Tidal variations in bacterial biomass, productivity and oxygen uptake rates in a shallow channel in the Oosterschelde basin, The Netherlands*

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ABSTRACT: Distribution of bacterial biomass, productivity, and oxygen uptake rates were studied in a shallow tidal channel during 3 tidal cycles in spring and late summer. Maxima in concentrations of suspended particles, bacterial biomass and productivity were always observed around the turn of low tide. Numbers of attached bacteria, but not numbers of free-living bacteria, were always significantly correlated with amounts of suspended matter. In spring, the percentages of attached bacteria were correlated with the tide, whereas in late summer the percentages of attached bacteria were more uniformly distributed during the tidal cycle. A significant correlation was always observed between the numbers of attached bacteria and bacterial productivity. The numbers of free-living bacteria and bacterial productivity were only correlated in late summer. In spring, bacterial doubling times were most dependent upon the tide compared to late summer. In comparison to bacterial biomass and productivity, oxygen uptake rates were less regularly distributed during the tidal cycles, probably by the influence of primary production. A mean bacterial carbon conversion coefficient of 40 % was calculated from the data on bacterial productivity and oxygen uptake rates.

INTRODUCTION

Planktonic bacteria in shallow waters may be stimulated by the close vicinity of highly productive sediments. Elevated numbers of bacteria were found during outgoing tides in surface microlayers at the air-water interface of water returning from salt marshes (Harvey et al. 1983). Bacterial growth rates, determined by incorporation of tritiated thymidine into DNA, were relatively high over coral reef flats and seagrass beds (Moriarty & Pollard 1982, Moriarty et al. 1985). Here, we report a study on the effect of an intertidal mudflats on bacterial numbers and productivity in the overlying water. The study was done in a tidal channel surrounded by extensive intertidal mudflats. At high tide, the channel is flooded with water from the deeper parts of the basin. At low tide, the channel is filled with water from above the mudflats.

In order to measure specifically the bacterial component of total pelagic activity, incorporation of tritiated thymidine was applied. Since determination of oxygen consumption rates provide a simple and hence widely used method for measuring heterotrophic activity in aquatic systems (Straskrabova 1979) these measurements were also included in the study. However, the method is not very selective with regard to heterotrophic activity (Williams 1981).

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. However, the discharge of freshwater from the rivers Rhine and Meuse is regulated by sluices. The maximum total input of freshwater amounts to 50 m³ s⁻¹, whereas the mean volume of the basin is 3050×10^6 m³. As a consequence the basin is well mixed with a salinity of ca 27 ‰. The mean depth of the basin is 8 m and the total surface area amounts to 380 km², of which 44 % is intertidal.

The Sampling Station was chosen in the Mossel-

kreek, a tidal channel in the most eastern, shallow part
of the basin. It is surrounded by extensive mudflats.
The tidal amplitude at the sampling station was 4 m
and the minimum depth was 1.5 m. Maximum current
velocities, up to 50 cm s^{-1}, were recorded around the
turn of low tide.

Sample collection. Samples were collected from
half-depth during 3 tidal cycles in June and August
1984. Incubations were done on board at *in situ* tem-
peratures. Samples for analyses were filtered directly
on board and stored at 4 °C.

Biomass estimates. Samples for enumeration of free-
living and attached bacteria were preserved by addi-
tion of 2 % formaldehyde (final concentration). Direct
counts of free-living and attached bacteria and simul-
taneous determinations of mean cell volumes were
performed on black-stained 0.2 μm polycarbonate fil-
ters using the fluorescent dye acridine orange (Hobbie
et al. 1977). Bacterial biomass was obtained from
bacterial number and mean cell volume by applying a
conversion factor of 560 fg C μm^{-3} (Bratbak 1985).

Bacterial production rates. Bacterial production rates
were determined with (methyl-^{3}H) thymidine incorpo-
ration 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, U.K.) 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 % form-
aldehyde (final concentration). The rate of thymidine
incorporation into DNA was converted to bacterial
production rates by assuming that 2.1×10^{18} cells
were produced per mole thymidine incorporated
(Fuhrman & Azam 1982). Cell production was con-
verted to carbon production by applying a conversion
factor of 560 fg C μm^{-3} (Bratbak 1985).

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

RESULTS AND DISCUSSION

Bacterial biomass

The first experiments were done in June 1984 in 2
periods of 15 h. Each experiment included a complete
tidal cycle. The first experiment started at sunrise at
low tide and lasted until sunset at low tide. The second
experiment started 22 h later at low tide in the evening
and lasted until low tide the following morning. The

third and last experiment was done in August 1984 and
also included a complete tidal cycle. This last experi-
ment started at sunrise at high tide and lasted until
sunset at high tide. During the first experiment, water
temperature increased steadily from 15 °C in the morn-
ing to 21 °C in the evening, whereas during the second
experiment water temperature decreased again from
20 °C to 16 °C. During the third experiment, water
temperature was fairly constant and fluctuated
between 20 and 21 °C.

Due to the high current velocities around low tide
(up to 50 cm s^{-1}), large amounts of particles, mainly
mineral, were resuspended from the intertidal mud-
flats. At this time particle concentrations up to 150 mg
l^{-1} were recorded. Around the turn of high tide, on the
other hand, particle concentrations were only 10 mg
l^{-1}. Amounts of dissolved organic carbon were inde-
pendent of the tide. Analogous to the maxima in parti-
cle loading, maxima in bacterial numbers were also
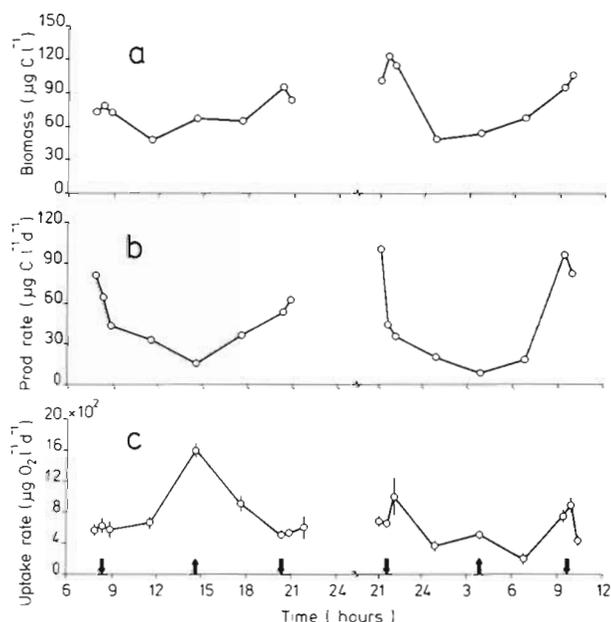recorded around low tide (Fig. 1a & 2a). Total numbers

Fig. 1. Distribution of (a) bacterial biomass, (b) bacterial pro-
ductivity, (c) oxygen uptake rates in a tidal channel of the
Oosterschelde basin during 2 tidal cycles in Jun 1984. ↓ Low
tide; ↑ high tide

of bacteria were indeed significantly correlated with
the concentrations of suspended particles (Table 1).
The numbers of attached bacteria were also signifi-
cantly correlated with the concentrations of suspended
particles. However, the numbers of free-living bacteria
were not always significantly correlated with the con-
centrations of suspended particles. In the Humber
estuary, significant correlations between numbers of
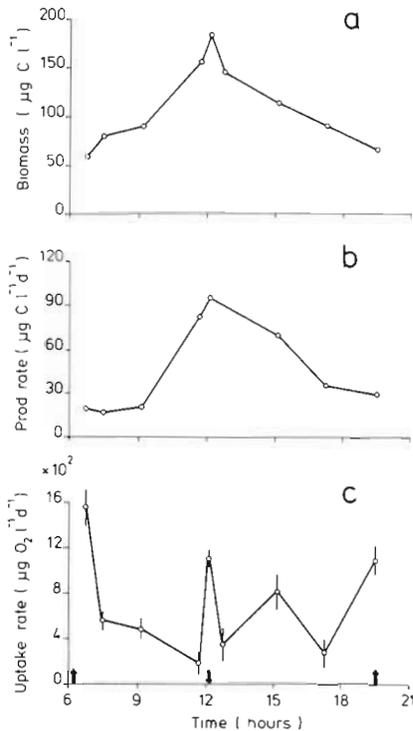attached bacteria and concentrations of suspended

Fig. 2. Distribution of (a) bacterial biomass, (b) bacterial productivity, (c) oxygen uptake rates in a tidal channel of the Oosterschelde basin during a tidal cycle in Aug 1984. ↓ Low tide; ↑ high tide
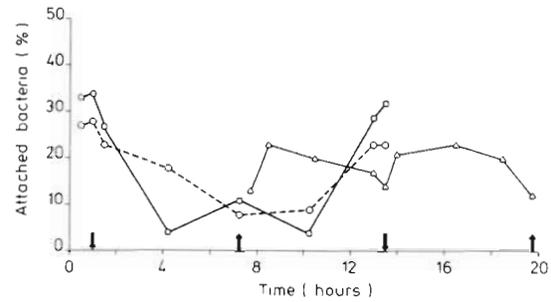

Fig. 3. Distribution of percentages of attached bacteria in a tidal channel of the Oosterschelde basin during 2 tidal cycles in Jun 1984 (□, ○) and 1 tidal cycle in Aug 1984 (△). ↓ Low tide; ↑ high tide

Table 1. Correlation coefficients of the relation between bacterial numbers and concentration of suspended particles observed in a tidal channel during 3 different tidal cycles

Log no. of bacteria	Log conc. of suspended particles		
	26 Jun 1984	27/28 Jun 1984	29 Aug 1984
Total	0.816**	0.901***	0.777**
Free-living	NS	0.874***	0.750**
Attached	0.925***	0.848***	0.754**

NS Not significant
* $p \leqslant 0.05$ ** $p \leqslant 0.01$ *** $p \leqslant 0.005$

particles were also found, whereas the numbers of free-living bacteria were independent of particle concentration (Goulder 1976, Bent & Goulder 1981). The same was observed in some coastal zones (Cammen & Walker 1982, Fukami et al. 1983).

During the first experiments in June, relatively low percentages of attached bacteria were determined around high tide (Fig. 3). Low percentages of attached bacteria are a common phenomenon in the deeper parts of the Oosterschelde basin in spring (Laanbroek & Verplanke unpubl.). During the August experiment,

however, the percentages of attached bacteria were more uniformly distributed in relation to the tide, but with distinct minima at tide turns. The observed difference in distribution of attached bacteria between the experiments in June and August could not be explained from physical or chemical environmental conditions. That in the deeper parts of the basin may be caused by a difference in bacterial preference for suspended particles in spring and late summer.

Bacterial productivity

Simultaneously with the measurements of bacterial biomasses, bacterial productivities were determined by incorporation of (methyl-^{3}H) thymidine. Bacterial productivity determined during the experiments agreed well with production rates found in other coastal zones (see Ducklow 1983). Maxima in productivity were always observed around low tide (Fig. 1b, 2b). Tide-dependent heterotrophic activity has also been observed in a salt marsh estuary (Hanson & Wiebe 1977). Since maxima in total bacterial biomass occurred also around these points of tide, bacterial productivity and biomass seemed to be correlated. However, a significant correlation between bacterial productivities and total numbers of bacteria was not always observed (Table 2). In contrast, bacterial productivities were always significantly correlated with numbers of attached bacteria.

Although the percentages of attached bacteria might be low, they may incorporate comparatively large amounts of substrates (Bell & Albright 1981, Kirchman & Mitchell 1982). Numbers of free-living bacteria were significantly correlated with bacterial productivities only during the August experiment. A shift in activity between free-living and attached bacteria depending upon the particle-loading or upon the season has been observed before in coastal zones (Bell & Albright 1982, Cammen & Walker 1982). Bacterial doubling times

Table 2. Correlation coefficients of the relation between bacterial numbers and bacterial productivities observed in a tidal channel during 3 different tidal cycles

Log no. of bacteria	Log bacterial productivity		
	26 Jun 1984	27/28 Jun 1984	29 Aug 1984
Total	NS	0.754*	0.878***
Free-living	NS	NS	0.896***
Attached	0.840***	0.789**	0.652*

NS Not significant
* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$

were calculated from bacterial biomasses and corresponding productivities (Fig. 4). It should be kept in mind that the calculated doubling times are minimum values, since the actual bacterial productivities may have been underestimated due to non-equilibrium of
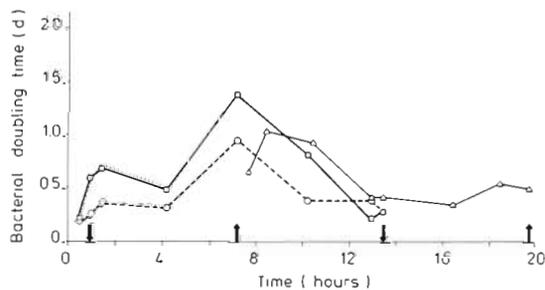

Fig. 4. Distribution of bacterial doubling times in a tidal channel of the Oosterschelde basin during 2 tidal cycles in Jun 1984 (□, ○) and 1 tidal cycle in Aug 1984 (△). ↓ Low tide; ↑ high tide

the (methyl-³H) thymidine used (Moriarty & Pollard 1981, 1982, Riemann & Søndergaard 1984). However, a possible underestimation by non-equilibrium of the nucleotide may be even more severe around low tide when the water in the channel is loaded with particles resuspended from the intertidal mudflats. Compared to the distribution of bacterial doubling times during the tidal cycles in June, the distribution of bacterial doubling times in August was apparently more dependent on diurnal changes than on tidal changes. Hence, the effect of intertidal mudflats on bacterial productivity in the water column is more pronounced in spring than in late summer.

Oxygen consumption rates

Since determinations of oxygen uptake rates provide a simple and hence widely used method for measuring heterotrophic activities in aquatic environments (Stras-

krabova 1979), such measurements were included in the experiments. Unlike bacterial biomasses and productivities, oxygen uptake rates were less regularly distributed during the tidal cycles (Fig. 1c, 2c). Bacterial productivities and oxygen consumption rates were never significantly correlated. The absence of correlation between bacterial productivities and oxygen consumption rates can partly be explained by the effect of primary production and partly by the oxygen-consuming activity during incubation in the dark by the algae themselves. During the first experiment in June, primary production was high (80 mg C m⁻² h⁻¹, P. R. M. de Visscher pers. comm.) in the middle of the day, since high tide, with low concentrations of suspended particles, occurred at that time of the day. Consumption of oxygen in samples taken around high tide may have been stimulated by primary production in the previous light period. Such a stimulation, however, was not observed in bacterial productivity (Fig. 1b). Two explanations can be given for the observed disagreement between bacterial productivity and oxygen uptake rates. (1) The bacteria *in situ* were utilizing and oxidizing primary products, but this was not coupled to growth. However, this is very unlikely. (2) Incubation of samples loaded with primary products for longer times in the dark caused high oxygen consumption rates not encountered *in situ* either by the algae or by the bacteria. Algal respiration in the dark can be high depending upon the previous light period (Stone & Ganf 1981, Verity 1982). Hence, great care must be taken to extrapolate oxygen consumption rates determined by incubations in the dark to *in situ* activities in the light. When samples were taken in the dark, as happened in the second June experiment, overestimation of *in situ* oxygen consumption rates by dark incubations may be less severe. During the experiment in August, primary production was low (25 mg C m⁻² h⁻¹, P. R. M. de Visscher pers. comm.) in the middle of the day, since low tide, with large amounts of suspended matter, occurred at that time of the day. The small maximum in oxygen uptake rate observed during that experiment (Fig. 2c) may have been caused by a short period of high primary production allowed by a rapid sedimentation of suspended particles just at the turn of tide when the current velocity was zero.

Oxygen consumption rates can be converted to carbon mineralization rates by applying a carbon to oxygen ratio of 0.29 (Sepers 1979). From these calculated carbon mineralization rates and the bacterial carbon production rates, a bacterial carbon conversion coefficient can be determined. Assuming that oxygen consumption rates determined by incubation in the dark were similar to *in situ* rates in the dark and assuming that all the oxygen was used by the bacteria, a mean bacterial carbon conversion coefficient of 20 % was

determined. However, according to size fractionation experiments, approximately 50 % of total oxygen consumption rates could be attributed to bacterial activity in the Oosterschelde basin in spring (Laanbroek & Verplanke in press). Hence, the mean bacterial carbon conversion coefficient will be 40 %, which is in between the value recorded by Newell et al. (1981) for bacterial utilization of algal cell debris and the value determined by Jensen (1983) for bacterial growth on algal exudates. The calculated value of 40 % represents a lower limit, since actual bacterial productivity may have been underestimated by a possible non-equilibrium of the specific activity of (methyl-^{3}H) thymidine as mentioned above.

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