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Activity of heterotrophic bacteria and its coupling to primary production during the spring phytoplankton bloom in the southern bight of the North Sea

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Abstract

Parallel measurements were made of particulate and dissolved products of primary production, utilization rate of amino acids, monosaccharides, and glycollate, thymidine incorporation into DNA, and exoproteolytic activity before and during the spring bloom at different stations in the southern bight of the North Sea and in the English Channel. High correlations were found between the three methods used for estimating the activities of heterotrophic bacteria. A reasonable quantitative agreement was found between the estimate of bacterial production based on thymidine incorporation into DNA and the estimate of total carbon utilization based on the sum of the utilization rates of amino acids, monosaccharides, and glycollate. A close coupling between microheterotrophic activity and primary production was demonstrated. Examination of the nitrogen balance during the spring bloom shows that microheterotrophic activity could play an important role in food-web dynamics by partly satisfying the nitrogen needs of phytoplankton, which represents about twice the mineral nitrogen stock initially present in the water column.

Growing evidence leads to the conclusion that a major part of the organic carbon produced by photosynthesis in marine environments cycles through bacterial activity (Pomeroy 1974; Williams 1981). In most recently published studies of coastal and upwelling areas, zooplankton grazing has been shown to consume <30–40% of the net particulate primary production (*see* Joiris et al. 1982). Recently developed methods for measuring heterotrophic activity of bacterioplankton show that it accounts for at least 20–50% of primary production (Fuhrman and Azam 1980, 1982; Larsson and Hagström 1979, 1982).

A budget of carbon cycling in the Belgian coastal zone of the North Sea (Joiris et al. 1982) revealed that only 20% of the annual net primary production is grazed by zooplankton, while 40% is consumed by planktonic microheterotrophs and 40% settles to the sediments and is degraded by benthic microorganisms. About 70% of the total annual primary production occurs during the spring bloom, which is dominated from mid-April to mid-May by the haptophyceaen *Phaeocystis poucheti* (Hariot) Lagerheim. The very peculiar physiological characteristics of this colony-forming species (Lancelot 1984*a*) may be responsible for some ecological features of the Belgian coastal ecosystem, namely the low zooplankton grazing efficiency (as this 3-μm organism is either too small singly or too large when in colonial form to be grazed by copepods: Joiris et al. 1982), the importance of dissolved products of primary production (probably linked to the production of the mucous envelope: Lancelot 1984*a*), and the importance of the flux of phytoplankton material to the sediments (due to the large size of *Phaeocystis* colonies).

To better understand the relationship between phytoplankton and microheterotrophs in the southern bight of the North Sea, we made parallel measurements of primary production and heterotrophic activity of planktonic bacteria in 1982 before and during the spring bloom. We report here the results of these measurements.

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Biotopes and methods

Collection of samples—The sampling stations in the southern bight of the North Sea and the eastern part of the English Channel are shown in Fig. 1. Samples were collected at a depth of 3 m with 5-liter Niskin bottles.

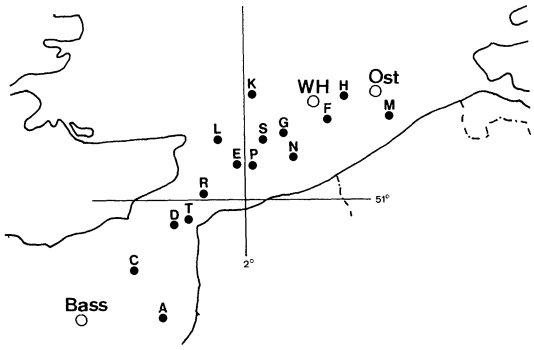

Fig. 1. Location of the sampling stations in the southern bight of the North Sea. Stations Ostend (Ost), West Hinder (WH), and Bassurelle (Bass) were chosen for a more detailed study of seasonal variations.

At least in the Belgian coastal zone, the water column is well mixed (Nihoul and Polk 1977).

All microbiological measurements began within an hour of collection. Subsamples for chemical analysis, filtered through decontaminated Whatman GF/C filters, were immediately frozen.

Primary production—Primary production of particulate and dissolved materials was measured according to the procedure described in detail by Lancelot (1983) during 4-h incubations centered around noon at ambient temperature and light intensity. Bottles covered by screens of known light transmission allowed the parameters of the photosynthesis-light relationship to be measured.

Vertical extinction coefficients were measured with an immersible quantameter and variations of solar irradiance were measured hourly at the meteorological station of Ostend. From this information, the daily primary production was calculated by using Vollenweider's (1965) model modified for a well mixed water column.

Activity of heterotrophic bacteria—Three independent methods were used for measuring the activity of planktonic heterotrophic bacteria.

The relative rate of uptake and respiration of glucose, amino acids (protein hydrolysate: Amersham), and glycollate was determined by adding tracer amounts of high specific activity (150 mCi·mmol^{-1}) ^{14}C-labeled substrates, after the procedure of Billen et al. (1980). The natural concentrations of these substrates determined on several occasions (Billen et al. 1980) do not display large seasonal or geographical variations, in agreement with the observations of others (Andrews and Williams 1971; Crawford et al. 1974) and with the hypothesis of the control of substrate concentration by rapidly growing microorganisms (Billen 1984). The variations in the absolute rates of substrate utilization are thus entirely reflected by the variations in relative rates. We therefore estimated the absolute rates of monosaccharides, amino acids, and carboxylic acids utilization by multiplying measured relative rates of glucose, amino acids, and glycollate utilization by their mean total in situ concentrations: 0.8 μmol·liter^{-1} (60 μg C·liter^{-1}) for total free monosaccharides, 0.5 μmol·liter^{-1} (25 μg C·liter^{-1}) for free amino acids, and 2 μmol·liter^{-1} (50 μg C·liter^{-1}) for carboxylic acids resulting from phytoplankton excretion. As organic matter of high molecular weight cannot be directly taken up by bacteria but can only be absorbed after exoenzymatic hydrolysis into monomers (Rogers 1961; Somville and Billen 1983) the total uptake of monomers must account for all the utilization of organic matter, whether it is primarily produced as small molecules or as macromolecules. On the other hand, since polysaccharides and proteins are by far the most abundant polymers in phytoplankton-derived organic matter, and since the products of phytoplankton and zooplankton excretion comprise free and combined amino acids, carbohydrates, and carboxylic acids, it is reasonable to think that the sum of the utilization rates of amino acids, monosaccharides, and carboxylic acids accounts for the major part of organic matter utilization. A much greater diversity of organic compounds obviously exists in seawater, but the fluxes through the pools of monosaccharides, amino acids, and carboxylic acids are probably by far the most important. This line of argument suggests that the sum of the utilization rates of these three classes of monomeric substrates can be used as an estimate of the total rate of carbon utilization by heterotrophic bacteria.

[Methyl-^{3}H]thymidine incorporation into cold TCA-insoluble material, an index of bacterial production, was determined according to the procedure of Fuhrman and Azam (1980, 1982). By comparing radioactivity incorporated into cold and hot TCA-insoluble fractions, we estimated that radioactivity incorporated into proteins represented only about 10% of the former.

The potential activity of proteolytic exoenzymes was determined using L-leucyl-βnaphthylamide, following the procedure of Somville and Billen (1983). The measurements reported here were made on unfiltered samples and therefore represent total (free and attached) exoproteolytic activity. Parallel measurements after filtration of the samples through 0.2-μm membranes showed that the "free" dissolved exoproteolytic activity represents as a mean only about 10% of the total activity (range 0–30%) (Vives-Rego et al. in prep.).

Chemical methods—Chlorophyll *a* was determined spectrophotometrically (Lorenzen 1967). Ammonium was measured by the phenolhypochlorite method (Slawyk and MacIsaac 1972). Nitrate and nitrite were determined according to the procedure of Armstrong et al. (1967). Dissolved organic nitrogen was evaluated by determining nitrate after persulfate digestion and subtracting the value of total mineral nitrogen concentration (Smart et al. 1981). Particulate nitrogen was determined by a Kjeldahl procedure.

Results

Table 1 shows the data on bacterial activities, phytoplankton biomass and production, and nutrient concentrations obtained during transects from the Belgian coast to the eastern English Channel carried out from November 1981 to October 1982.

Figure 2 shows the geographical distribution of bacterial activities, chlorophyll *a* concentrations, and particulate nitrogen in May 1982. This figure clearly illustrates the contrast between the rather oligotrophic water masses coming from the channel and the eutrophic water of the Belgian coastal zone which receives nutrient inputs of continental origin.

Seasonal variations of nutrient concentration, bacterial activities, phytoplankton biomass, and total and dissolved products of primary production are shown in Fig. 3 for the three stations (Ostend, West Hinder, and Bassurelle) representative of the different nutritive conditions encountered from the Belgian coast to the eastern English Channel. From mid-October to March, total primary production is rather constant at the three stations, ranging from 100 to 250 mg $C \cdot m^{-2} \cdot d^{-1}$; dissolved primary production is very low. The phytoplankton bloom starts by the beginning of April. Particulate production is first the most important part of total primary production, but when nitrate is exhausted, dissolved production becomes the dominant component (Lancelot 1983), at least at the Ostend and West Hinder stations, where *P. poucheti* largely dominates the phytoplanktonic community. The maximum level of primary production reached during the bloom is higher, and its duration is longer in the Belgian coastal zone than in the channel.

The changes in bacterial activity at the three stations parallel the development of the phytoplankton, with only 10 days lag. In fact, during the bloom, all measures of bacterial activity closely follow the variations in dissolved primary production. Outside the bloom, they remain reasonably constant at much lower values. Higher activities (on a m^3 basis) are found at the more coastal stations than at the offshore ones or in the channel.

Discussion

Measurement of heterotrophic activity—The present data allow comparison between three methods for measuring heterotrophic activity in seawater. Each of these methods suffers from specific drawbacks, and no universally accepted method has as yet been proposed.

As explained above, the use of the sum of monosaccharides, amino acids, and carboxylic acids utilization rates as a measure of total heterotrophic activity rests on reasonable but still unproved assumptions. First, the hypothesis is made that only organic molecules of low molecular weight can be directly taken up by bacteria, while macromolecular organic compounds must first

Table 1. Heterotrophic bacterial activity, primary production (PP), and nutrient concentrations from data collected in 1982 in the southern bight of the North Sea.

	Direct substrate utilization				Thy†	EA‡	Chl pigments ($\mu g \cdot liter^{-1}$)		PP§ ($mg\ C \cdot m^{-2} \cdot d^{-1}$)		Nutrients (μM)			
	Glu	PH	Gly	Total*										
Station		($\%\cdot h^{-1}$)					Chl	Pheo	Part	Diss	NO_3^-	NH_4^+	DON	PON
17 Nov 81														
M		2.9				1.89								
Ost	0.46	1.6		16.2		1.74								
H		1.2				1.62								
N	0.41	1.9		17.3		1.96								
8–9 Feb 82														
Ost	0.117	0.13	0.52	8.7	<1	0.98	0.93	1.4	209	0	36	1.5	28	7.3
WH	0.036	0.062	0.60	8.1	3.7	0.63	0.22	0.3			16.8	0.3	19	3.4
P	0.118	0.33		3.7		0.89	0.47	0.44			16.1	0.5	8	2.9
T	0.092	0.43		3.9		0.71	0.63	0.65			11	0	9.5	1.5
Bass	0.014	0.10	0.002	0.8	<1	0.29	0.19	0	156	0	6.6	4.4	17	1.5
14 Apr 82														
Ost							3.85	1.75	(352)	(62)	33	9		
WH							7.75	1.75	(576)	(384)	0.5	13		
21–23 Apr 82														
A	0.17	0.17	1.3	19	6.5	3.5	5.74	2.2			2	0.5		7.5
Bass	0.015	0.024	0.69	8.6	3	1.97	0.32	0.34	413	130	3	0		10.1
C	0.83	1.17	0.91	29.9	9.2	5.6	2.9	1.5			0	0		8.1
D	0.305	2.75	0.81	30.6	7.0	8.86	5.4	2.4			0	0		8.6
E	1.03	1.68	0.39	29.6	4.5	8.48	7	2.1			0	0		6.1
F	0.18	2.6	0.78	27.6	4.9	6.35	5.2	1.1			16	1.6		12.8
M	0.43	1.8	0.36	21.3	9.1	4.26	7.2	3.1			48	0		11.3
Ost	0.35	1.97	—	21.2	4.9	9.49	9.3	5	618	206	19	0		23
H	0.96	2.11	0.32	30.3	10.7	9.30	9.9	2.1			5	5.7		19.4
WH	1.3	3.88	0.3	45.6	7.38	12.1	14.5	3	545	320	3	3		22
11 May 82														
WH							4.4	0.87	(125)	(83)				

Table 1. Continued.

Station	Direct substrate utilization				Thy†	EA‡	Chl pigments (μg liter^{-1})		PP§ (mg C m^{-2}·d^{-1})		Nutrients (μM)			
	Glu	PH	Gly	Total*			Chl	Pheo	Part	Diss	NO_3^-	NH_4^+	DON	PON
		(%·h^{-1})												
19–21 May 82														
C	0.024	2.2	0.16	15.4	1.9	3.9	0.39	0			0	0		2.9
Bass	0.25	0.56	0	3.9	1.54	2.2	0.09	0.04	126	107	0	0		2.3
R	0.88	1.87	0.18	26.1	7.5	14.0	0.7	0			0	0		13.6
S	1.23	3.61	0.67	47.4	13.2	13.1	0.11	0.24			0	0		2.6
F	0.18	10.2	0.67	71.8	—	27.8	4.7	1.3			0.8	0		15.6
M	2.47	2.86	0.36	57.0	61.0	33.2	4	2.4			25	2	56	21.7
Ost	1.0	6.66	0.18	56.5	53.5	25.1	6.9	3.7	279	1,116	2	0	66	21
H	2.3	0.024	1.27	48.5	46.0	31.2	6.7	6.4			,0	0		24.2
WH	1.8	0.42	0.01	28.5	11.7	14.1	4	2.4	158	387	0	0.3	35	14
28 May 82														
Ost	2.62	1.75	0.31	52	—	1.57								
7 Jun 82														
Ost							5.5	1.1	(505)	(25)	—	—		
WH							2.7	0.8	(151)	(8)	—	—		
22–24 Jun 82														
Ost	0.92	2.44	0.51	34	12.9	3.9	1.9	1.8	432	48	6.5	2.4		4.6
WH	0.49	0.27	0.16	10.6	0.77	4.8	0.3	2.2	740	39	3	5.0		6.1
G	0.82	0.89	0.19	19.4	—	3.1	0.7	0.7	795	42	0.4	2.9		11.2
E	0.91	0.93	—	20	3.3	2.7	0.5	0.3	881	98	0	0		4.9
18 Oct 82														
M	0.86	1.27	0.53	26.4	5.0	2.5	2.59	3.11	103	5	6	3	33	6.4
Ost	0.44	0.53	0.32	13.3	1.27	1.8	1.8	1.8	180	2	5	12	52	2.4
WH	0.19	0.26	0.035	4.7	1.47	1.3	1.4	0.8	267	51	7	3		1.2
K	0.014	0	0	0.2	0.72	1.2					7.5	6		1.2
L	0.01	0.001	—	0.15	0.3	1.0	0.4	1.4			8			1
D	0.28	0.43	0	6.6	5.9	3.7	2.5	3.1			9.5	16	27	—
Bass											7	2.4	4	—

* Total in mg C·m^{-3} d^{-1}.

† Thymidine incorporated into DNA in pmol·liter^{-1}·h^{-1}.

‡ Exoproteolytic activity in nmol βN liter^{-1} min^{-1}.

§ Primary production (mg C·m^{-2}·d^{-1}) values in parentheses were calculated from chlorophyll data as follows: Particulate primary production is calculated assuming that the parameters of the photosynthesis–light relationship are similar to those of the nearest phytoplankton population. Dissolved primary production is estimated from the inorganic nitrogen concentrations using the relationship P.E.R. (percentage of extracellular release) – inorganic nitrogen defined for each phytoplankton population present (Lancelot 1983).

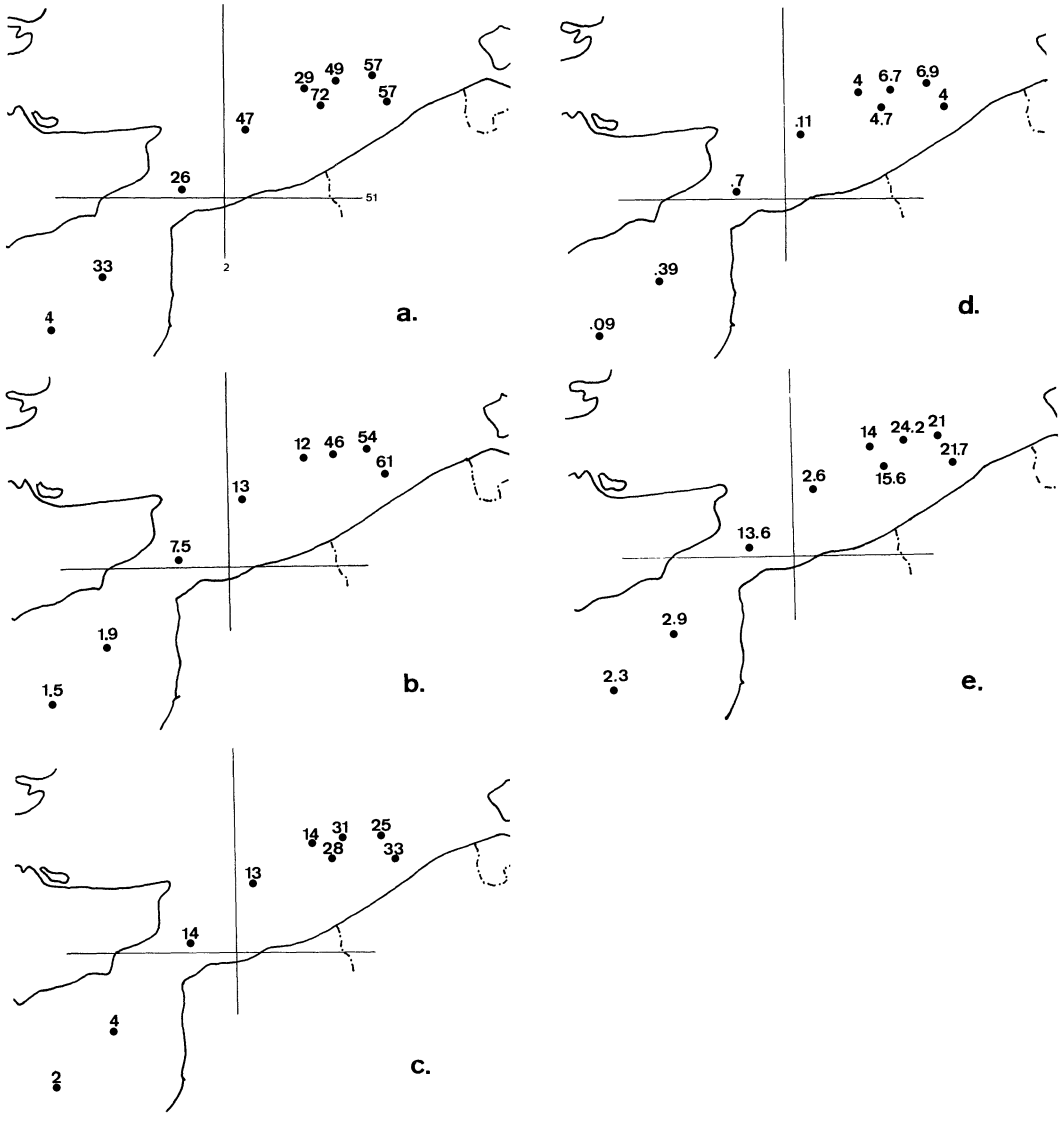

Fig. 2. Geographical distribution of some parameters on 19–21 May 1982. a. "Direct" utilization of substrate (mg C·m^{-3}·d^{-1}); b. thymidine incorporation into DNA (10^{-3} nmol·liter^{-1}·h^{-1}); c. exoproteolytic activity (nmol N·liter^{-1}·h^{-1}); d. chlorophyll *a* (Lorenzen 1967) (μg·liter^{-1}); e. particulate organic nitrogen (μmol·liter^{-1}).

be hydrolyzed by exoenzymatic activity. Second, it is assumed that no other classes of directly usable substrates than monosaccharides, amino acids, or small organic acids make an important contribution to the total flux of carbon utilization by heterotrophic bacteria. Moreover, the use of this estimate ignores the possibility that some substrates measured as "free" could, in some circumstances, be unavailable to the microheterotrophs or that microenvironments enriched in organic substrates could exist in the proximity of algae or detrital particles (Azam and Ammerman 1984).

As pointed out by Fuhrman and Azam (1980, 1982) and by others (Karl 1982), measurements of [^{3}H]thymidine incorporation into the cold TCA-insoluble fraction

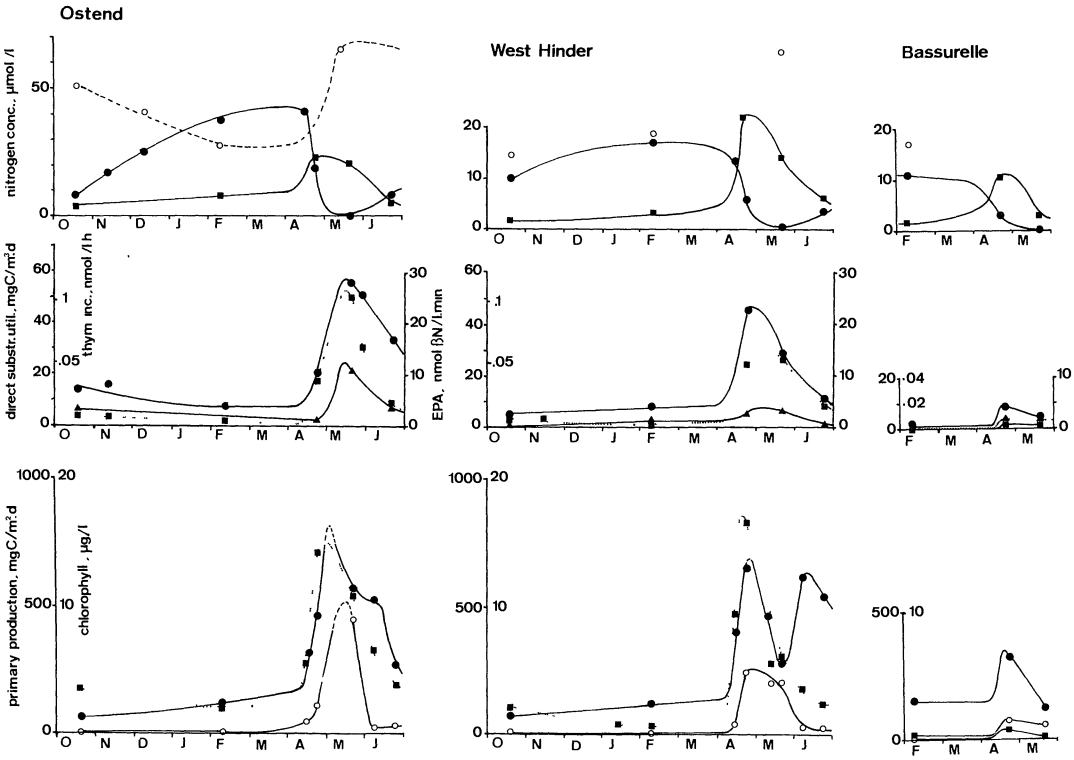

Fig. 3. Seasonal variations in nutrient concentration [NO_3^- + NH_4^+ (●), dissolved organic nitrogen (○), and particulate organic nitrogen (■)], bacterial activities [direct substrate utilization (●), thymidine incorporation (■), and exoproteolytic activity (▲)], phytoplankton biomass and production [Chl *a* + pheopigments (■), total primary production (●), and dissolved primary production (○)] at stations Ostend, West Hinder, and Bassurelle.

do not account for isotope dilution of the intracellular pool of labeled thymidine by de novo synthesis. This could lead to underestimation of the true rate of DNA synthesis.

Exoproteolytic activity measurements as described by Somville and Billen (1983) cannot a priori be considered a general index of heterotrophic bacterial activity, because they represent only the potential ability of the microbial community to hydrolyze peptide bonds. Moreover, the validity of the activity measured by an artificial substrate has yet to be demonstrated.

In view of these considerations, the close similarity of the results obtained with the three methods for both geographical variations (Fig. 2) and seasonal variations (Fig. 3) is quite remarkable. High correlations are obtained between thymidine incorporation (y in 10^{-3} nmol Thy·liter^{-1}·h^{-1}) and direct substrate utilization (x in µg C·liter^{-1}·d^{-1}) ($y = 0.25x + 0.37$, $r = 0.82$, $n = 27$) on the one hand and between exoproteolytic activity (y in nmol βnaphthylamine·liter^{-1}·min^{-1}) and direct substrate utilization (x in µg C·liter^{-1}·d^{-1}) ($y = 0.43x - 2.3$, $r = 0.87$, $n = 36$) on the other hand. This suggests that in spite of the uncertainties concerning the methods used for measuring the activities of heterotrophic bacteria, at least they provide reliable indexes for coastal marine waters.

A further check on the quantitative validity of our estimates can be obtained by comparing the absolute values of thymidine incorporation and direct substrate utilization. The slope of the correlation we found indicates that 6.25×10^{-3} nmol thymidine is incorporated into DNA for every µg C of

Table 2. Mean daily rates of primary production (PP) and activity of heterotrophic bacteria, as evaluated by graphical integration of the data of Fig. 3, for the spring bloom (1 April–30 June 1982) and the winter (February 1982) period (all in mg $C \cdot m^{-2} \cdot d^{-1}$).

	Spring bloom		Winter	
Belgian coastal zone	Ost	WH	Ost	WH
Part PP	444	344	200	–
Diss PP	356	211	0	–
Total	800 (1,000)*	555 (778)*	200	–
Activity†	355	378	87	120
Eastern channel (Bass)				
Part PP	293		156	
Diss PP	92		0	
Total	385		156	
Activity†	158		30	

* Values in parentheses are maximum values calculated by considering that primary production values of 2 g $C \cdot m^{-2} \cdot d^{-1}$ early in May were not observed (*see text*).

† Calculated from the data of direct substrate utilization, by considering a mean depth of 10, 15, and 35 m for the water column in the surrounding regions of stations Ostend, West Hinder, and Bassurelle.

total substrate utilization. Fuhrman and Azam (1982) estimate the number of bacterial cells produced for 1 nmol thymidine incorporated into DNA as $1.7–2.4 \times 10^9$. Assuming a mean carbon content per bacterial cell of $1–2 \times 10^{-8}$ μg C (Hagström et al. 1979), our data would thus indicate a bacterial production of 0.1–0.3 μg C per μg of total carbon utilization, i.e. a growth efficiency of 10–30%. Although this is at the low side of the range generally cited for the growth yield ratio in pure culture or in enriched samples (Williams 1981; Billen 1984), it is not unrealistic in natural situations where nitrogen deficiency can limit bacterial production. For instance, the data of Lucas et al. (1981), Newell et al. (1981, 1983), and Linley et al. (1983), who measured directly the production of bacterial biomass from the heterotrophic utilization of various organic materials in seawater, show growth efficiencies in the range of 7–37%.

The correlation that we found between potential exoproteolytic activity and direct substrate utilization needs specific comment. Because proteolytic enzymes of marine bacteria are inducible enzymes, produced only when proteins are present in the medium (Vives-Rego et al. in prep.), this correlation suggests that most organic nitrogen available to bacteria is originally in the form of proteins rather than being directly produced as free amino acids, in good agreement with the work of Billen (1984) and Somville and Billen (1983). In this context, it is noteworthy that phytoplanktonic excretion in the Belgian coastal zone has been shown (by ultrafiltration) to consist mainly of high molecular weight organic compounds (Lancelot 1984*b*).

Relationship between heterotrophic activity and primary production—Figure 3 shows a very close parallelism between activity of heterotrophic bacteria and primary production. The correlation between direct substrate utilization (y in mg $C \cdot m^{-2} \cdot d^{-1}$) and total primary production (x in mg $C \cdot m^{-2} \cdot d^{-1}$) is $y = 0.37x + 46.0$, $r = 0.72$ ($n = 15$).

Primary production of total, particulate and dissolved organic matter, and activity of heterotrophic bacterioplankton during the spring bloom can be evaluated by graphical integration of Fig. 3, as shown in Table 2. The data from the two stations of the Belgian coastal zone give very similar estimates of total primary production (72 and 50 g $C \cdot m^{-2}$ from 1 April to 30 June at Ostend and West Hinder).

These values of total primary production are rather low when compared with the mean spring bloom value of 215 g $C \cdot m^{-2}$ from 15 March to 15 July given for this area by Joiris et al. (1982) for the years 1971–1980. This discrepancy may be explained by our lack of data for the first days of May, when primary production is generally highest. If we assume that particulate primary production had reached 2 g $C \cdot m^{-2} \cdot d^{-1}$ and

dissolved production 1 g $C \cdot m^{-2} \cdot d^{-1}$ at the beginning of May at Ostend and West Hinder (reasonable maximum values reported for this area), our estimates would be too low by 20 and 29%. It seems, therefore, either that spring 1982 was rather unproductive compared with the mean of the previous decade or that the production figures of Joiris et al. (1982) were overestimated.

In the Belgian coastal zone, direct substrate utilization by heterotrophic bacteria amounts to 32–34 g $C \cdot m^{-2}$ from 1 April to 30 June, i.e. 44–68% of the total primary production during the same period. Similar percentages of primary production consumed by bacterioplankton are found in the eastern channel (Table 2). Dissolved primary production accounts for an important part of the needs of the bacterioplankton (up to 100% during the spring bloom at Ostend), in good agreement with the data reported by Larsson and Hagström (1982) for a eutrophicated coastal area of the Baltic Sea. Clearly, however, a significant part of the particulate primary production is also used rapidly by microheterotrophs. Our data thus provide a good indication of a rapid response of bacteria not only to dissolved materials associated with the spring phytoplankton bloom, but to particulate material as well.

The ecological significance of this close coupling between bacterioplankton activity and primary production becomes obvious when the nutrient balance of phytoplankton is considered. The decrease in mineral nitrogen concentration (mainly nitrates) at the beginning of the spring bloom (Fig. 3) is obviously due to phytoplankton uptake. This drop, of 4.7 and 2.8 g $N \cdot m^{-2}$ at Ostend and West Hinder, is approximately balanced by a simultaneous increase of particulate and dissolved organic nitrogen (Fig. 3). However, if the C:N ratio of 6, characteristic of phytoplankton from the southern bight of the North Sea in winter and spring (Lancelot 1982), is applied to our estimates of primary production during the spring bloom (Table 2), the nitrogen needs of phytoplankton during this period can be calculated to be 12 and 8.3 g $N \cdot m^{-2}$ at Ostend and West Hinder (15 and 11 if the maximum primary production values are used). This shows that the nitrogen needs of phytoplankton during the spring bloom are more than twice the stock of inorganic nitrogen initially present in the water. Thus, primary production can only proceed at the rate it does because of efficient and rapidly adjusting mechanisms of nutrient recycling. Planktonic bacterial activity is the most obvious such mechanism. Because of their short generation times, bacteria are able to follow phytoplankton growth closely even during the first outburst of the spring bloom. Whether bacteria themselves are directly mineralizing organic nitrogen or whether this mineralization is the result of a microbial loop involving grazing of bacteria by heterotrophic flagellates and microzooplankton (Azam et al. 1983) cannot be decided on the basis of our present data. Nutrient regeneration by benthos or macrozooplankton probably has much longer reaction times.

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