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Dynamics of a *Phaeocystis*-dominated spring bloom in Belgian coastal waters. II. Bacterioplankton dynamics

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ABSTRACT: Measurements of dissolved organic matter, bacterial biomass, exoproteolytic activity, uptake of direct substrates, bacterial production and mortality were carried out in the Belgian coastal zone during the wax and wane of the 1984 spring phytoplankton bloom. These data offer an accurate description of bacterioplankton dynamics in response to phytoplankton development. A model, taking into account the basic processes involved in bacterial utilization of organic matter (HSB model), provides a good simulation of the observed variations of bacterial biomass and activity.

INTRODUCTION

In the first paper of this series, Lancelot & Mathot (1987) presented data concerning the dynamics of phytoplankton in the Belgian coastal zone, collected during the spring bloom in 1984. Measurements of biomass and activity of bacterioplankton were simultaneously carried out and are presented in this paper.

The existence of a close coupling between phytoplankton and bacterioplankton during the spring bloom in the Southern Bight of the North Sea has already been demonstrated by Lancelot & Billen (1984). The data presented here, however, were obtained with a much closer sampling program, and allow a more detailed analysis of bacterial dynamics.

As a guideline for this analysis, we present in Fig. 1 a diagrammatic representation of our general view of the basic processes involved in bacterial organic matter utilization, as evolved from our previous works (see e.g. Billen et al. 1980, Billen 1984, Servais 1986). The main features of this conceptual model (referred to as the 'HSB model') can be summarized as follows.

Biodegradable organic matter in the sea is mostly supplied by excretion or lysis of phytoplankton in the form of macromolecular biopolymers (H) (Billen 1984). These cannot be directly taken up by bacteria and have first to be hydrolysed through the action of exoenzymes and converted into monomeric substrates (S). Exoenzymatic hydrolysis therefore often constitutes the limiting step of the whole process of organic matter utilization (Somville & Billen 1983).

On the other hand, the uptake of 'direct' monomeric substrates is very rapid, so that their concentration is maintained at a steady low value (Billen et al. 1980, Billen 1984). Once taken up by bacteria, direct substrates can be either catabolized and respired or used for biosynthesis. The ratio between biosynthesis (i.e. production of bacterial biomass [B]) and total rate of organic matter utilization defines the growth yield (Y). This ratio, along with the C/N ratio of the substrates used determines the extent of ammonification, or of mineral nitrogen uptake when nitrogen-deficient organic substrates are used (Billen 1984, Lancelot & Billen 1985).

The bacterial biomass (B) formed is subject to mortality, caused either by microzooplankton grazing or by spontaneous or virus-induced lysis (Servais et al. 1985).

This view of the interactions between bacteria and organic matter is admittedly idealized. Its usefulness, however, lies in the fact that powerful methods have been developed for directly measuring the rate of most of the basic processes listed above: exoenzymatic

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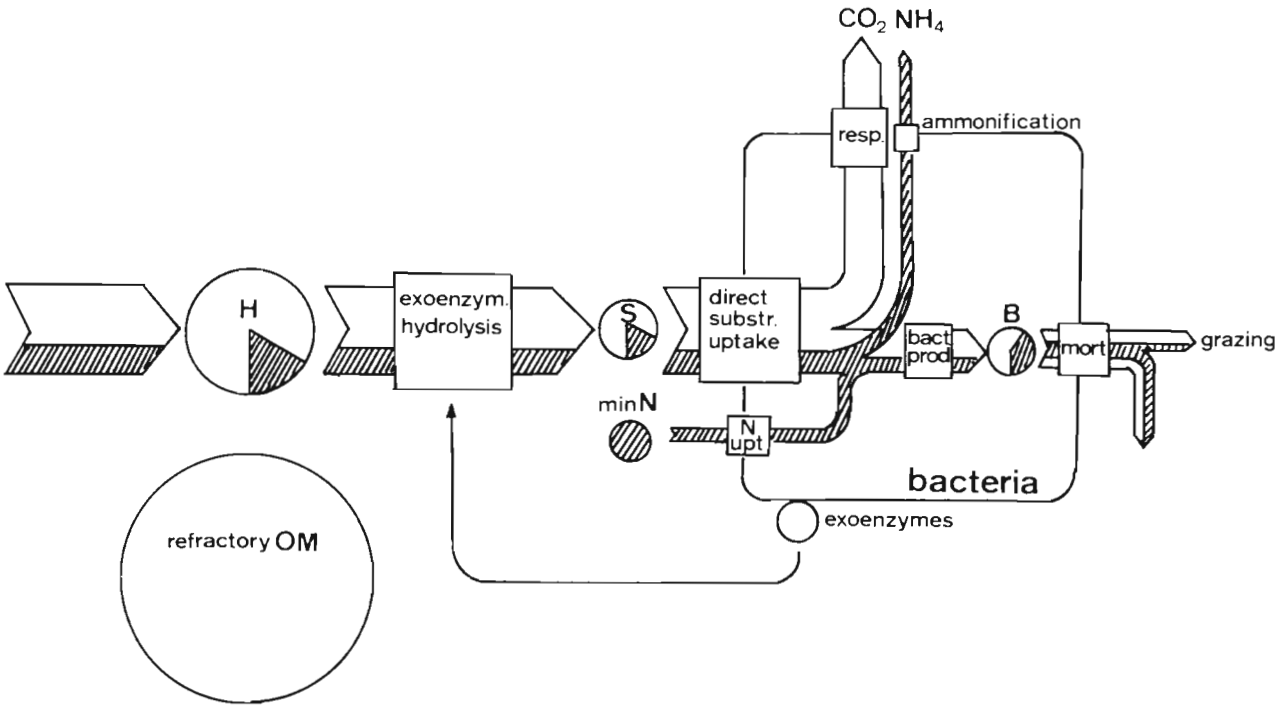

Fig. 1. Diagrammatic representation of the basic processes involved in bacterial utilization of organic matter according to the HSB model. Shaded arrows represent nitrogen fluxes. H: high molecular weight biodegradable organic material (biopolymers); S: directly usable low molecular weight organic substrates (direct substrates); B: bacterial biomass; min N: mineral forms of nitrogen (nitrate, nitrite, ammonium)

activity (Somville & Billen 1983, Vives-Rego et al. 1985), total uptake of direct substrates (Lancelot & Billen 1984), bacterial biomass production (Fuhrman & Azam 1982), and bacterial mortality (Servais et al. 1985).

This paper presents the first account of the simultaneous application of all these methods during a 2 mo period in a single marine environment. On basis of these measurements, a budget of heterotrophic bacterial activity during this period is established. A simulation, based on the HSB model presented above, allows prediction of the major trends of the observed variations of bacterial biomass and activity.

METHODS

Samples were collected at a station located 10 km off Ostend (51°26'05''N, 2°49'08''E) in the Belgian coastal zone of the North Sea. A general description of the sampling site and procedures was given by Lancelot & Mathot (1987), along with a discussion of nutrient chemistry and phytoplankton dynamics.

Dissolved organic carbon was measured with a Dohrman 80, wet combustion carbon analyser, after filtration of the sample through pre-ashed GF/C filters (nominal pore size about 1 µm). Dissolved proteins and amino acids were determined by the fluorescamine method adapted from North (1975) as described by Fontigny et al. (unpubl.). Peptone and glycine were used as standards for proteins and amino-acids determinations respectively.

Bacterial biomass was determined by epifluorescence microscopy after acridine orange staining, following the procedure of Hobbie et al. (1977). Cells numbers were counted and converted into biovolume by considering the mean size of the bacteria, estimated visually by comparison with a calibrated grid. A conversion factor of 1.2×10^{-7} µg C μm^{-3} was used for calculating biomass from biovolume. This value is the median of those cited in the literature which vary in the range 0.87 to 1.65×10^{-7} (Ferguson & Rublee 1976, Williams & Carlucci 1976, Watson et al. 1977, Hagström et al. 1979, Jordan & Likens 1980, Pedros-Alio & Brock 1982).

Heterotrophic ciliate counts kindly provided by Drs C. Lancelot and S. Mathot were obtained by inverted microscopic examination after Lugol fixation and sedimentation of the samples.

Exoproteolytic activity was essayed by the L-leucyl-β-Naphthylamide method, described by Somville & Billen (1983). The enzyme unit (e.u.) is defined as the amount of enzyme catalyzing the cleavage of 1 µmole β-Naphthylamine min^{-1}. Several studies have shown that this assay provide a good index of the overall capacity of natural bacterial populations to hydrolyse

proteins (Somville & Billen 1983, Lancelot & Billen 1984, Vives-Rego et al. 1985, Fontigny et al. unpubl.).

Uptake rates of monosaccharides, glycolic acid and amino acids were determined by tracer additions of ^{14}C-labelled substrates, as explained by Lancelot & Billen (1984). As justified in the latter paper, the sum of the utilization rates of these 3 classes of direct substrates is considered to be an estimate of the total rate of carbon utilization by heterotrophic bacteria.

Bacterial biomass production was estimated from measurements of thymidine incorporation into cold TCA-insoluble material, performed according to the procedure proposed by Fuhrman & Azam (1982). On the basis of both theoretical and empirical arguments, these authors suggested the use of a factor of 1.7×10^9 bacteria formed per nmole thymidine incorporated in coastal seawater. However, there has been much controversy in the literature regarding the validity of this factor. Other authors have shown that the ratio between bacterial biomass produced and thymidine incorporation can vary widely in different environments, the range of values cited covering one order of magnitude (0.5 to 6.2×10^9 bact. $nmol^{-1}$) (Riemann et al. 1982, Bell et al. 1983, Fallon et al. 1983, Riemann et al. 1984) or even more (0.5 to 68×10^9) (Kirchman et al. 1982). These differences can be partly explained by the fact that a significant proportion of thymidine is sometimes incorporated into other TCA-insoluble macromolecules beside DNA; partly also by variations in the DNA content of bacteria, in the extent of intracellular isotope dilution of thymidine, and by other factors (Karl 1982, Witzel & Graf 1984). We verified that the hot TCA-insoluble fraction (often considered to be made only of proteins) contained only a negligible percentage (less than 10 %) of the incorporated radioactivity.

In order to determine the conversion factor for our own site, the following experiment was carried out on 4 occasions. A seawater sample filtered through a 0.2 µm membrane was inoculated at 20°C. Rapid bacterial growth was observed. Bacterial numbers and thymidine incorporation were followed every 4 to 5 h for 24 h. The increase in cell numbers was plotted against cumulated thymidine incorporation. As shown in Fig. 2, the results are in close agreement with the factor 1.7×10^9 bact. $(nmole\ thy)^{-1}$, proposed by Fuhrman & Azam (1982).

Rate of bacterial mortality was estimated according to the procedure developed by Servais et al. (1985), consisting of following the disappearance of radioactive tracer from the DNA of (^3H)thymidine-labelled natural assemblages of bacteria. Filtration of a subsample through 2 µm Nuclepore membrane after labelling of the bacteria allowed distinction of bacterial grazing by >2 µm micro-zooplankton from 'residual' mortality due to other causes, such as smaller grazers, bacterial predators, and spontaneous lysis.

RESULTS

For the sake of comparison, seasonal variations of phytoplankton biomass, as described in the preceeding paper of this series, are presented in Fig. 3, expressed in cellular protein content (Lancelot & Mathot 1987). These data clearly show a first peak of diatoms in mid-April, followed by a large bloom of *Phaeocystis pouchetii* in early May.

Dissolved organic carbon remained within the typical range for coastal water (1 to 2 mg C l^{-1}) during April (Fig. 4). A rapid increase was observed during

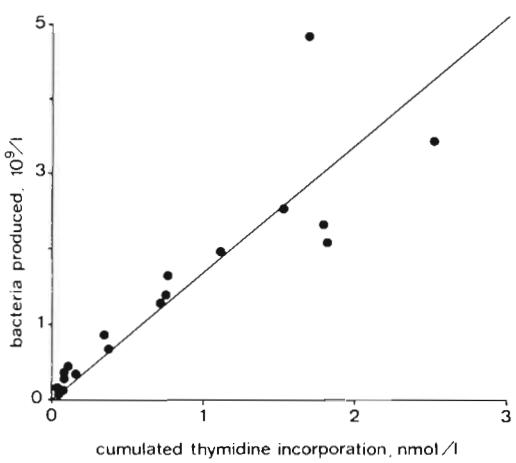

Fig. 2. Relation between number of bacteria produced during short-term incubation of reinoculated 0.2 µm filtered seawater samples and cumulated thymidine incorporation into cold TCA-insoluble material. The straight line shows the theoretical relation proposed by Fuhrman & Azam (1982) (1.7×10^9 bact. [nmol thy.]$^{-1}$)

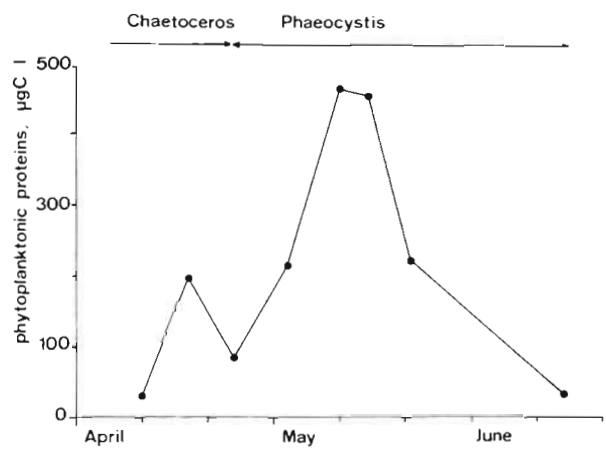

Fig. 3. Seasonal variations of proteic phytoplankton biomass during the 1984 spring bloom in the Belgian coastal zone (from Lancelot & Mathot 1987)

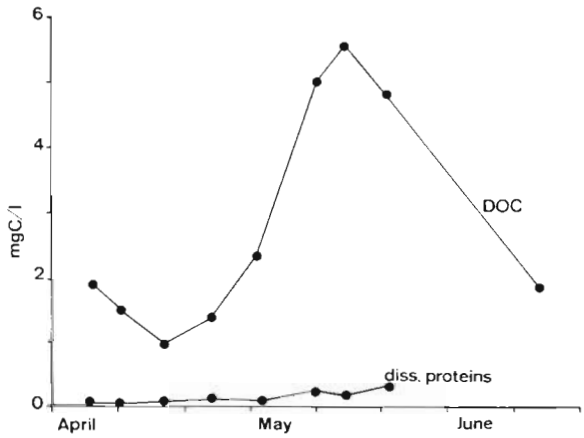

Fig. 4. Seasonal variations of dissolved organic carbon and protein concentrations during the 1984 spring bloom in the Belgian coastal zone

the *Phaeocystis pouchetii* bloom and high concentrations (near to 5 mg C l^{-1}) remained some time after the decline of cellular *P. pouchetii* biomass. Free amino-acid concentrations remained fairly constant around a mean value of 25 µg C l^{-1}, as expected from previous measurements in the same area and theoretical considerations (Billen et al. 1980). Dissolved proteins showed a regular increase from about 50 µg C l^{-1} at the beginning of April to 350 µg C l^{-1} at the end of May. Combined dissolved amino acids always represented a very small fraction of dissolved organic carbon, suggesting that the latter material was mainly made up of nitrogen-deficient organic compounds.

Bacterial biomass (Fig. 5) showed a distinct peak in mid-May, following the *Phaeocystis pouchetii* bloom with only a few days delay. This peak corresponds to a 3-fold increase in numbers (1.3 to 3.5×10^9 bact. l^{-1}) and 2-fold increase in biovolume (0.65 to 0.13 μm^3 bact.$^{-1}$).

The variations of exoproteolytic activity (Fig. 6) also closely followed phytoplankton biomass variations,

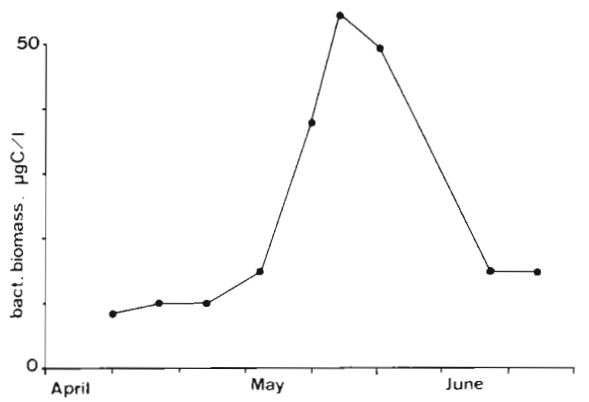

Fig. 5. Seasonal variations of planktonic bacterial biomass during the 1984 spring bloom in the Belgian coastal zone

with 2 well-marked successive maxima. Exactly the same pattern was observed for direct substrates utilization (Fig. 7) and bacterial production (Fig. 8).

The flux of bacterial mortality is shown in Fig. 9. The part of this process attributable to micro-zooplankton (> 2 µm) grazing is also shown. It is seen that grazing explains most of the mortality during May while mortality must be explained by other processes in April. The importance of grazing mortality in May, but not in April, is consistent with the observations that a significant density of ciliates only developed from the beginning of May (Fig. 9). Unfortunately, data on hetero-

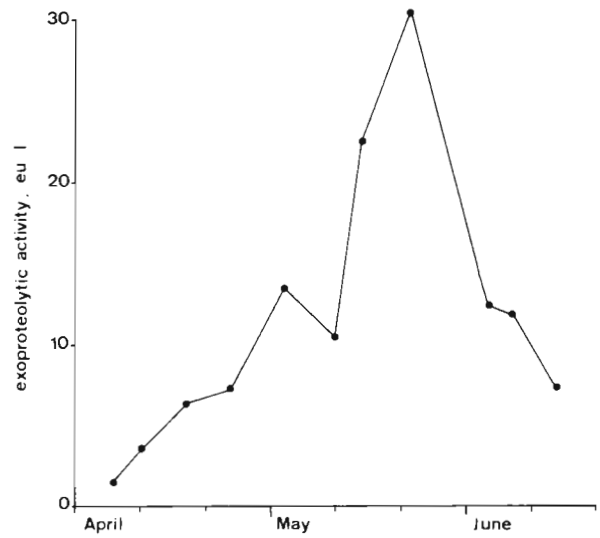

Fig. 6. Seasonal variations of exoproteolytic activity during the 1984 spring bloom in the Belgian coastal zone

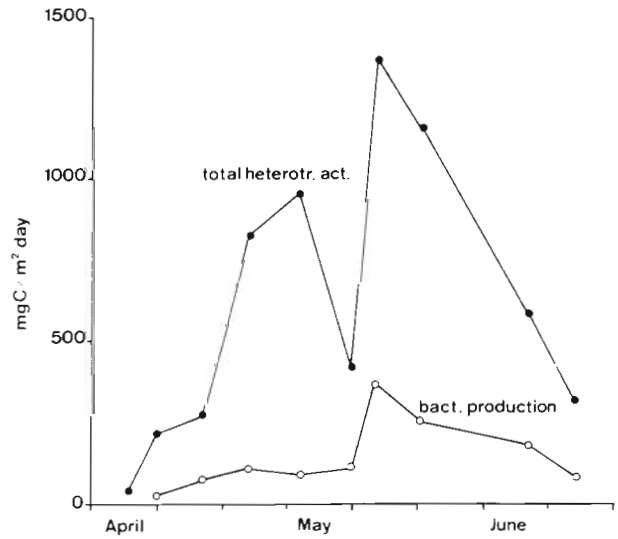

Fig. 7. Seasonal variations of direct substrates utilization (monosaccharides + amino-acids + glycolate utilization) by planktonic bacteria during the 1984 spring bloom in the Belgian coastal zone

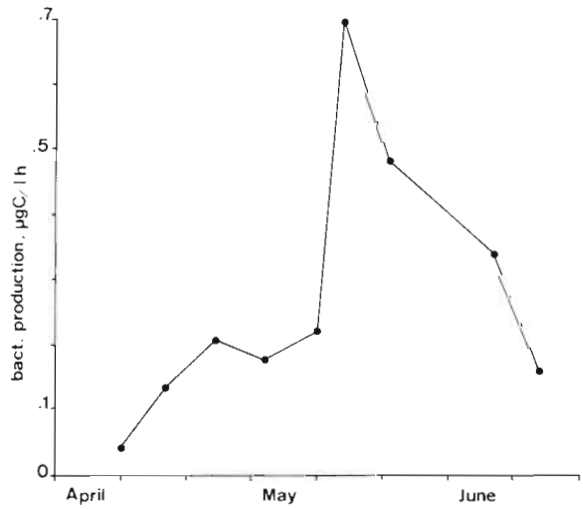

Fig. 8. Seasonal variations of bacterial production during the 1984 spring bloom in the Belgian coastal zone

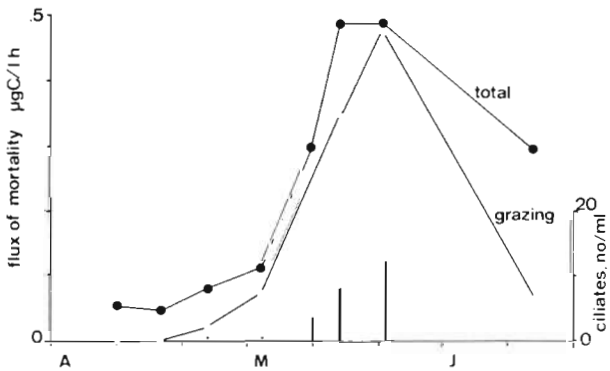

Fig. 9. Seasonal variations of total (●) and grazing (○) bacterial mortality during the 1984 spring bloom in the Belgian coastal zone. Bars indicate the density of heterotrophic ciliates taken as an index of the existence of a microzooplankton population

trophic flagellates (which are likely the major bacterial grazers) are not available for the period studied. We used here ciliates counts as an index of the occurrence of a micro-zooplankton population.

DISCUSSION

Phytoplankton/bacterioplankton coupling and accumulation of dissolved organic matter

Apart from our previous observations in the same site (Lancelot & Billen 1984), and similar data from the nearby Oosterschelde recently reported by Laanbroek et al. (1985), there have been few reports in the literature of a distinct bacterial bloom in close coupling with the spring phytoplankton bloom.

The data presented here on seasonal variations of 3 measurements of bacterial activity – exoproteolytic activity (Fig. 6), direct substrates utilization (Fig. 7) and bacterial production (Fig. 8) – show a distinct 2-peak pattern. Each of these peaks probably corresponds to one of the 2 successive peaks of phytoplankton biomass shown in Fig. 2. The delay with respect to the first diatom peak appears a little longer than with respect to the *Phaeocystis pouchetii* peak. These delays, however, never exceeded 10 d and were often shorter.

Our data thus demonstrate a rapid response of bacterioplankton to the production of organic matter by phytoplankton. This rapid response could appear contradictory to the observation of large accumulation of dissolved organic matter during the *Phaeocystis pouchetii* bloom (Fig. 4). Such unusually high concentration of dissolved organic carbon have been frequently reported as a general characteristic associated with *Phaeocystis* blooms (Bölter & Dawson 1982, Eberlein et al. 1985). It has sometimes been suggested that this accumulation of dissolved organic matter results from an inhibition of bacterial activity by antibiotic substances produced by the algae (Sieburth 1960, Eberlein et al. 1985). No evidence of bacterial inhibition is apparent from our data. Indeed the ratio of bacterial activity (either thymidine incorporation, exoproteolytic activity or direct substrates utilization) to bacterial biomass does not show significant variations which could be related to such an effect. In fact, a part of the organic matter, determined as dissolved, may be constituted by the mucilaginous products composing the matrix of *P. pouchetii* colonies, passing through the filter and dissolving in the filtrate (Bölter & Dawson 1982, Lancelot & Mathot 1987). It therefore does not only represent strictly 'dissolved' organic matter. The fact that this material is largely deficient in nitrogen further suggests that it mainly originates from the mucous envelope of *P. pouchetii*, while the cellular material released after cell lysis does not accumulate in the dissolved phase as it is rapidly used by bacteria.

Growth yield and ammonification rate

Comparison of direct substrate utilization (Fig. 7) with bacterial production (Fig. 8) allows determination of the bacterial growth yield (Y). Its value varies between 0.1 and 0.3. This range may seem extremely low with respect to values as high as 0.6 to 0.8 cited during the 70's by several authors (e.g. Williams 1970, Crawford et al. 1974, Gocke 1976, Williams et al. 1976, Williams 1981). The evidence for these high yields lay in the low apparent respiration of individual ^{14}C-labelled organic compounds by natural bacterial assem-

blages during short-term experiments. It has now been shown (Billen et al. 1980, King & Berman 1984) that these low respiration rates are an artefact due to the lack of isotopic equilibrium of the internal pools of respiratory metabolites. Direct determination of the conversion of detritic organic matter into bacterial biomass during decomposition experiments, reported by Lucas et al. (1981), Newell et al. (1981, 1983) and Linley et al. (1983), provides efficiencies in the range 0.29 to 0.37 in the absence of nitrogen limitation, and in the range 0.07 to 0.13 with N-deficient material in low nutrient water (see also discussion by Lancelot & Billen 1985).

If we consider that amino-acid uptake represents by far the most important flux of organic nitrogen utilization by bacteria, comparison between this flux and bacterial production rate allows determination of the rate of ammonium production or uptake by bacteria. Thus, the rate of ammonification (or ammonium uptake) is given by the difference between the amount of organic nitrogen taken up and the amount of nitrogen immobilized as biomass. The former term can be calculated from the measurements of amino-acid uptake, assuming a mean C/N ratio of 3.3, while the latter is estimated from the bacterial production figures, considering a C/N ratio of 4 for bacterial biomass (Luria 1960, Lucas et al. 1981). Both terms are plotted in Fig. 10. Except on 14 May, the flux of amino-acid uptake is more than enough for meeting the nitrogen requirements of biomass synthesis. Ammonification is, however, much more important during the first part of the bloom, corresponding to the utilization of organic matter produced by diatoms, than during the second one, corresponding to the utilization of *Phaeocystis pouchetii* material. This should probably reflect a lower mean nitrogen content of the organic material used by bacteria during the second peak than during the first one.

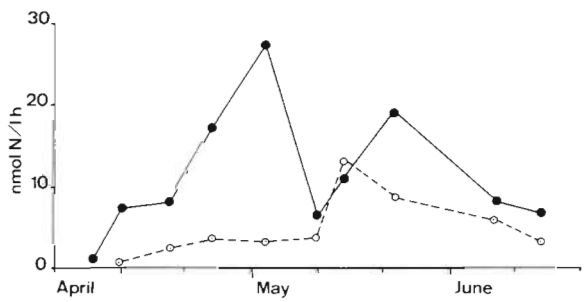

Fig. 10. Seasonal variations of total amino-acid uptake (●) and the nitrogen requirements of bacterial biomass production (○) (calculated from production figures by considering a C/N ratio of 4 for bacterial biomass) during the 1984 spring bloom in the Belgian coastal zone. Higher figures for amino-acid uptake indicate net ammonification, while the reverse would indicate mineral nitrogen uptake by bacteria

Balance of bacterial production and mortality

The variations of bacterial biomass (Fig. 5) obviously result from the balance between the rates of production and mortality. As shown by comparing Fig. 8 & 9, the rates of production and mortality estimated by completely independent methods are very close to each other. As expected, the difference is positive during the increasing phase of bacterial biomass, and negative during the decline of the bloom. These differences are plotted in Fig. 11 and compared with the observed net rate of increase or decrease of bacterial biomass deduced from the data of Fig. 5. The agreement is striking and gives confidence in the validity of our rate measurements.

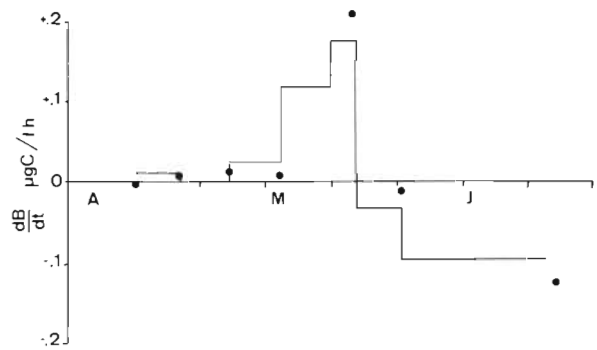

Fig. 11. Comparison between the net rate of change of bacterial biomass deduced from the observed variations shown in Fig. 5 (——), and the difference between bacterial production and mortality figures (Fig. 8 & 9 respectively) (●)

CONCLUSIONS

Budget calculation and modelling

The data presented in this paper offer an accurate description of bacterioplankton dynamics in response to a phytoplankton bloom. They allow establishment of a budget for the different metabolic activities represented in Fig. 1. Considering a mean depth of 18 m for the well-mixed coastal area under study, this budget has been established as shown in Table 1 for the period from 10 April to 14 June.

We will now show with the aid of an idealized model that very simple assumptions regarding bacterioplankton dynamics (HSB model, Fig. 1) on the one hand, and phytoplankton/bacteria relations on the other hand, can account for the major trends of the observed seasonal variations of bacterioplankton biomass and activities reported in this paper.

This model is based on the dynamics of proteins alone, taken here as the best indicator of biomass at successive levels of the food web, in accordance with

Table 1. Budget of bacterial metabolic activities in the Belgian coastal zone during the period 10 Apr to 14 Jun. Depth 18 m

Process	Activity	
	gC m^{-2}	gN m^{-2}
Direct substrate utilization	39	
Amino acid utilization	17	5
Bacterial production	8	2
Ammonification	–	3
Mortality: Total	7.5	
Grazing	5.2	

the ideas developed by Lancelot et al. (1986) for phytoplankton.

We consider that dissolved proteins (H) are released by lysis of phytoplankton cells at a rate proportional to their protein biomass (F). By comparing the net rate of change of phytoplanktonic biomass, expressed in protein content, with daily protein production figures, Lancelot & Mathot (1987) have been able to determine an overall first order rate constant of phytoplankton mortality (k_f) of about 0.009 h^{-1}. Combining this result with the data of Fig. 3 on phytoplankton protein biomass gives an integrated flux of phytoplankton mortality of about 50 g C protein m^{-2} for the period from 10 April to 14 June. Comparison with total amino-acid utilization by bacteria during the same period (see Table 1) indicates that the part (α) of phytoplanktonic protein production which is utilized by bacteria in the water column is about 0.34. As zooplankton grazing is of minor importance at this time of year (Joiris et al. 1982, Fransz & Gieskes 1984, Lancelot & Mathot 1987), we interpret this ratio as reflecting the part of phytoplanktonic material which is released after lysis in the form of dissolved material, more easily available to bacterioplankton – the rest being probably deposited onto the sediments. For comparison, artificial disruption of phytoplanktonic cells by freeze-drying (Otsuki & Hanya 1972) or by freezing and thawing cycles (Newell et al. 1981) results in the production of respectively 25 and 34 % of the cellular material in the form of dissolved organic matter.

Dissolved proteins (H) released by phytoplankton mortality are assumed to be hydrolysed into free amino acids by exoproteases according to Michaelis-Menten kinetics. Fontigny et al. (unpubl.) determined the half-saturation constant (K_H) as 80 µg C l^{-1} and the maximum rate (e_{max}) was shown to be directly related to bacterial biomass (B), with a proportionality coefficient of 0.04 to 0.15 µg C (µg bacterial C. h)$^{-1}$.

The amino acids (S) produced are rapidly taken up by bacteria, also according to Michaelis-Menten kinetics. The half-saturation constant (K_S) is arbitrarily fixed at a value of 0.1 mg C l^{-1} and the maximum rate of uptake (b_{max}) is fixed at a value of 0.5 h^{-1} which is the median of the values derived from the data of Jannasch (1968) for maximum growth rates of marine bacteria.

The value of growth yield (Y') to be considered for the purpose of the present model, which takes only amino-acid uptake into account, is different from the carbon conversion efficiency discussed above. We will define it here as the ratio of bacterial biomass produced to total amino acid taken up (both expressed in mg C). For the whole period from 10 April to 14 June, this ratio is 0.47.

Bacterial biomass (B) is subject to mortality with a mean first order rate constant (k_d) of 0.009 h^{-1}.

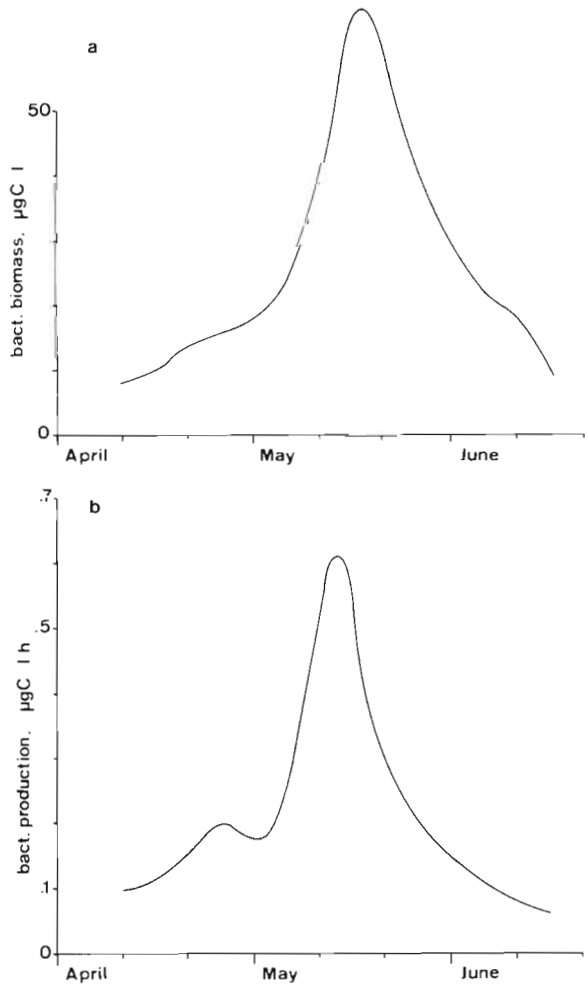

Fig. 12. Simulation of the seasonal variations of (a) bacterial biomass and (b) production according to the HSB model. The system receives an imposed variable flux of dissolved proteic material released through phytoplankton mortality, which is calculated from the observed variations of phytoplankton biomass (Fig. 3) by considering a constant mortality rate (K_f = 0.009 h^{-1}) and a constant fraction of dissolved organic material (α = 0.34). The variations of H, S and B in response to this variable input are described by the differential equations (1), (2) and (3) (see text)

These considerations lead to the following systems of equations:

$$\frac{dH}{dt} = \alpha \cdot k_f \cdot F - e_{max} \cdot \frac{H}{H + K_H} \cdot B \quad (1)$$

$$\frac{dS}{dt} = e_{max} \cdot \frac{H}{H + K_H} \cdot B - b_{max} \cdot \frac{S}{S + K_S} \cdot B \quad (2)$$

$$\frac{dB}{dt} = Y \cdot b_{max} \cdot \frac{S}{S + K_S} \cdot B - k_d \cdot B \quad (3)$$

where all symbols and numerical values of the parameters have been defined above.

This system has been numerically solved for simulating the variations of bacterial production and biomass during the period under study, by imposing the values of H, S and B observed on 10 April as initial conditions, and the observed variations of phytoplanktonic protein biomass (F) as a control variable.

The results of this simulation are shown in Fig. 12a, b. The agreement with the observed variations of bacterial biomass and production (Fig. 5 & 8) is excellent. Of particular significance in our opinion is the fact that the model correctly predicts a single bacterial biomass peak and a double production peak, in response to the 2 successive phytoplankton peaks. Also the delays of bacterial response to phytoplankton variations are correctly simulated. The good agreement between simulation and observations suggests that the HSB model presented here, although idealized, provides reasonably accurate description of bacterioplankton dynamics.

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