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Distribution of phyto- and bacterioplankton growth and biomass parameters, dissolved inorganic nutrients and free amino acids during a spring bloom in the Oosterschelde basin, The Netherlands*

H. J. Laanbroek, J. C. Verplanke, P. R. M. de Visscher & R. de Vuyst**

Delta Institute for Hydrobiological Research, Vierstraat 28, 4401 EA Yerseke, The Netherlands

ABSTRACT: Distribution of phyto- and bacterioplankton growth and biomass parameters were followed during a spring bloom in the marine Oosterschelde basin, Netherlands. Several other biotic and abiotic parameters were measured. During the measuring period from 4 April until 4 June, concentrations of the dissolved nutrients silicate, ammonium, and nitrate decreased, while dissolved phosphate tended to increase. Concentrations of chlorophyll *a* increased with a distinct maximum in mid May during a bloom of the haptophycean *Phaeocystis pouchetii*. In the shallower part of the basin maxima of particulate primary production and bacterioplankton production were reached at the end of April during a mixed bloom of diatoms of the genera *Cryptomonas, Skeletonema* and *Thalassiosira* as well as small flagellates. In the central basin, the most pronounced particulate primary production was observed during the *P. pouchetii* bloom. Relatively low percentages of extracellular release of total primary production were observed during and after this bloom. Percentage of bacterial incorporation of total exudates was high at the beginning of the experiment and decreased until the *P. pouchetii* bloom, when it increased again. Maximum bacterial productivities and biomasses were recorded during the decline of the *P. pouchetii* bloom. Overall oxygen consumption rates seemed to be determined by phytoplankton biomass and by bacterioplankton production, but not by phytoplankton production or bacterioplankton biomass. Concentrations of dissolved free amino acids (DFAA) tended to decrease until the end of the large *P. pouchetii* bloom when an increase was observed; lowest concentrations were during this bloom. Alanine, aspartate, glycine, leucine and serine were always quantitatively the most important amino acids; during the *P. pouchetii* bloom also glutamate. Mole percentages of aspartate and methionine increased at the end of this bloom.

INTRODUCTION

It is generally assumed that algae release dissolved organic matter either by excretion or by decay of senescent cells (Fogg 1983). Grazing zooplankton may increase the release of this matter from phytoplankton (Lampert 1978). Algal exudates may contribute up to 50 % of the bacterioplankton energy requirements (Wiebe & Smith 1977, Lancelot 1979, Larsson & Hagström 1979, Keller et al. 1982, Larsson & Hagström 1982, Wolter 1982, Jensen 1983, Lancelot & Billen 1984). The release of small as well as large compounds has been reported (Wiebe & Smith 1977, Mague et al. 1980, Jørgensen et al. 1983, Lancelot 1984). In short-time experiments, Lancelot (1984) showed that small molecules in particular were rapidly reconsumed. Dissolved free amino acids (DFAA) may form a substantial part of the small fraction (Jørgensen 1982). The amount of DFAA released in the marine environment and the contribution of the individual amino acids depend on the algal species involved (Macko & Green 1982, Poulet et al. 1984) and on the physiological condition of the algae or the algal population (Riley & Segar 1970, Crawford et al. 1974, Hammer & Eberlein 1981,

* Delta Institute for Hydrobiological Research, Communication No. 317

** Addressee for correspondence; present address: Institute for Ecological Research, Boterhoeksestraat 22, 6666 GA Heteren, The Netherlands

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Ittekot 1982, Hammer & Brockmann 1983, Lancelot 1983, Poulet & Martin-Jézéquel 1983). Amino acids are submitted to rapid recycling (Laane 1983). Bacteria are well-known consumers of DFAA (Crawford et al. 1974, Dawson & Gocke 1978, Billen et al. 1980, Sepers 1981, Amano et al. 1982, Bölter & Dawson 1982, Jørgensen 1982, Keller et al. 1982, Hagström et al. 1984). Besides bacteria, algae may also be involved in the consumption of DFAA (Bonin & Maestrini 1981, Admiraal et al. 1984). Thus, the production and consumption of DFAA is a dynamic process. A net *in situ* production of DFAA may only be demonstrable during or shortly after an algal bloom.

The aim of the present study was to determine the distribution of algal and bacterial biomass and growth parameters as well as of dissolved inorganic nutrients and free amino acids during a spring bloom. The study was carried out in the Oosterschelde basin as part of an intensive ecosystem research initiated on account of the construction of a storm surge barrier.

METHODS

Description of the area. The Oosterschelde basin in the south-western part of the Netherlands is a former estuary still in open connection with the North Sea (Fig. 1). Mean depth of the basin is 8 m, of which 53 % is less than 5 m deep, maximum depth is 55 m and total surface area amounts to 380 km^2, of which 44 % is intertidal. A hundred years ago the connection between the basin and the river Scheldt was closed by dams and since 1969 the discharge of freshwater from the rivers Rhine and Meuse is regulated by sluices. The maximum total input of freshwater amounts to 50 m^3s^{-1}, whereas the mean tidal volume is approximately 1250×10^6 m^3 with a mean volume of 3050×10^6 m^3 for the total basin. Consequently the Oosterschelde basin is a well mixed system with a salinity of ca 27 ‰ except close to the freshwater sources.

Sampling Stations 01 and 02 (Fig. 1) were selected in the main tidal channel of the basin, both at a mean depth of 20 m. Station 02 was surrounded by extensive intertidal mudflats.

Sample collection. During spring 1984, samples were collected weekly at half-tide around 0900 h. Mixed samples from the euphotic zone (up to 8 m depth) were maintained at *in situ* temperature until analysis in the laboratory, which occurred always within 1 h. Samples for amino-acid analysis were filtered immediately on board and stored at −20 °C.

Amino acid analysis. Sub-samples for DFAA analysis were gently filtered through a 0.2 µm polycarbonate

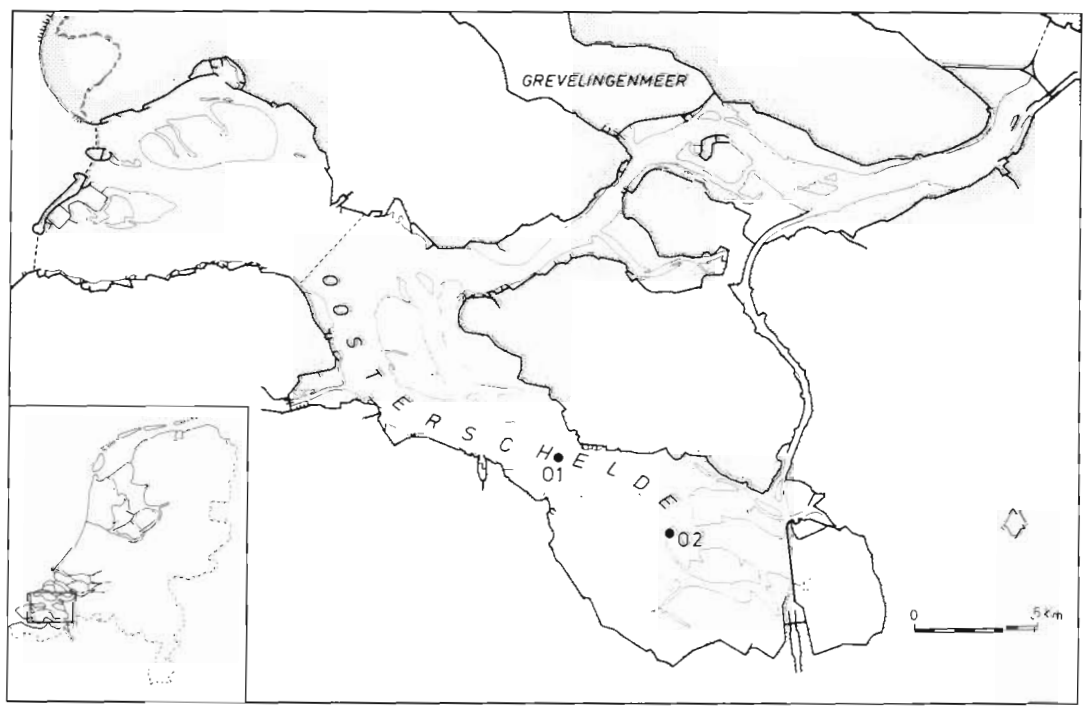

Fig. 1. Map of Oosterschelde basin in the south-western part of The Netherlands showing sampling stations; —— mean level of low tide, salt-marsh edge

filter (Nuclepore, 25 mm diameter) and collected in amounts of 4 ml in ultra-cleaned vials and stored at −20 °C. Within 3 wk, DFAA analysis was made by precolumn fluorescence derivatization with O-phthaldialdehyde using reversed-phase high performance liquid chromatography (HPLC) according to a slightly modified method of Lindroth & Mopper (1979). Since concentrations of some amino acids appeared to decrease during storage at −20 °C, DFAA analysis was repeated after 3 mo. Initial amino acid concentrations at time of sampling were calculated by linear extrapolation. By the method used the common amino acids, except cysteine, proline, lysine and ornithine, could be detected to levels of 1 to 10 nM without further pretreatment of the samples. The HPLC used was Waters controlled equipment in combination with a Perkin Elmer LC-10 fluorescence detector and a 5 μ resolve spherical C18 column (Waters, type P/N 85711). Amino acids were identified and quantified by spiking with standards. Depending upon the amino acid, coefficients of variation ranged from 3 to 13 % for standards at the 500 nM level.

Primary production and excretion. The classical radiocarbon method of Steeman-Nielsen (1952) was used to measure primary production. *Semi situ* experiments were performed in an incubator described by Fee (1973). Identical samples were illuminated at a series of irradiances from 0 to 139 W m^{-2}. Integral daily production values were calculated from the relations between applied irradiance and measured rate of photosynthesis. Five μCi $NaH^{14}CO_3$ were added to 50 ml seawater. After 5 to 6 h incubation at *in situ* temperature, samples were gently filtered through 3.0, 0.8 and 0.2 μm membrane filters (Schleicher and Schull). Five ml of filtrate was acidified with 0.01N HCl to pH 2.5 and gassed with air for 1 h to remove gaseous CO_2. Excretion by algae with simultaneous bacterial incorporation was estimated by a slightly modified method of Wolter (1982). (Methyl-^{3}H)thymidine instead of ^{14}C-glucose was used as tracer of bacterial size distribution. Samples of 50 ml containing 5 nM (methyl-^{3}H)thymidine (80 Ci $mmole^{-1}$) were incubated at *in situ* temperature at an irradiance of 24 W m^{-2} for 5 to 6 h, after which they were treated in the same way as the $NaH^{14}CO_3$ samples mentioned above. The irradiance of 24 W m^{-2} corresponds to the average irradiance in the water column of the Oosterschelde basin in spring. Since this irradiance equals approximately half the optimum irradiance for primary production in *semi-situ* experiments, excretion by photo-inhibition could be excluded. The size fraction <0.8 μm was regarded as being free of algal primary production after correction by means of a 0.8 μm prefiltered $NaH^{14}CO_3$-incubated sample. After 3 h incubation, the specific activity of the excreted ^{14}C-labeled carbon was determined by dividing the ^{14}C-labeled fraction of algal particulate material by the total phytoplankton biomass determined according to Mullin et al. (1966). Total bacterial utilization of ^{14}C-labeled compounds, including the amount used for dissimilation, was calculated by assuming a bacterial carbon conversion efficiency of 75 % for bacteria growing on algal exudates (Jensen 1983).

Daily solar radiation was measured at the Institute, 3 and 5 km from Stations 01 and 02, respectively.

Bacterial production. Bacterial production was determined with (methyl-^{3}H)thymidine incorporation into cold TCA-insoluble material according to the method of Fuhrman & Azam (1980, 1982). Samples of 5 ml containing 5 nM (methyl-^{3}H)thymidine (40 Ci $mmole^{-1}$, Amersham UK) were incubated in 30 ml screw-cap bottles in the dark at *in situ* temperature for 3 h. Blanks were prepared by addition of 2 % formaldehyde (final concentration). The rate of thymidine incorporation into DNA was converted to bacterial production by assuming that 2.1×10^{18} cells were produced per mole thymidine incorporated (Fuhrman & Azam 1982). Cell production was converted to carbon production by applying the factor 1.21 fg C μm^{-3} (Watson et al. 1977).

Oxygen consumption rates. As an index of overall heterotrophic activity, bacterial as well as algal, oxygen consumption rates were determined. Ten oxygen bottles were filled with water, 5 bottles immediately fixed and 5 incubated in the dark at *in situ* temperature for 24 h and then fixed. Oxygen concentrations were determined by Winkler titration according to Bryan et al. (1976).

Biomass estimates. Chlorophyll *a* concentrations were used as an index of phytoplankton biomass. Samples for chlorophyll *a* analysis were obtained by filtration of water samples over fiber-glass filters (Schleicher and Schull No. 6). Chlorophyll *a* analysis was by high performance liquid chromatography using a slightly modified method of Gieskes & Kraay (1983). The method was calibrated by using standards of pure chlorophyll *a* (Sigma), the concentration of which was determined spectrophotometrically (Lorenzen 1966).

Direct counts of bacteria and simultaneous determinations of mean cell volumes were performed on black stained 0.2 μm polycarbonate filters using the fluorescent dye acridine orange (Hobbie et al. 1977). Bacterial cell-carbon was obtained from the bacterial number and mean cell volume by applying the conversion factor of 1.21 fg C μm^{-3} (Watson et al. 1977).

Chemical analyses. Ortho-phosphate, reactive silicate, ammonium-nitrogen and nitrate-nitrogen were measured on a Technicon AA II auto-analyser, according to slightly modified techniques described by Strickland & Parsons (1972).

RESULTS AND DISCUSSION

Dissolved inorganic nutrients

A period with steady weather and a relatively high level of daily solar radiation (average 8.36×10^6 J m^{-2}, Fig. 2a) during the last weeks of April caused a rapid increase of water temperature from 6 to 12 °C. (Fig. 2b).

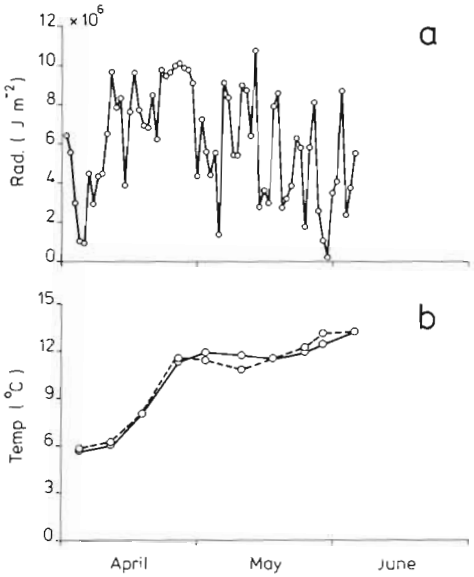

Fig. 2. Distribution of (a) solar radiation and (b) water temperature at Station 01 (——) and Station 02 (- - -)

From the beginning of May the weather became less steady, average daily solar radiation decreased to 5.24×10^6 J m^{-2}, and the increase of water temperature was subsequently less pronounced. There was no consistent difference between the 2 sampling stations.

Concentrations of nutrients measured at both stations differed only slightly. Concentrations of dissolved phosphate tended to increase at both stations (Fig. 3a). A distinct minimum was observed during a bloom of the haptophyceae *Phaeocystis pouchetii* in the middle of May (Table 1). A low concentration of dissolved phosphate in spring followed by an increase in June was also observed in the saline Lake Grevelingen, a former estuary in the direct vicinity of the Oosterschelde basin (Fig. 1) (Lindeboom & Merks 1983). In Lake Grevelingen, the increase in concentration of dissolved phosphate was not dependent on water temperature, but seemed to be a result of changing conditions in the sediments. Unlike dissolved phosphate, silicate concentration in the water decreased rapidly during the last weeks of April. At this time diatoms of the genera *Skeletonema* and *Thalassiosira* were quantitatively important in the Oosterschelde basin (Table 1). The concentration of silicate remained

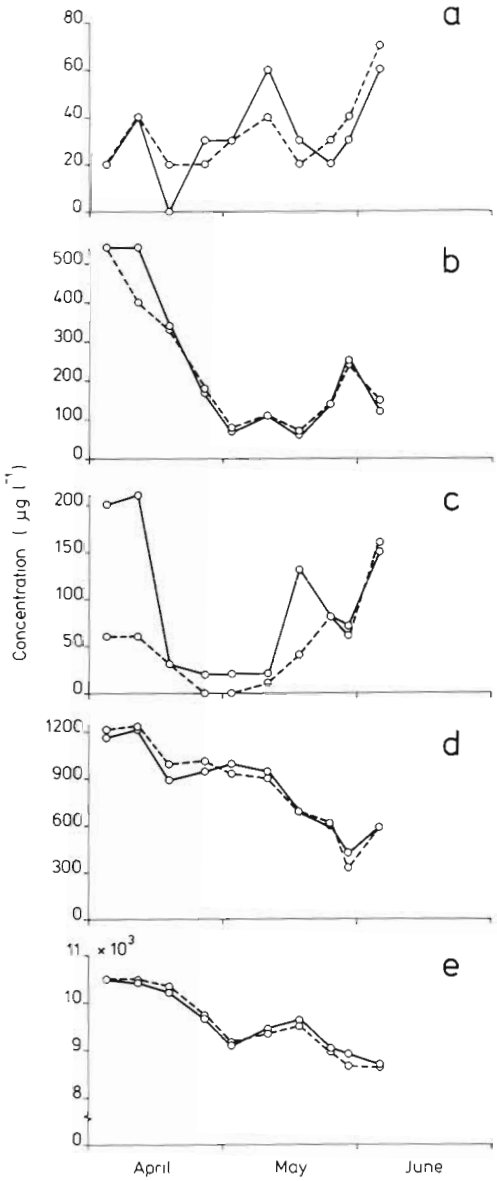

Fig. 3. Distribution of abiotic parameters (µg l^{-1}) in the water column of the Oosterschelde basin in spring 1984 at Station 01 (——) and Station 02 (- - -). (a) dissolved phosphate; (b) reactive silicate; (c) ammonium-nitrogen; (d) nitrate-nitrogen; (e) dissolved oxygen

at a low level in May until the end of the *P. pouchetii* bloom when a temporary increase of silicate was observed at both stations (Fig. 3b). Apparently, *P. pouchetii* had been outcompeting diatoms for nutrients leaving dissolved silicate in the water column of the basin. The concentration of dissolved ammonium in the water also decreased rapidly during the last weeks of April (Fig. 3c), probably through the activity of primary producers. At the end of the measuring period, the amount of dissolved ammonium increased again, possibly through the activity of heterotrophic,

Table 1. Dominant phytoplankton species enumerated at Station 02 in spring 1984; cell numbers ml^{-1} (C. Bakker & J. C. M. Weststrate-Rijk unpubl. results)

Species	Sampling date								
	4 Apr	11 Apr	18 Apr	26 Apr	2 May	10 May	17 May	24 May	4 Jun
Biddulphia aurita	4	1	11	nd	nd	nd	nd	nd	nd
Cryptomonas spp. (large)	263	340	312	732	508	599	286	496	315
Cryptomonas spp. (small)	68	185	99	794	1379	678	284	75	416
Flagellates (0 to 4 µm)	154	385	554	933	675	251	140	nd	339
Phaeocystis pouchetii	nd	nd	nd	nd	54	2603	14842	2174	nd
Rhizosolenia spp.	nd	nd	nd	nd	10	nd	5	nd	52
Skeletonema costatum	227	178	1158	1253	396	238	81	97	70
Thalassiosira spp.	99	39	130	130	33	16	8	7	5

nd = not detectable

ammonifying bacteria (Sepers 1981). The concentration of dissolved nitrate decreased steadily during the period of study (Fig. 3d). Relatively large decreases were observed in the middle of April when the concentrations of dissolved ammonium and silicate also decreased rapidly, and during the second half of May, a period characterized by the large *P. pouchetii* bloom. The concentration of oxygen decreased slowly during the period of study (Fig. 3e). However, a small increase in oxygen concentration was observed during the first half of May at the time of the *P. pouchetii* bloom. The water was always slightly oversaturated with oxygen, but saturation tended to decrease towards the end of the measuring period.

Phytoplankton biomass, particulate production and excretion

The concentration of chlorophyll *a* determined by HPLC was taken as an index of phytoplankton biomass. Dynamics in phytoplankton developments in coastal waters could approximately be followed by measurements of chlorophyll *a* (Côté & Platt 1983). However it should be kept in mind that no quantitative aspects of total phytoplankton carbon biomass could be extracted from such measurements, since carbon to chlorophyll *a* ratios are dependent on temperature and light conditions (Bienfang et al. 1983, Redalje 1983, Redalje & Laws 1983), on nutrient limitations (Laws & Bannister 1980) and on algal species involved (Perry et al. 1981). All these biotic and abiotic conditions changed throughout the period of study. A moderate increase in chlorophyll *a* concentration was observed up to mid May at both stations (Fig. 4a). A mixed bloom of diatoms and small flagellates at Station 02 in the second half of April (Table 1) was not reflected in the concentrations of chlorophyll *a* determined by HPLC. On May 17 a large increase in chlorophyll *a* concentration was observed at both stations. The increase was most pronounced at Station 01. Within a week the chlorophyll *a* concentrations decreased again to levels measured before the bloom. Particulate phytoplankton production increased from the beginning of observations at both stations (Fig. 4b). However, while particulate primary production reached a more or less stable level in the middle of April at Station 01, particulate production at Station 02 continued to increase for another week and reached a relatively high value, after which it declined again to a level comparable to values measured at Station 01. At Station 02, maximum numbers of *Cryptomonas, Skeletonema* and *Thalassiosira* species as well as small flagellates were observed at the end of April (Table 1). The observed maximum in phytoplankton particulate production on April 26 at Station 02 coincided with a temporary maximum in bacterioplankton production at the same date and at the same station (Fig. 4e). At Station 01, such a maximum in bacterioplankton production was not observed. A second increase in particulate primary production was observed at May 17 at both stations during the bloom of *Phaeocystis pouchetii* in colonial stage (Table 1). At that time the maxima in particulate production coincided with maxima in chlorophyll *a* concentrations. After May 17 both phytoplankton biomass and particulate production declined again to lower levels.

Phytoplankton extracellular release and a concomitant incorporation by bacterioplankton was determined in samples taken at Station 01. Total excretion rates were relatively high in April before and during a mixed bloom of diatoms and small flagellates, but low during the *P. pouchetii* bloom in the middle of May (Fig. 3c). Since excretion rates were determined only at one irradiance (24 W m^{-2}) we consider it inappropriate to use these rates for calculations of daily excretion

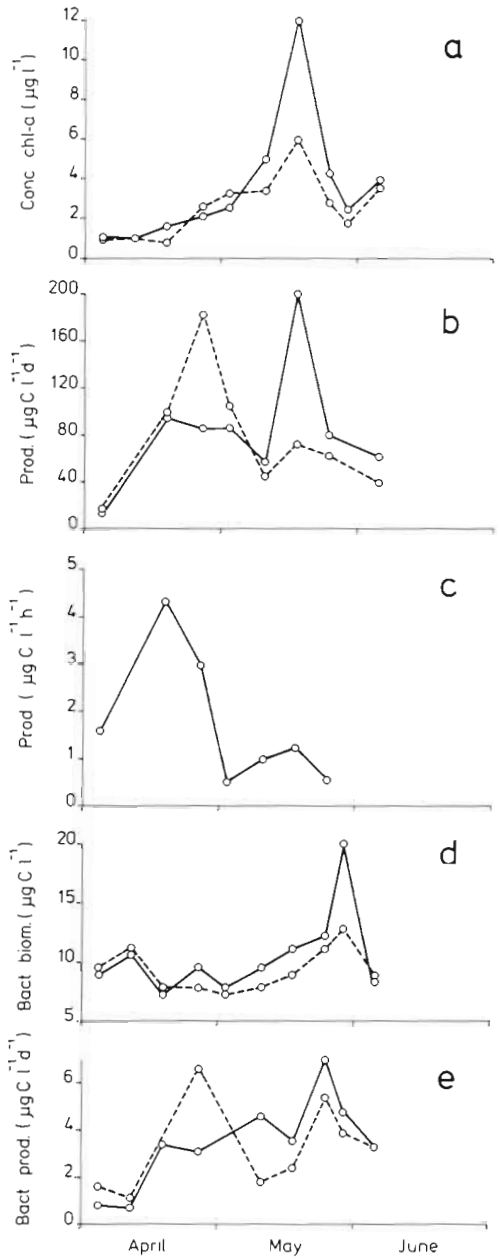

Fig. 4. Distribution of biotic parameters in the water column of the Oosterschelde basin in spring 1984 at Station 01 (——) and Station 02 (- - -). (a) chlorophyll *a* concentration (µg l^{-1}); (b) particulate primary production rate (µgC l^{-1} d^{-1}); (c) rate of algal extracellular release (µgC l^{-1} h^{-1}); (d) bacterioplankton biomass (µgC l^{-1}); (e) bacterioplankton production rates (µgC l^{-1} d^{-1})

rates as was done in the case of particulate primary production. So, excretion rates were only compared with production rates determined at the same irradiance. No correlation was found between extracellular release and particulate primary production. The percentage extracellular release (PER) was high during the mixed bloom in April and low during the mid-May *P. pouchetii* bloom (Table 2). As in the Kiel Fjord (Wolter 1982), PER values were apparently dependent upon the dominant phytoplankton species in the Oosterschelde basin. With respect to the *P. pouchetii* bloom, our results are contradictory to the observations of Lancelot (1983). PER values as high as 80 % were recorded by her during the decline of a bloom of *P. pouchetii* in the southern bight of the North Sea. A bacterioplankton carbon conversion coefficient for algal exudates of 75 % (Jensen 1983) was assumed for calculation of PER values in the Oosterschelde basin. These PER values would increase when assuming a bacterial carbon conversion efficiency less than 75 %. Lancelot & Billen (1984) calculated a conversion coefficient of 10 to 30 % during a *P. pouchetii* bloom. Bacterial carbon conversion efficiencies as low as 10 % have also been recorded by Newell et al. (1981), but these values concerned the bacterial incorporation of phytoplankton cell debris, which are more refractory than labile compounds released by living phytoplankton. PER values as low as 5 % were also determined by Veldhuis & Colijn (pers. comm.) during a bloom of *P. pouchetii*. So, correct PER values during blooms of *P. pouchetii* are still obscure.

Percentages of bacterioplankton utilization of total exudates fluctuated during the measuring period (Table 2). High percentages of bacterial utilization of total exudates were found in early April when the total amounts of excreted organic carbon were also relatively high. The percentages of bacterial utilization of total exudates decreased when primary production increased. An increase in the percentage of bacterial utilization of total exudates was observed during the decline of the *Phaeocystis pouchetii* bloom.

Relatively high percentages of total bacterioplankton activities, as determined by (methyl-^{3}H)thymidine incorporation during *semi-situ* incubation, were retained by 3 µm membrane filters (Table 3). The percentage of total activity retained by the 3 µm membrane increased during the experimental period. A possible explanation for this phenomenon could be found in an increase of the percentage bacteria attached to larger particles. However, rather the opposite was observed. The percentage of epibacteria decreased during the experimental period at both stations. So, once again the inefficiency of membrane filters for separation of suspended matter has been shown (Hobbie et al. 1977, Danielsson 1982).

Bacterioplankton biomass and production

Bacterioplankton biomass was fairly constant at both stations until the end of the *Phaeocystis pouchetii* bloom at the end of May when maxima in bacterio-

Table 2. Percentages of algal extracellular release of total primary production, and of bacterial utilization of total exudates, both determined with $NaH^{14}CO_3$ during 5 to 6 h incubation experiments at 24 W m^{-2} and *in situ* temperature

	Sampling date							
	4 Apr	18 Apr	26 Apr	2 May	10 May	17 May	24 May	4 Jun
Extracellular release	24 %	23 %	26 %	5 %	6 %	2 %	3 %	nd
Bacterial utilization	93 %	85 %	82 %	40 %	6 %	36 %	58 %	50 %

nd = not determined

Table 3. Size distribution of bacterial activity as determined by incorporation of (methyl-^{3}H)thymidine during 5 to 6 h incubation at 24 W m^{-2} and *in situ* temperature

Sampling date	Percent total bacterial activity at following pore sizes		
	0.2 µm	0.8 µm	3.0 µm
4 Apr	24	35	41
18 Apr	21	46	33
26 Apr	16	59	25
2 May	26	39	35
10 May	25	33	42
17 May	14	23	63
24 May	21	13	66
4 Jun	21	14	65

plankton biomass were observed (Fig. 4d). These maxima in biomass were preceeded by maxima in bacterioplankton carbon production (Fig. 4e). For the whole measuring period, bacterioplankton biomass and production were not significantly correlated, neither were these bacterioplankton parameters significantly correlated with the corresponding phytoplankton parameters. However, from the middle of May a distinct succession of events could be observed in the water column (Fig. 4). The bloom of *P. pouchetii* with high production levels but low PER values was followed by an increase of bacterioplankton production, which gave rise to bacterioplankton biomass maxima. Such a time-lag between phyto- and bacterioplankton parameters after a bloom of *P. pouchetii* was also observed in the coastal zone of the southern North Sea by Lancelot & Billen (1984). A high production level of bacterioplankton with a corresponding high production level of particulate primary production was observed at April 26 at Station 02. Incorporation of (methyl-^{3}H)thymidine into phytoplankton DNA can be excluded (Findlay et al. 1984, Pollard & Moriarty 1984). The rates of (methyl-^{3}H)thymidine incorporation measured in the Oosterschelde basin (2.3 to 22.9 pMole $l^{-1}h^{-1}$) were within the range of incorporation rates measured in the coastal zone of the southern North Sea by Lancelot & Billen (1984). The bacterial carbon production rates also agreed well with production rates found in other coastal zones (see Ducklow 1983).

However, if the bacterioplankton production rates measured in the Oosterschelde basin are correct and a bacterioplankton carbon conversion coefficient of 75 % is assumed for phytoplankton exudates, only a minor part (average 5 %) of the primary production could simultaneously be utilized by the bacterioplankton. In the Baltic Sea, approximately 15 % of primary production is used by bacterioplankton (Larsson & Hagström 1982, Wolter 1982). In the Belgian coastal zone, direct substrate utilization by heterotrophic bacteria amounted to 44 to 68 % of total primary production (Lancelot & Billen 1984). The rate of bacterioplankton production in the Oosterschelde basin as determined by the incorporation of (methyl-^{3}H)thymidine is also low compared to the rate of bacterioplankton utilization of phytoplankton exudates as can be calculated from Fig. 4c and Table 2. This means that the amount of algal extracellular release was possibly overestimated by underestimating the specific activity of released organic compounds. This is not entirely unlikely, since released metabolic compounds may have been labeled at a higher rate than more structural compounds used to determine specific activity. On the other hand, incorporation of (methyl-^{3}H)thymidine may have underestimated the actual bacterioplankton production rates due to the non-equilibrium of the specific activity of the nucleotide (Moriarty & Pollard 1981, 1982, Riemann & Søndergaard 1984).

Overall oxygen consumption

Overall oxygen consumption rates were measured to determine total heterotrophic activity including dark respiration by phytoplankton (Straskrabova 1979). Two maxima in overall oxygen consumption rates could be distinguished (Fig. 5), one on April 26 and one around May 17 both at times of maximum phytoplankton numbers (Table 1). Overall oxygen consumption rates were well correlated with water temperature, chlorophyll *a* concentrations and bacterioplankton production rates.

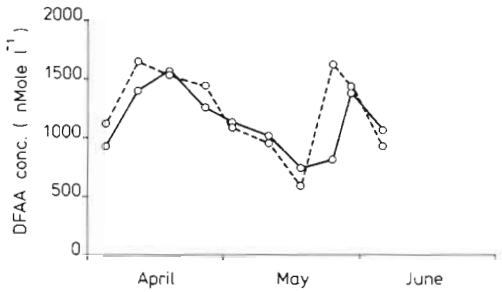

Fig. 5. Distribution of total oxygen uptake rates (μg l^{-1} d^{-1}) in water samples taken at Station 01 (——) and Station 02 (- - -) in the Oosterschelde basin in spring 1984

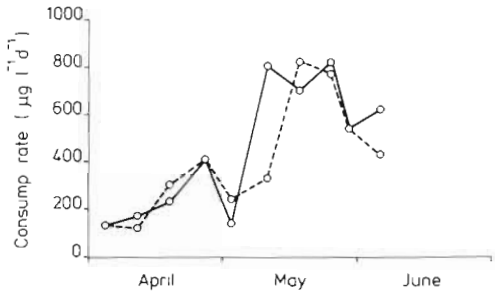

Fig. 6. Distribution of dissolved free amino acids (nMole l^{-1}) in water samples taken at Station 01 (——) and Station 02 (- - -) in the Oosterschelde basin in spring 1984

No significant correlation was found between overall oxygen consumption rates on the one hand and the particulate phytoplankton production rates and the bacterioplankton biomasses on the other hand. So, overall oxygen consumption rates were apparently determined by phytoplankton biomass and not by phytoplankton production, whereas the opposite was found for bacterioplankton parameters. Bacterioplankton production and not biomass determined overall oxygen consumption rates.

According to size fractionation experiments, approximately 50 % of total pelagic oxygen consumption rates could be attributed to bacterioplankton activity in the Oosterschelde basin in spring (Laanbroek et al. unpubl.). Assuming a carbon:oxygen ratio of 0.29 (Sepers 1981) as well as a bacterioplankton carbon conversion coefficient of 75 %, bacterioplankton production rates varied between 52 and 357 μg C $l^{-1}d^{-1}$ during the measuring period. These values are of the same magnitude as the values found for particulate primary production (Fig. 4b). However, the carbon conversion coefficient of 75 % used for exudates may be too high for other organic compounds used by the bacterioplankton as was discussed above. A conversion coefficient of 10 % would yield bacterioplankton production rates between 2 and 13 μg C $l^{-1}d^{-1}$, which is of the same magnitude as the bacterioplankton production rates as determined with (methyl-^{3}H)thymidine incorporation.

Distribution of dissolved free amino acids

A temporary increase in DFAA was observed in the beginning of April at both stations (Fig. 6), which agreed well with the initial production of algal extracellular organic compounds (Fig. 4c). From April 11 on, the concentration of DFAA decreased steadily until the end of the *Phaeocystis pouchetii* bloom (Fig. 4b, Table 1). Minimum amounts of DFAA were found during this bloom. Increases in the concentration of DFAA were observed at the end of this bloom. These increases in DFAA corresponded with increases in bacterioplankton biomass (Fig. 4d) and bacterioplankton production (Fig. 4e). The difference of some days between the times of observed maximum concentrations of DFAA at both stations may be a result of the sampling frequency. The real maxima in the water column may have been missed by this sampling frequency. But there may also have been some effect of the intertidal mudflats around Station 02 on the distribution of DFAA. Release from the sediment surface appeared to be the major source of DFAA in the water of a shallow Danish fjord (Jørgensen 1982) and the presence of a possible aspartic acid-hydroxyproline dimer gave some evidence of sediment resuspension at a deeper water-layer of Chesapeake Bay (Sigleo et al. 1983). However, no influence of intertidal mudflats on the distribution of individual amino acids at Station 02 was observed. No quantitatively important differences were observed between stations. The *in situ* amino acid concentrations were slightly high compared to those measured in coastal waters elsewhere (Dawson & Gocke 1978, Billen et al. 1980, Keller et al. 1982, Mopper & Lindroth 1982, Poulet et al. 1984). Concentrations comparable to those detected in the Oosterschelde basin were found by Jørgensen (1982) in spring and autumn in a shallow estuary on the coast of Denmark.

The percentage distribution of DFAA observed at Station 01 is summarized in Fig. 7. During most of the measuring period, the distribution of individual amino acids was fairly constant. Alanine, aspartate, glycine, leucine and serine were always quantitatively the most important amino acids. These amino acids constituted at least 60 % of total DFAA. The predominance of these amino acids, sometimes together with ornithine and lysine, is a commonly observed phenomenon in marine or estuarine waters (Bohling 1970, Dawson & Gocke 1978, Billen et al. 1980, Amano et al. 1982, Jørgensen 1982, Keller et al. 1982, Macko & Green 1982, Mopper & Lindroth 1982, Sigleo et al. 1983, Poulet et al. 1984). Unfortunately, ornithine and lysine were not detected by our HPLC procedure. These more or less fixed patterns of individual amino acids

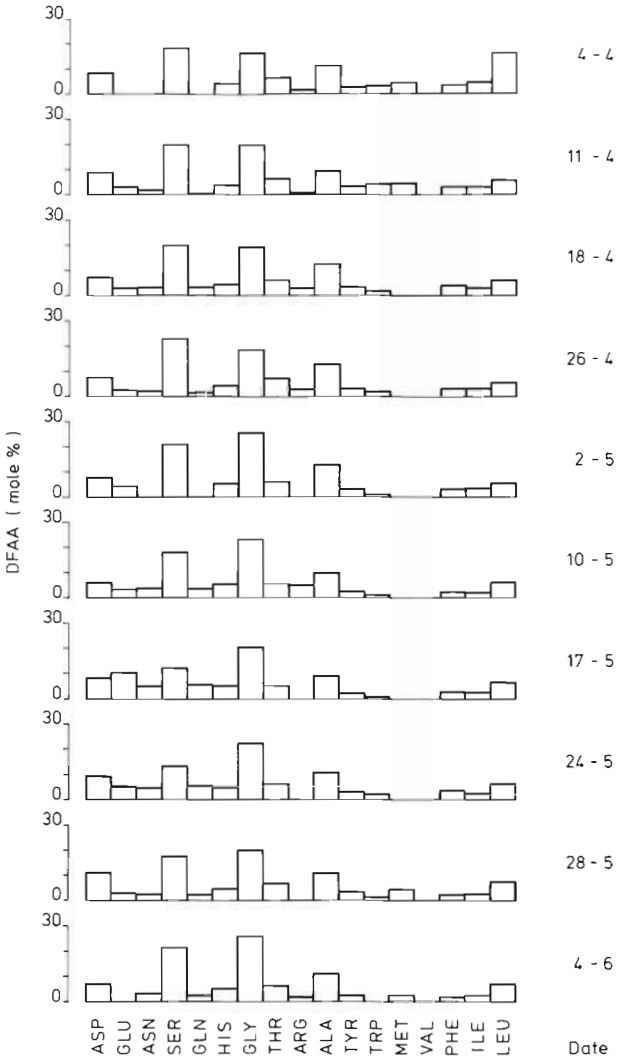

Fig. 7. Percentage distribution of individual dissolved free amino acids in water samples taken at Station 01

encountered in many marine and estuarine water bodies suggest a fixed pattern of amino acids released by different phytoplankton species or suppose the presence of an adequate population of heterotrophic bacteria consuming released DFAA. Maximum mole percentages of aspartine, glutamine and especially of glutamate were measured at the peak of the *Phaeocystis pouchetii* bloom (Fig. 7). On the other hand maximum mole percentages of aspartate and methionine were observed after this bloom. Although gentle filtration was applied for DFAA analysis, a part of the measured amino acids may have been originated from mucilaginous compounds of the *P. pouchetii* colonies. These DFAA were indistinguishable from excreted amino acids in our experiments.

Different behaviour in distribution of individual amino acids in relation to algal blooms has been observed before. Seasonal variations in DFAA were observed in coastal waters by Bohling (1970) and Riley & Segar (1970). In the northern North Sea, a glutamate maximum was also registered in the early stages of a *Chaetoceros* bloom, whereas histidine was found in maximum concentrations towards the end of the bloom (Ittekot 1982). Macko & Green (1982) mentioned highly significant correlations between glutamate and lysine on the one hand and blooms of *Asterionella* and *Chaetoceros* on the other hand in the Damariscotta River estuary. However, as in our experiments, the presence of a selective population of bacteria as well as zooplankton could not be excluded from the data in literature. And finally, it should be kept in mind that the amounts of chemically detectable DFAA may not necessarily be identical to biologically significant amino acids (Dawson & Gocke 1978).

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