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# Annual variation of bacterial number, production and activity in Central Kiel Bight

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### Abstract

In connection with the international monitoring programme of the Baltic Sea, supervised by the Helsinki Commission (HELCOM) microbiological investigations were carried out at 5 stations situated in the Western Baltic Sea. Data from the 3 most frequently monitored stations are presented with a special regard to station Kiel Bight.

Total bacterial number shows a maximum value of  $3 \times 10^6$  cells x ml<sup>-1</sup> in July 1988 and  $3.8 \times 10^6$  cells x ml<sup>-1</sup> at 2 m depth in June 1989 and exhibits nearly the same pattern in annual variation as bacterial production, measured by thymidine incorporation. Number of saprophytic bacteria turned out to be a fraction of  $\approx 0.1$  % of total bacterial number. While maximum of total bacterial number was found to coincide with shortest turnover times of glucose and maximum of bacterial production, this was not observed with the number of saprophytes.

## Introduction

In October 1985 microbiological investigations of the Western Baltic Sea and Kiel Bight started. This investigations were carried out within the scope of the Baltic Monitoring Project, supervised by the Helsinki Commission (HELCOM). The first period of investigations which ranged from Oct. 1985 to Dec. 1987 has been spent mainly with adjusting the methods and the sampling conditions. Since the beginning of 1988 five sampling stations are routinely investigated. The stations are Boknis-Eck, Kiel Bight, Fehmarn-Belt, Mecklenburger-Bight and Gedser Rev. While at the first 3 stations sampling takes place every month, Mecklenburger-Bight and Gedser Rev are sampled only twice a year (April and September). On each sampling cruise besides the microbiological parameters chemical (nutrients), zoo- and phytoplanktological parameters are measured.

#### Material and methods

Samples were taken at each station from 3 depths, which are 2 m, 10 m, and 20 m as well as 15 m (only station Kiel Bight). The methods which are used for the microbiological monitoring are:

- Total bacterial number and biomass of bacteria
- Production of bacteria
- Colony-forming bacteria (colony count)
- Heterotrophic activity (turnover time of glucose)

The method for measuring total bacterial number and bacterial biomass was done according to the method described by HOBBIE et al. (1977). For filtration of the sample Irgalan Black stained Nuclepore filters with poresize 0.2  $\mu$ m were used. The staining of bacteria was done with acridine orange (AO) solution (0.5 mmol). Determination of cell number and volume was done by epifluorescence microscopy with a New Porton Grid (Graticules Ltd., England).

Production of bacterial biomass was determined according to the modified TTImethod (tritiated thymidine incorporation) described by FUHRMAN and AZAM (1982). The method was adopted for routine use by LARSEN et al. (1985).

For the determination of the number of colony forming bacteria (saprophytes) ZoBell's medium 2216E with 1.5 % agar was used by performing the "pour plate method". Colonies were counted after 14 days incubation at 20  $^{\circ}$ C.

Detailed descriptions of these methods are given in Baltic Sea Environment Proceedings 27 B (1988).

Heterotrophic activity was determined according to GOCKE (1977) by measuring incorporation rates of <sup>14</sup>C-labelled glucose, expressed as turnover time of glucose. 100 ml of the sample were supplemented with an activity of 0.1  $\mu$ Ci of labelled glucose and incubated at *in situ* temperature for 1 - 3 hours (dependent of the *in situ* temperature). Reaction was stopped by addition of 1 % of unbuffered formol, afterwards the sample was filtrated through a cellulose-nitrate filter (SARTORIUS) with 0.2  $\mu$ m poresize and after addition of 5 ml scintillation liquid counted in the scintillation counter.

## **Results and discussion**

The highest level of bacterial biomass with an amount of 220  $\mu g$  C  $1^{-1}$  was observed during June - July 1988 in the surface layer at the coastal-near station Boknis-Eck (Fig. 1). In 1989 the bacterial biomass at all 3 stations (Boknis-Eck, Kiel Bight, Fehmarn-Belt) showed a distinct maximum at the same months without significant differences between the stations. The reason might be that 1988 was a year with unique atmospheric conditions (summer gales) and because of the greater coastal influence at Boknis-Eck biological parameters respond much faster and with more intensity to extreme hydrographic conditions (GOCKE et al. 1990).

Fig. 2 shows the average cellvolume and bacterial production at Kiel Bight in the mixed surface layer (2 m). While the average cellvolume shows no visible pattern in annual variation, bacterial production goes down from 0.3 mg C x m<sup>-3</sup> x h<sup>-1</sup> to values below 0.1 mg C x m<sup>-3</sup> x h<sup>-1</sup>, so that a significant correlation between average cellvolume and bacterial productivity is not given, neither at this station nor at the other sampling stations.

Annual variation of total bacterial number (TBN) exhibits a nearly similar pattern as the bacterial production. The maximum number of cells was found with  $3 \times 10^6$  cells  $\times$  ml<sup>-1</sup> in July 1988 and  $3.8 \times 10^6$  cells  $\times$  ml<sup>-1</sup> at 2 m depth in June 1989. The number of cells reaches the minimum always during the winter months. This coincides more or less with the number of colony forming bacteria

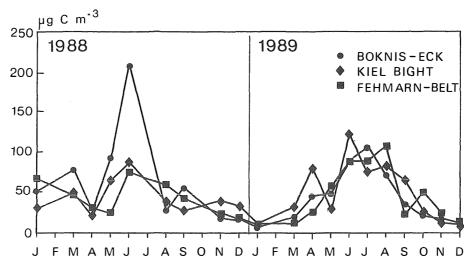


Fig. 1. Bacterial biomass found at 3 monitoring stations in the mixed surface-layer (sampling depth = 2 m). Carbon conversion factor = 0.3 pg C x  $\mu^{-3}$ .

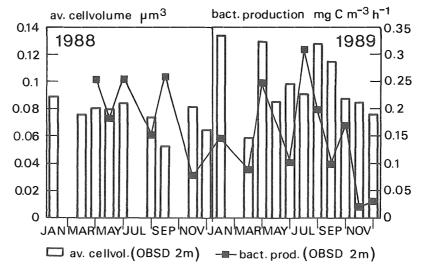


Fig. 2. Comparison of average cellvolume and bacterial production in the mixed surface-layer (sampling depth = 2 m) at station Kiel Bight.

(saprophytes), at least in 1989 (Fig. 3). The graph shows that the number of colony forming bacteria is in average a fraction of 1  $^{0}/_{00}$  of the total bacterial number. In 1988 annual variation of saprophytic bacteria shows not a quite regularly pattern. The reason might be, as mentioned above, specific hydrographic conditions. Long time investigations in the area of Kiel Bight show, that the

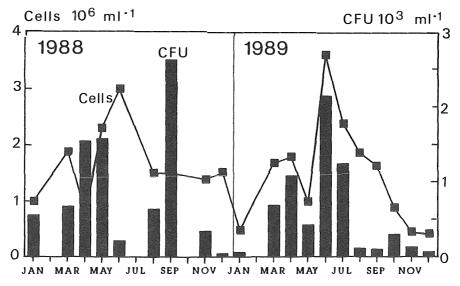


Fig. 3. Comparison of total bacterial number (TBN) and number of colony forming (saprophytic) bacteria, expressed as colony forming units (CFU) in the mixed surface-layer (sampling depth = 2 m) at station Kiel Bight.

number of saprophytic bacteria responds much faster to a change in environmental conditions than the total bacterial number does. This may sometimes result in a bad correlation between those two parameters.

Turnover time of glucose reaches the smallest value (6 h) at late spring till early summer while the longest turnover times (>300 h) occur during December -February. During the same time bacterial production reaches the highest level. Fig. 4a shows the connection between physiological activity of a bacterial population, expressed as turnover time of glucose, production of bacterial biomass, and standing stock of bacteria expressed as total bacterial number. The 3 dimensional graph shows clearly that high cell numbers correspond high physiological activity as well as with maximum bacterial production. In Fig. 4b number of saprophytic bacteria (CFU) is plotted against turnover time and bacterial production. In analogy to Fig. 4a the maximum number of saprophytes coincides with shortest turnover times. Whereas the bacterial production is not at maximum rate ( $\approx$  170 mgC x m<sup>-3</sup> x h<sup>-1</sup>). This phenomenon can be explained by the different growth behaviour of two groups of organisms: During spring time, after the first algal bloom has passed its highest level, high concentrations of POC and DOC are present in the water column. At this time water temperature has not yet reached its annual maximum as well as bacterial production being positively related to water temperature. Because saprophytic bacteria are only a small fraction of the standing stock of bacteria increased production rates of saprophytic bacteria do not measurably contribute to the production. Saprophytes are known to have the highest physiological activity. This may be the reason for highest number of saprophytes at low turnover times but not at maximum bacterial production. So we can say that the peaks in Figs. 4a and 4b represent two different bacterial populations which occur at different times of the year.

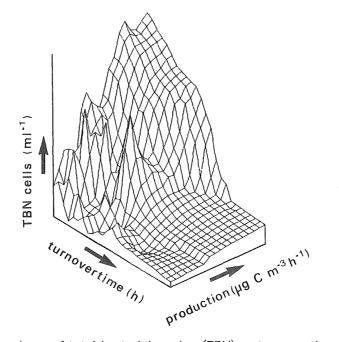


Fig. 4a. Dependency of total bacterial number (TBN) on turnover time of glucose and bacterial production.

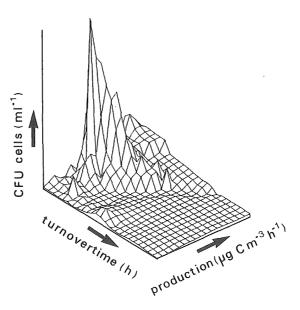


Fig. 4b. Dependency of the number of colony forming units (CFU) on turnover time of glucose and bacterial production.

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