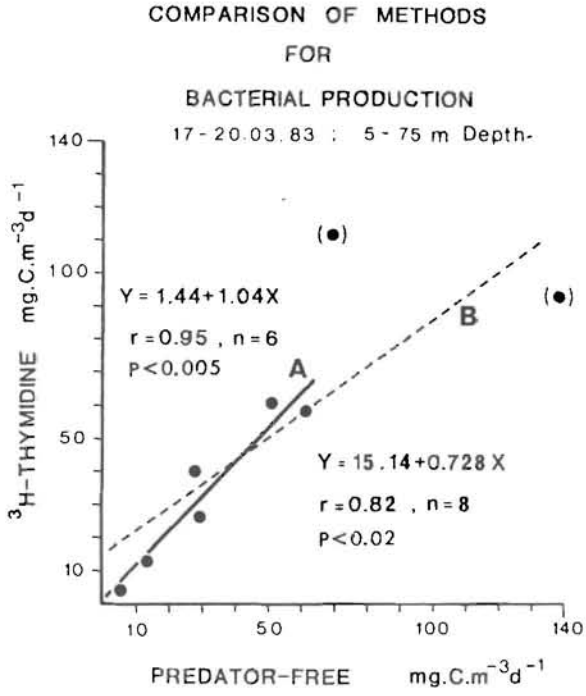

Figure 3 : A linear regression plot of the lower estimate of ^{3}H-Thymidine production calculations versus production estimates based on $< 3\mu m$ predator-free incubations (data from Table 1). Regression slope A is fitted after omitting the data points shown in brackets.

1982 ; Cole *et al.*, 1982 ; Lancelot and Billen, 1984). In such a situation the net growth efficiency of bacteria is likely to be high (60-90 %). For a naturally decaying phytoplankton assemblage where bacteria utilised both detrital particulates and PDOC, Newell *et al.*, (1981) recorded a net growth efficiency of 31 %. Thus bacterial consumption of carbon (C_C) required to sustain measured bacterial production (Pc) rates can be calculated from : Cc = Pc / 0.31. Clearly, an overestimate of bacterial production will very significantly increase the estimate of carbon passing through the microheterotrophic decomposer pathway.

From Table 1 it is evident that bacterial carbon production at the 25 % irradiance depth for the three profiles (17th, 19th and 20th March) amounts to 14.4 %, 98 % and 58 % respectively of photosynthetically fixed carbon production. For the first profile (17.3.83), the percentage of bacterial carbon production relative to phytoplankton production (14.4 %) at the 25 % irradiance depth is in good agreement with similar estimates of Laake *et al.* (1983). At this time it would seem that phytoplankton PDOC exudation (124 mg.C.m^{-3}d^{-1}*ca.* 30 % of 414 mg.C.m^{-3}d^{-1} total fixed carbon) could entirely meet bacterial carbon requirements (99.7mg.C.m^{-3}d^{-1}) even if a relatively low net growth yield of 60 % was used for such soluble molecules (Cc = 59.8/0.60 = 99.7mg.C.m^{-3}d^{-1}).

However, as the bloom decays (19th and 20th March) bacterial production throughout the water column generally equals or exceeds (x4 - x12) phytoplankton production. Bacterial carbon consumption requirements here considerably exceed PDOC exudation estimates even if bacterial net growth yield was estimated to be 100 % efficient. Bacterial production at this depth and also in the aphotic zone must therefore be supported by

detrital POC utilisation, (see Newell *et al.*, 1985, 1986). Elsewhere, we have found that in the unproductive Antarctic system encountered in May 1984, bacteria also utilised detrital POC as a carbon source (Painting *et al.*, 1985).

In conclusion , calculations of carbon flux through the bacterioplankton depend greatly upon accurate estimates of bacterial production and net growth yield. While the ^{3}H-Thymidine incorporation method of Fuhrman and Azam (1980 and 1982) for measuring bacterial production is convenient to use, our evidence suggests that their conservative calculation of bacterial production agrees with the predator-free incubation method and would seem the more realistic (but see also Lucas, 1986). On this basis, it appears that carbon flow through heterotrophic bacteria can readily be accounted for by PDOC utilisation in the euphotic zone during a spring bloom and detrital POC utilisation in the euphotic or aphotic zones when phytoplankton blooms senesce, decay and sink through the water column.

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