The abundance, biomass and production of bacterioplankton in the Pomeranian Bay*

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Abstract

A microbiological investigation was carried out in the Pomeranian Bay in 1996–1997 to determine the spatial and seasonal changes in the numbers, biomass and productivity of bacterioplankton. Substantial differences in the spatial distribution of bacterioplankton populations were found. At the stations in the coastal zone of the Pomeranian Bay numbers, biomass and production of bacteria were high, with maximum values noted at the mouth of the river Świna. This is indicative of the significant impact of riverine waters on the bacterioplankton in the Pomeranian Bay.

Seasonal fluctuations and bacterial microflora activity were recorded. The dynamics of the changes showed that most of the bacteriological parameters examined reached their maximum in summer while minimum values were noted in winter.

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1. Introduction

In aquatic ecosystems the abundance, biomass and productivity of bacterioplankton correspond closely to the concentration of organic matter. Owing to the very high level of primary production (Ochocki *et al.* 1995), the Pomeranian Bay accumulates a large quantity of autochthonous organic matter. At the same time, all the rivers discharging their waters into the Bay carry large amounts of allochthonous matter (Beszczyńska-Möller 1995), which could endanger the natural balance of this area of the Baltic Sea. Microbiological studies in the Pomeranian Bay (Maciejowska 1995, Pollehne *et al.* 1995) have shown that bacteria play a key role in preserving its homeostasis. These organisms are very active in the decomposition and transformation of dissolved and suspended auto- and allochthonous organic matter and in the distribution of the mineral compounds thus formed (Künnis & Saava 1990, Goosen *et al.* 1995). Through the induction of their enzymatic systems, bacteria can quickly respond metabolically to any new organic compound introduced into the basin (Wright & Coffin 1983). Earlier investigations carried out in the Baltic Sea (Gocke *et al.* 1987, 1990, Rheinheimer *et al.* 1989, Heinänen 1991, 1992) provided evidence of the temporal and spatial variability of the bacteriological parameters. Hence, the aims of the present study were to estimate the spatial distribution of bacterioplankton in the Pomeranian Bay, to determine the seasonal dynamics of variations in the abundance and biomass of bacteria, and to measure their productivity.

2. Material and methods

The bacteriological investigations were carried out in the Pomeranian Bay in 1996–1997. During that period four voyages on board r/v 'Baltica' covered the annual quarter cycle. The voyages enabled samples to be taken at 25 marine stations, the locations of which are indicated in Fig. 1. The water was sampled by means of a rosette of twelve bathometers, each 5 litres in volume.

The samples were subjected to bacteriological analysis, which included a determination of the following parameters: total bacteria number, biomass and volume; thymidine and leucine incorporation rates; secondary bacterial production.

The total bacteria number (TBN), volume (V) and biomass (BBM) were determined by the acridine orange direct count method (Hobbie *et al.* 1977). To do so, 1–5 cm^3 water samples fixed in formaldehyde were filtered on 25 mm irgalan–black–stained polycarbon filters (0.2 μm diameter). The bacteria were stained with a 0.01% solution of acridine

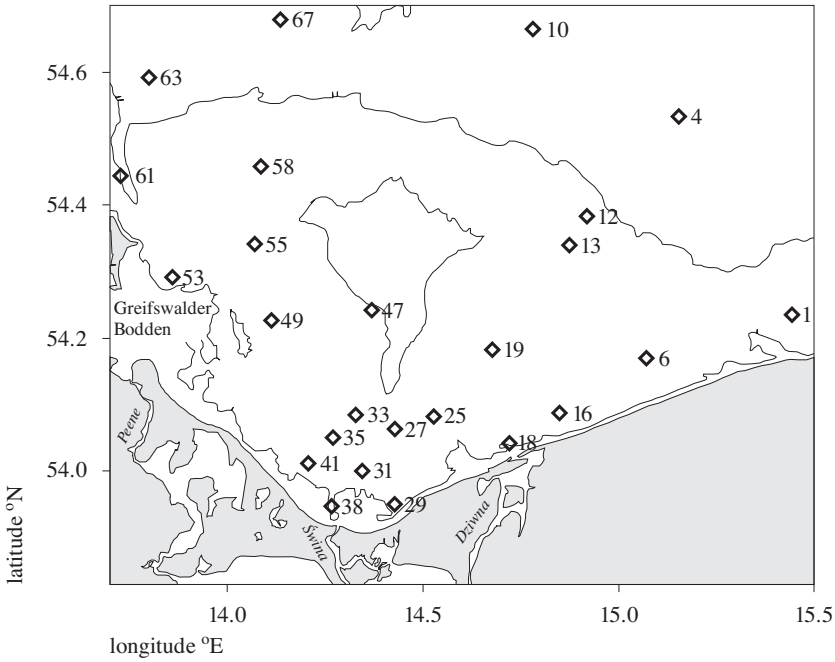

Fig. 1. Map of the Pomeranian Bay area and the location of 25 sampling sites

orange for 10 min. The specimens were then analysed by means of an Olympus BX60 epifluorescence microscope. A Porton G12 eyepiece insert (Walton, Beckett) was used for counting bacteria. The bacteria cells were counted in 20 fields (about 10 to 20 cells field^{-1}) and 50 bacteria cells per sample dimensions were measured. The number and biomass were calculated with the use of computer programs created on the basis of a calculation sheet. A conversion factor of 0.35 pgC μm^{-3} (Bjørnsen 1986) was used to calculate biomass from cell volumes.

The thymidine incorporation rate (TTI) was determined on the basis of the method described by Fuhrman & Azam (1982). In order to determine this parameter, 20 μl [methyl – ^{3}H] thymidine (specific activity 1.61 TBq mmole^{-1}) was added to 10 cm^3 water samples in three replications and a control sample to give a final concentration of 11 nM. The control was treated with formalin before the addition of thymidine in order to obtain blank values. Depending on the *in situ* temperature, the incubation time was 1–2 hours. After this period the incubation was interrupted in order to add 200 μl of 37% formaldehyde to the sample. The samples were filtered on a 10-station filtering device on 25 mm diameter polycarbon filters (pore diameter 0.2 μm). The filters were rinsed 5 times with 1 cm^3 of 5% trichloroacetic acid (TCA). The filters were then transferred to scintillating

vessels containing $5\,cm^3$ of scintillating cocktail and the reading was taken on a Beckman LS 6000 IC scintillation counter.

The leucine incorporation rate (TLI) was determined according to the method described by Kirchman *et al.* (1985). For TLI measurements, samples were incubated with [$4,5$ – ^{3}H] L-leucine (specific activity $1.34\,TBq\,mmole^{-1}$) together with unlabelled leucine to reach saturation level. $12\,\mu l$ of a mixture of ^{3}H-leucine and L-leucine were added to $10\,cm^3$ water samples to give a final concentration of 10.3 nM. The extraction, filtration and radioassay procedures were the same as described above for the TTI measurement.

The calculation of bacterial production (BP) was based on the thymidine incorporation technique (Fuhrman & Azam 1982) using a factor of 1.1×10^{18} cells mol^{-1} (Riemann *et al.* 1987) and a carbon content of $0.35\,pgC\,\mu m^{-3}$ (Bjørnsen 1986).

3. Results

The data obtained on plankton bacteria number and biomass in the Pomeranian Bay are indicative of a substantial temporal and spatial variability in the occurrence of the investigated microflora populations. Very distinct seasonal fluctuations in bacterioplankton numbers were noted there. The dynamics of these changes indicate seasonal maxima in the summer and autumn months (3.0–$3.27 \times 10^6\,cm^{-3}$) and minima during winter and spring ($1.94 \times 10^6\,cm^{-3}$) (Table 1). The bacteria biomass underwent an identical seasonal fluctuation. This parameter was highest in summer and autumn (41.52–$43.76\,mgC\,m^{-3}$) and lowest in winter ($17.51\,mgC\,m^{-3}$) (Table 1). Variations in bacteria biomass were found to correspond strictly with numbers (Fig. 2).

The results in Table 1 indicate great differences in average bacteria cell volume in particular seasons: this varied from $0.026\,\mu m^3$ in winter to $0.04\,\mu m^3$ in autumn.

Numbers, biomass and cell volumes of bacteria along with TLI and TTI were all affected by distinct seasonal changes. TTI, indicating the rate of bacterial cell division, was most active in spring and summer (16.72–$18.74\,pmol\,dm^{-3}\,h^{-1}$) and least active in winter ($2.74\,pmol\,dm^{-3}\,h^{-1}$) (Table 1). An identical seasonally-dependent cycle was noted in leucine incorporation by bacteria, which is an indicator of bacteria protein production. The highest rate of leucine build-up in bacterial cell structures was observed in summer ($50.62\,pmol\,dm^{-3}\,h^{-1}$), whereas in winter the process was found

Table 1. Seasonal fluctuation of bacteriological parameters investigated in the Pomeranian Bay (averaged for all stations)

Parameter	Units	Season			
		winter (44)*	spring (61)	summer (59)	autumn (49)
total bacteria number (TBN)	cells $\times 10^6$ cm^{-3}	1.94 (0.84–3.46)	1.94 (0.78–5.97)	3.27 (1.81–5.67)	3.00 (1.27–5.17)
biomass (BBM)	mgC m^{-3}	17.54 (6.61–37.86)	21.87 (6.83–63.83)	43.76 (16.77–109.1)	41.52 (19.3–74.04)
volume (V)	μm^3	0.026 (0.016–0.039)	0.032 (0.022–0.058)	0.038 (0.021–0.065)	0.040 (0.025–0.053)
thymidine incorporation (TTI)	pmol dm^{-3} h^{-1}	2.74 (0.5–12.09)	18.74 (1.47–104.67)	16.72 (2.38–73.01)	14.58 (3.76–34.65)
leucine incorporation (TLI)	pmol dm^{-3} h^{-1}	10.55 (1.08–30.57)	33.86 (2.79–284.92)	50.62 (7.46–211.49)	25.00 (6.51–91.49)
bacterial production (BP)	mgC m^{-3} d^{-1}	0.70 (0.1–3.29)	5.48 (0.33–26.73)	5.90 (0.68–25.9)	5.31 (1.34–13.55)

* in parentheses – number of samples studied

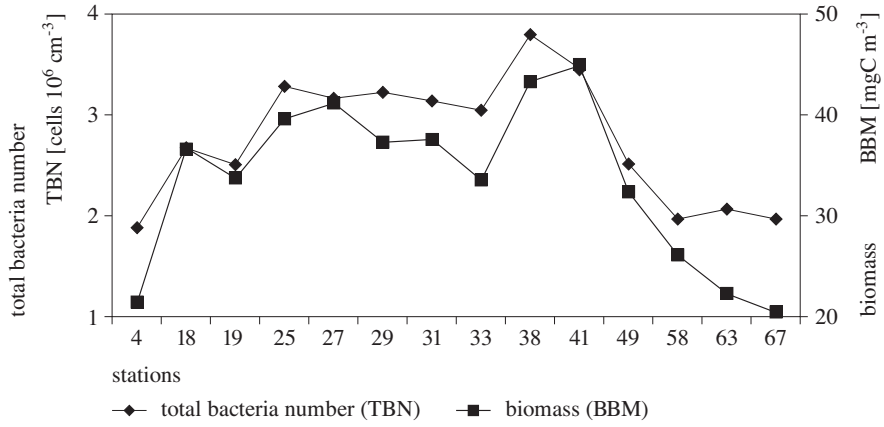

Fig. 2. Relationship between total bacteria number and bacterial biomass in the Pomeranian Bay

to be five times slower (10.55 pmol dm^{-3} h) (Table 1). Fig. 3 clearly shows that there is a strict, directly proportional relationship between TTI and TLI.

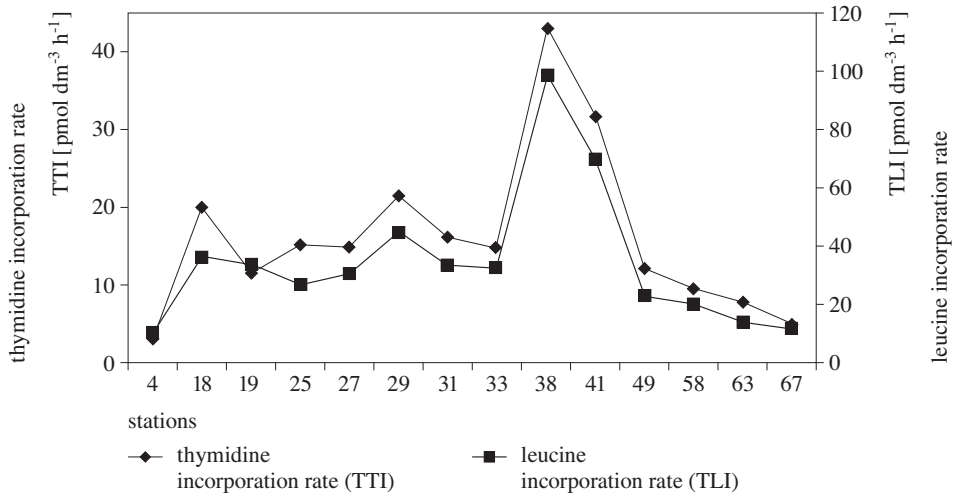

Fig. 3. Comparison of the thymidine and leucine incorporation rates at different stations

In spring, summer and autumn the secondary bacterial production levels in the Pomeranian Bay were similar, ranging from 5.32 to 5.90 mgC m^{-3} d^{-1}, while in winter they were half as high (2.74 mgC m^{-3} d^{-1}) (Table 1). Secondary bacterial production corresponded strictly to the abundance of bacteria (Fig. 4).

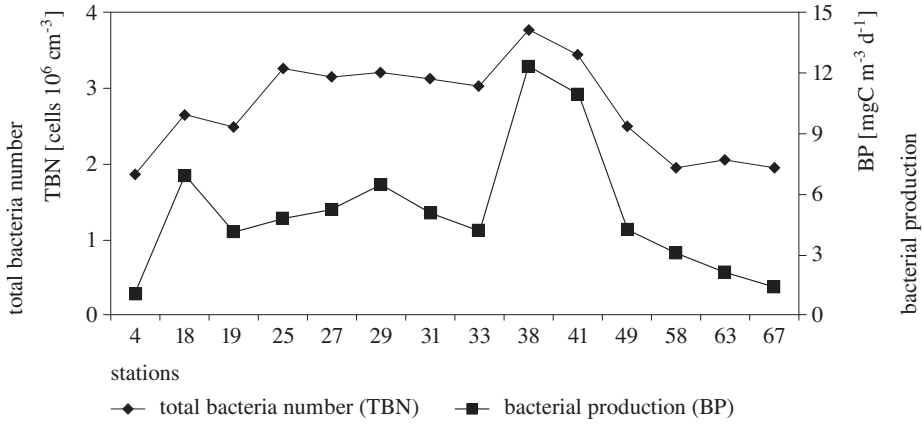

Fig. 4. Secondary bacterial production in relation to the total bacteria number

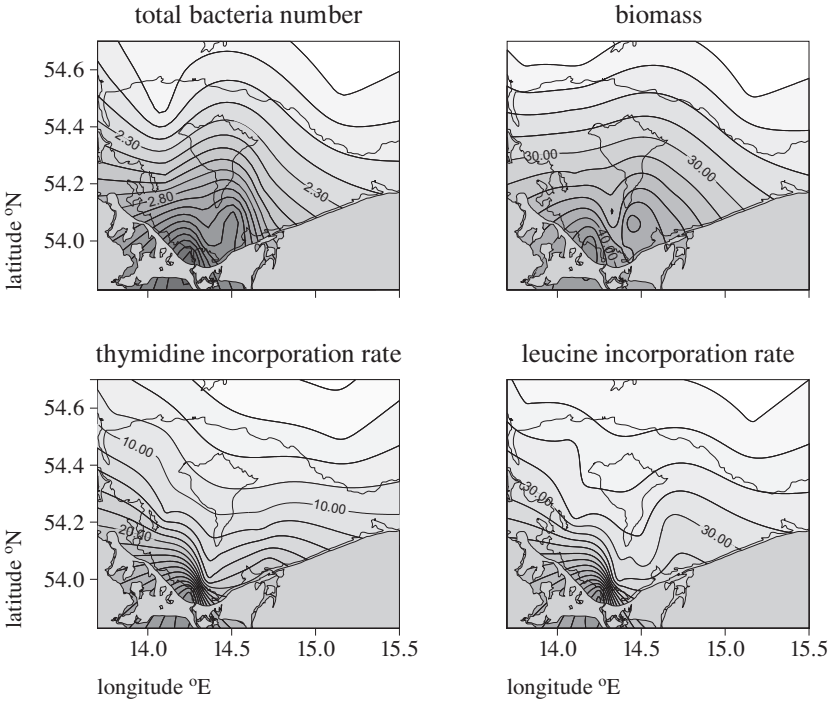

Fig. 5. Spatial distribution of total bacteria number [cells $\times 10^6\,\mathrm{cm}^{-3}$], biomass [mgC m^{-3}], thymidine incorporation rate [pmol $\mathrm{dm}^{-3}\,\mathrm{h}^{-1}$] and leucine incorporation rate [pmol $\mathrm{dm}^{-3}\,\mathrm{h}^{-1}$] in the Pomeranian Bay (integrated values, all seasons)

Analysis of the distribution of the planktonic bacterial populations pointed to significant differences in the numbers and biomass of bacterioplankton in some parts of the Pomeranian Bay. In the course of the investigation the greatest numbers and biomass of bacteria were noted at the sampling stations at the mouth of the river Świna (Figs. 2 and 5). In addition, bacterioplankton metabolic activity, measured by TTI and TLI, reached a maximum there (Figs. 3 and 5). Thus, the results clearly demonstrate the impact of the rivers entering the Pomeranian Bay on its waters, and obviously, on the bacterial populations inhabiting it.

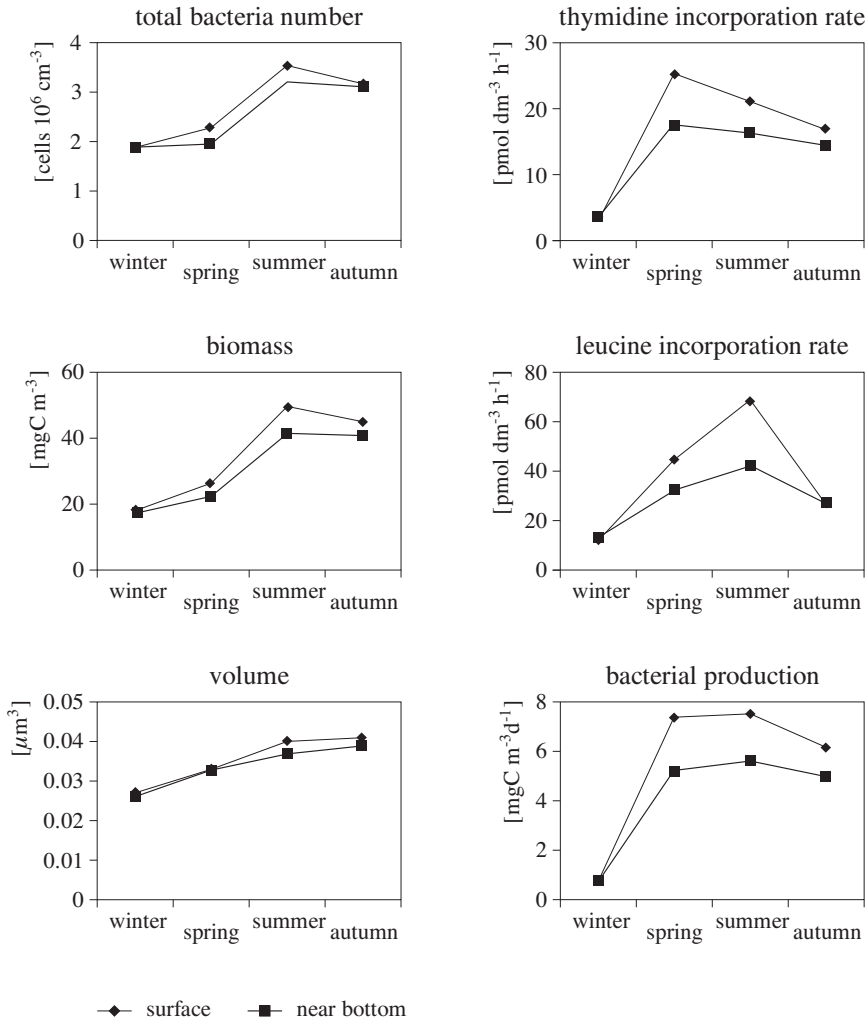

Fig. 6. Seasonal variations in bacteriological parameters in surface water and near-bottom layer

The data in Fig. 6 show that during the investigation period there were substantial differences between the bacteria inhabiting surface waters and those in the demersal waters. As a rule, apart from winter, the bacteria living in the surface waters were more abundant and had a greater biomass and productivity as measured by TTI and TLI than those inhabiting the demersal zone. No significant differences were found between the two layers with reference to bacteria cell volume.

4. Discussion

Significant temporal and spatial fluctuations in salinity, temperature, oxygen, pH, and organic matter concentration in particular, are common in brackish ecosystems, of which the Pomeranian Bay is an example (Barbosa 1991, Gericke & Rheinheimer 1991, Beszczyńska-Möller 1995). Hence, the ability of the organisms living there to compensate and neutralise the continuous amplitude of abiotic factors (Kinne 1971). Ecologically highly flexible, the bacterioplankton displays outstanding adaptive capabilities. It undergoes qualitive and quatitative autocorrelation when ecological conditions change dramatically (Painchaud *et al.* 1987).

The results of many earlier investigations carried out in the Baltic Sea (Jost & Ballin 1984, Gocke *et al.* 1987, Kirsten 1991, Heinänen 1992) point to very characteristic periodic oscillations in the numbers and activity of the bacterioplankton populations. The typical picture of the seasonal variations in bacteriological parameters in the Baltic Sea is the occurrence of maximum values in summer and minima in winter. In the Gotland Basin (Gocke *et al.* 1990) numbers, biomass and production of bacteria were half as great in winter than in summer. However, in the Bornholm Basin the differences in these parameters were tenfold (Gocke & Hoppe 1982). The present investigation also resulted in the discovery that all the bacteriological parameters examined, in the Pomeranian Bay and elsewhere in the Baltic Sea, reached maximum values in summer and minimum ones in winter. Extreme differences were noted in the case of metabolic activity of bacteria. Thymidine and leucine incorporation rates and secondary bacterial production in the Pomeranian Bay in winter were five times lower than in summer. The summer maxima of bacteria numbers and activity in the Pomeranian Bay may have been due to the simultaneous occurrence of several different factors, the most important of which appeared to be the presence of phytoplankton.

Earlier studies (Lignell 1990, Heinänen 1992) carried out in the Baltic Sea revealed that in summer, algae very actively released dissolved organic matter, which made up to 80% of the assimilated carbon. In the Baltic Sea, in the assimilates released by algae, small molecules of 300–900

daltons are dominant (Lignell 1990). The organic matter released by algae consisted principally of monosaccharides, amino acids and peptides (Larrsson & Hagström 1982). These monomers are actively yet selectively incorporated by bacteria and inserted in their cell structures or used up as energy material (Lancelot 1984). Admiraal *et al.* (1985) pointed out that bacteria populations can actively adapt to the incorporation of the whole spectrum of organic compounds released by algae cells, including polymers, by a succession of different physiological types with modified enzymatic systems. The investigations carried out by Wolter (1982) with radioactively labelled algae excretions showed that these products were totally incorporated by bacteria after a few hours. According to Lignell (1990) 20–60% of phytoplankton excreta in the Baltic Sea enter the microbiological trophic chain, significantly contributing to the increase in bacteria number, biomass and production.

Earlier bacteriological investigations carried out in the Pomeranian Bay (Maciejowska 1995, Pollehne *et al.* 1995) provided evidence of substantial changes in the spatial distribution of plankton bacteria populations. It was found that the maximum values of all the bacteriological parameters examined occurred at the mouths of the rivers flowing into the Pomeranian Bay. Those investigations also showed that the bacterioplankton number, biomass and production measured by the thymidine and leucine incorporation rates were highest at the mouth of the Świna. This effect seems to have resulted from a significant enrichment of this part of the Pomeranian Bay by allochthonous matter carried there by the Świna. According to Heinänen (1992), large quantities of allochthonous matter continuously discharged into a water basin may give rise to the formation of a detritus-based food net totally independent of primary production. Some earlier studies (Coffin & Sharp 1987, Goosen *et al.* 1995) found that the discharge and high concentration of organic matter implied better access to food substrates, which may have caused the bacteria number, biomass and productivity of bacteria to increase. In the case of the Baltic Sea, Kremling & Petersen (1984) noted a significant direct influence of river waters on the bacteria microflora in the Gulf of Bothnia. According to Väätänen (1980) and Heinänen (1991), phytoplankton greatly affected populations of microorganisms in the open Baltic Sea, whereas in offshore waters it was more under the impact of land-borne allochthonous matter. It seems that the elevated bacteriological parameters noted at the Świna mouth were triggered by high concentrations of allochthonous matter and the high primary production occurring in this part of the Pomeranian Bay. Earlier studies (Ochocki *et al.* 1995) recorded the primary production in offshore waters as being 195–471 $\mathrm{mgC\,m^{-2}\,d^{-1}}$; at the Świna mouth,

however, it was as high as 1930 mgC m^{-2} d^{-1}. Lignell (1990) pointed out the close relationship between phytoplankton productivity and secondary bacterial production, which is partially confirmed by the present results.

Lahdes *et al.* (1988) were of the opinion that as much as 70% of bacterial production occurred in the surface waters of the Baltic. This was confirmed by studies on bacteria number and activity carried out in the Baltic Proper (Rheinheimer *et al.* 1989) and the Gulf of Bothnia (Heinänen 1992). The present study yielded similar results. Bacteria from the Pomeranian Bay surface water were more abundant and were more productive than the microflora inhabiting the demersal zone.

The authors of the present paper are fully aware of the fact that numerous abiotic and biotic factors not examined in this paper co-govern the dynamics of bacterioplankton development in the Pomeranian Bay. Thus a further bacteriological study seems necessary so that more complete evidence can be presented of the changes taking place under the impact of anthropogenic factors on the bacterial population, a basic trophic link in the marine ecosystem.

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