Impact of the inflow of Vistula river waters on the pelagic zone in the Gulf of Gdańsk*

Magdalena Wielgat-Rychert[1,*]
Anetta Ameryk[2]
Anna Jarosiewicz[1]
Janina Kownacka[2]
Krzysztof Rychert[1]
Lena Szymanek[2]
Mariusz Zalewski[2]
Alina Agatova[3]
Natalia Lapina[3]
Nadezhda Torgunova[3]

[1] Pomeranian University in Słupsk,
K. Arciszewskiego 22a, 76–200 Słupsk, Poland;

e-mail: wielgatm@wp.pl

*corresponding author

[2] National Marine Fisheries Research Institute,
H. Kołłątaja 1, 81–332 Gdynia, Poland

[3] Russian Federal Research Institute of Fisheries & Oceanography (VNIRO),
V. Krasnoselskaya 17, 107140 Moscow, Russia

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Abstract

The biomass, production, composition of autotrophic phytoplankton and heterotrophic bacteria were studied along with environmental and biological parameters. Samples were taken from Vistula river water (at Kiezmark) and from the river plume to the outer stations in the Gulf of Gdańsk (Baltic Sea) in June 2005. The phytoplankton biomass gradient appeared to be simply the result of dilution of the river water in the sea water, whereas the bacterial abundance and biomass dropped between the river station and the first sea water stations, a decrease that cannot be explained by the dilution effect. The Vistula water stimulated the production mainly of bacterioplankton but also of phytoplankton in the river plume as compared to rates measured in Vistula waters and at the open sea stations. However, this stimulation did not result in a measurable increase in biomasses, probably because of the short retention time of water in the river plume. Phytoplankton production was correlated with phytoplankton biomass (Chl a), while bacterial production was correlated with phytoplankton production and phytoplankton biomass (Chl a).

1. Introduction

The processes taking place in the mixing zone of fresh and sea waters have frequently been studied in coastal areas of seas and oceans (Findlay et al. 1991, Chin-Leo & Benner 1992, Troussellier et al. 2002). Such mixing zones in the Baltic Sea differ from those in oceanic regions because of the smaller difference in salinity – the Baltic Sea is a brackish water basin. The Gulf of Gdańsk is one of the largest bays in its central part, the Baltic Proper (Figure 1, see p. 862). It is fed by the second-largest river entering the sea, the Vistula (Wisła), with a mean annual flow of 1081 m^3 s^{-1} (HELCOM 2004); it is the only river of such size discharging into the Gulf of Gdańsk. Stratification is a typical feature of the entire Baltic Sea, where the deep water masses originate from the North Sea and the upper layer is strongly influenced by fresh water inflows. In the Gulf of Gdańsk, too, there is a stable halocline at 60–70 m depth, separating the upper, less saline layer (ca 7 PSU) from the bottom layer of higher salinity (10–14 PSU). The upper layer comprises 75–84% of the total volume of the Gulf of Gdańsk (Witek et al. 2003).

The Vistula flows directly into the Gulf of Gdańsk through a straight, man-made outlet. Other large branches of the river's delta were earlier cut off by locks, and the main river flow was directed to the sea by the shortest route. Therefore, we expect changes in the biological processes taking place in the Vistula river plume in the Gulf of Gdańsk, despite the smaller than oceanic salinity gradient. Other large rivers on the southern coast of the Baltic, the Nemunas and the Oder (Odra), empty into the sea indirectly through coastal lagoons. A lagoon usually acts as a transitional water body

(Pastuszak et al. 2005), so that the outer plume of mixed fresh and sea water leaving the lagoon contains a pelagic community pre-adapted to the new conditions. This has been demonstrated for the Oder river plume (Jost & Pollehne 1998). Previous studies (e.g. Grelowski & Wojewódzki 1996) indicated that the Vistula plume might extend up to 9–27 km from the river mouth and that its vertical extension might range from 0.5 m to 12 m, depending mainly on the wind speed and direction, as well as a combination of factors such as the river water discharge rate, sea level and the duration of their interactions (Matciak & Nowacki 1995).

The aim of the present work was to compare the river water, river plume and the open sea Baltic waters in the pelagic zone off the Vistula mouth with respect to autotrophic phytoplankton and heterotrophic bacteria, which are groups of organisms with the shortest generation time, responding rapidly to environmental changes. We studied the extent to which phytoplankton and bacteria differ in their response to the impact of the river water. Therefore, autotrophic phytoplankton and heterotrophic bacteria biomasses, phytoplankton composition, and production/consumption rates were measured concurrently. Additionally, enzyme activities, organic matter and nutrients were measured in order to identify possible factors governing these processes.

2. Material and methods

2.1. Sampling

Measurements were conducted from 14 to 24 June 2005. Sampling started in the Vistula river water at the Kiezmark measurement station (Figure 1), located 11.6 km upriver from the mouth, and was followed by measurements at 15 marine stations extending from the area close to the river mouth (2–10 km: ZN2 to E53) out into the open waters to a distance of 120 km (station P63) (Figure 1). Additionally, hydrological conditions were measured at 31 stations (data not given) in order to detect the position of the river water plume.

Samples were taken with a rosette 5 dm^3 water sampler (General Oceanics Multi Water Sampler) combined with a Niels Brown CTD. Several parameters were measured during the cruise: temperature, salinity, total bacterial number (TBN), bacterial biomass (BBM), bacterial respiration (BR), bacterial production (BP), gross primary production (GPP), chlorophyll a (Chl a), nutrient concentrations, particulate organic carbon (POC), enzymatic activity (APA and ETS) and phytoplankton composition and biomass.

All the parameters were measured at the following depths: 0, 2.5, 5, 10, 15, 20 or 25 m; this depth range corresponds approximately to the depth of
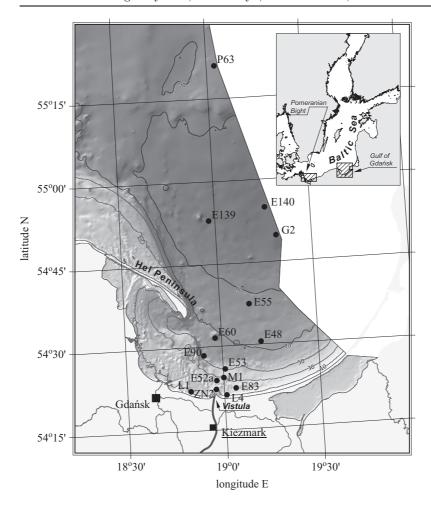
Figure 1. Location of sampling stations in the Gulf of Gdańsk. The inset shows the positions of the Gulf of Gdańsk and the Pomeranian Bight

the euphotic zone in the Baltic Sea. Phytoplankton and bacterial respiration were measured only in the integrated samples obtained by mixing equal water volumes ($1 \, \mathrm{dm}^3$) from discrete 0, 2.5 and 5 m layers. At Kiezmark, the Vistula water was sampled for all parameters only at the surface. Whenever average values are given for all stations in the Gulf of Gdańsk, river water is excluded from the calculations.

2.2. Nutrients, chlorophyll a and POC

Nutrients (nitrite (NO_2), nitrate (N-NO_3), ammonium (NH_4), orthophosphate (PO_4), total nitrogen (N_{tot}) and total phosphorus (P_{tot})) were analysed directly after sampling using the standard Baltic Monitoring

Programme's analytical methods recommended for the Baltic Sea (UNESCO 1983). Chlorophyll *a* was extracted from Whatman GF/F glass-fibre filters with a 90% acetone solution for 24 h in the dark at 4–6°C. After centrifugation, chlorophyll *a* concentrations were determined fluorimetrically (measurements of fluorescence before and after acidification; Evans et al. (1987)).

For POC analyses, GF/F filters containing particulate matter were loaded into a ceramic sample boat and combusted at 900°C by Solid Sample Module for TOC-5000 (carbon analyser). The resulting CO_2 was measured using a non-dispersive infrared detector. The instrument was calibrated with glucose standard dried at 60°C. Blank peaks were measured using normal measurement with an empty sample boat.

2.3. Phytoplankton

Phytoplankton was analysed at 14 stations. The 200 ml water samples were immediately fixed with acidic Lugol's solution to a final concentration of 0.5% (Edler 1979). The phytoplankton in 20 ml subsamples was analysed under an inverted microscope (Olympus IMT-2, magnification 400x, 200x and 100x with phase contrast and DIC) following the Utermöhl method.

The individual phytoplankton cells were counted and the biomass was calculated using the relevant stereometric formula according to HELCOM recommendations (HELCOM 2001). Conversion into carbon biomass was done according to Menden-Deuer & Lessard (2000).

Simulated primary production was measured at 12 stations with the radioisotope method (^{14}C) at 12 stations, as described by Renk et al. (2000), and primary production was measured in situ at four stations (P63, G2, E60, M1) (Steemann-Nielsen 1952). Incubations were performed in 100 cm^3 glass bottles (light and dark) at depths 0, 2.5, 5, 10, 15, and 20 m for 4 h at midday and in an incubator (in the same type of bottles, over a period of 2 h). The incubator ensured the same ambient temperature as in the sea and light at the level of 250 kJ m^{-2} h^{-1} (in the 400–700 nm range; PAR). Sample activity was measured with a Beckman LS 6000 IC scintillation counter. Following the BMB recommendations (Ærtebjerg Nielsen & Bresta 1984), the production value was multiplied by a correction factor of 1.06 to compensate for losses of organic ^{14}C due to phytoplankton respiration during incubation. Daily production was calculated by multiplying the production obtained during the entire in situ incubation time by the ratio of a full day's dose of light to the dose of light during the incubation period. Because the measurement time was relatively short (4 h, and a correction for respiration was introduced), the results were treated as gross primary production (GPP) (Witek et al. 1997). Simulated primary production in

the Vistula water was measured with surface plankton samples only and the measured underwater irradiance was used for calculations.

2.4. Bacteria

Total bacterial number (TBN), bacterial biomass (BBM) and the average bacterial cell volume were determined in samples fixed with formaldehyde (1% final conc.) by directly counting and measuring cell length and width under an Olympus BX50 epifluorescence microscope. Samples were stained with acridine orange (AO) (Hobbie et al. 1977). BBM was calculated from TBN and the average bacterial cell volume with a carbon conversion factor of 0.27 pg C μm^{-3} (Kuparinen 1988). Cell volume was determined by direct cell measurements using a 'New Porton' graticule and calculated using the relevant stereometric formula.

Bacterial production (BP) in unfiltered sea water samples was determined by measuring the bacterial uptake of tritium-labelled thymidine with a specific activity of 2.2–2.3 TBq mmol^{-1}. The thymidine was diluted four times, after which 30 μl were added to the 10 ml sample to produce a final concentration of 11–12 nM (Fuhrman & Azam 1980, Riemann et al. 1987). Samples were incubated at the in situ water temperature for 1 h when the temperature was $> 10°$C and for 2 h when $< 10°$C. Thymidine activity was measured with a Beckman LS 6000 IC scintillation counter. BP was calculated using the thymidine conversion factor 11×10^7 cell pmol^{-1} (Riemann et al. 1987) and the cellular carbon content.

Bacterial respiration (BR) was determined at 9 stations. Water was passed through the 0.8 μm filter, poured into a plastic container, then homogenized and siphoned for individual measurements into ca 100 ml borosilicate Winkler bottles acid-washed prior to use. For determining initial oxygen concentration, samples were fixed immediately with Winkler reagents. Other bottles were incubated for 2 days (in triplicate) at the in situ temperature. The dissolved oxygen content was determined with the Winkler method using an automated Titrino 702 SM burette with potentiometric endpoint detection. The respiratory quotient RQ = 1 was used to convert oxygen units to carbon. Our tests showed that TBN and BBM in 0.8 μm filtered water were not statistically different from the unfiltered samples.

Additionally, at two stations – ZN2 and E139 – the fraction of *Beta-* and *Gammaproteobacteria* was estimated using the staining method of molecular probes: Catalysed Reporter Deposition-Fluorescence In Situ Hybridization (CARD-FISH) (Pernthaler et al. 2002). Water samples were fixed with formaldehyde (1% final conc.), filtered through polycarbonate membranes, washed with water and frozen at $-20°$C. CARD-FISH hybridization was

performed according to the protocol by Sekar et al. (2003). Horseradish-labelled oligonucleotide probes EUB338 (Amann et al. 1990), BET42a, GAM42a (Manz et al. 1992) with competitors were used. A formamide concentration of 35% (vol/vol) in the hybridization buffers at a temperature of 46°C during hybridization was used. CARD-FISH preparation was evaluated by epifluorescence microscopy (Zeiss Axiophot). At least one thousand DAPI cells per sample were counted. Samples were tested in parallel with a NON-338 probe for assessing non-specific binding (Wallner et al. 1993). Detection rates of NON-338 to DAPI stained cells were subtracted from EUB338, BET42a and GAM42a rates. The relative abundance of bacteria (probe EUB338) was 64.5% at station ZN2 and 63.1% at station E139.

2.5. Enzyme activity

The GF/F filters with the collected particulate matter were homogenized and the resultant substances were used to prepare suspensions with distilled water. As a rule, the volume of suspensions was 10 ml. Before determining the activities of enzymes, glycine buffer with Triton X-100 was added to the suspensions to a final concentration of 0.3 volume per cent for the more complete release of intracellular enzymes. The determination of enzymatic activities was carried out no less than 2 h after the addition of reagent.

Alkaline phosphatase activity was determined using p-nitrophenyl phosphate (p-NPP) as substrate. The concentrations of p-nitrophenol formed following the splitting of the phosphate radical from p-NPP were measured spectrophotometrically at 420 nm (Agatova et al. 1985). The molar extinction coefficient of p-nitrophenol is 1.8×10^4 M^{-1} cm^{-1}. The reaction was initiated by the substrate and was stopped by adding 2 cm^3 1 N NaOH. The reaction mixture, to which 1 N NaOH was immediately added, served as a control. After stopping the reaction with 1 N NaOH, the reaction mixture was centrifuged for 10 min at 3000 RPM, and the supernatant absorption at 420 nm was compared with the corresponding control. Phosphatase activity was measured at the in situ water temperature. Alkaline phosphatase activity was given as specific (APA$_{sp}$) and total (APA) activities. The specific activity shows how many micromoles of phosphorus are cleaved in l h per l mg of particulate proteins, since enzymes are proteins. Particulate proteins were determined according to the method of Lowry et al. (1951). The total activity is given by the number of micromoles of phosphorus mineralized in 1 dm^3 of sea water.

$$\text{APA} = (\Delta D \times V \times v \times 1.1)/(18 \times S \times K \times t),$$

where ΔD is the corrected absorbance (absorbance of the assay minus the absorbance of the control), V is the crude homogenate volume [cm^3], v is

the quenched reaction mixture volume [cm^3], S is the aliquot volume of the homogenate used in the assay [cm^3], K is the filtered water volume, 18 is the absorption of 1 μm p-nitrophenol in 1 cm^3 reaction mixture in a l-cm cuvette equivalent of 1 μm phosphorus, t is the reaction time [h] and 1.1 is the dilution of crude homogenate by glycine buffer with Triton X-100.

The ETS activities were determined spectrophotometrically according to Packard & Williams (1981). This method used the concept that respiratory oxygen consumption was controlled by electron transport enzymes (ETS). Estimation of the ETS enzyme activity or the potential respiration was based on electron transfer from organic substrates to derivatives of phenyltetrazolium chloride, replacing oxygen as the electron acceptor for ETS. As a result, formazans were formed with an absorption maximum of 490 nm. In our case (0.1 M glycine buffer pH 8.0; 0.03% Triton X-100; 2.5 mM INT; 0.01% MgSO$_4$; 0.3 M succinate; 1.7 mM NADH; 0.48 mM NADPH; mixture 1:1 H$_3$PO$_4$:formalin) the molar extinction coefficient of INT-formazan was 4.7×10^3 M^{-1} cm^{-1}.

The reaction was started with the addition of 1 cm^3 of the supernatant of the respective suspensions. The same substances were placed in the control test tube with addition of 1 cm^3 of the clean GF/F filtered suspension supernatant. After incubation (60 min) at the in situ temperature, the reaction was quenched with 1 ml of a 1:1 (V/V) solution of 1 M H$_3$PO$_4$ and concentrated formalin. The ETS enzyme activities were characterized by the total activity (ETS) and specific activity (ETS$_\text{sp}$). ETS shows how much oxygen can be consumed by the seston in l dm^3 of sea water during l h; it has dimensions of μl O$_2$ h^{-1} dm^{-3}. ETS$_\text{sp}$ shows how much oxygen can be consumed by the enzyme in l mg protein during l h (dimensions μl O$_2$ h^{-1} mg protein^{-1}) (Agatova & Łapina 1994).

$$\text{ETS} = (\Delta D \times V \times v \times 1.1)/(0.21 \times S \times K \times t),$$

where ΔD is the corrected absorbance (absorbance of the assay minus the reagent blank), V is the crude homogenate volume [cm^3], v is the quenched reaction mixture volume [ml], S is the aliquot volume of the homogenate used in the assay [cm^3], K is the filtered water volume, 0.21 is the equivalent of the absorption of 1 μm O$_2$ in 1 cm^3 reaction mixture in a l-cm cuvette, and t is the reaction time [h].

2.6. Statistics

Linear regression was used to investigate the statistical correlations between the various water quality parameters and physical factors. Raw data or monthly averages were used in the regression analysis without log transformations.

3. Results

3.1. Hydrological conditions

In June 2005, the water column of the Gulf of Gdańsk was stratified as a result of temperature and salinity gradients. The outer region of the gulf (Gdańsk Deep) was stratified in the manner typical of the Baltic Proper: the permanent halocline lay at a depth of 65–80 m. The mean surface water salinity in the 0–5 m layer ranged from about 1.37–3.98 (salinity indicating freshwater addition) to 7.25 PSU, which is within the range of sea water salinity in this region of the Baltic Proper (stations E139, P63). The thermocline was measurable at a depth of about 17–25 m at the outer stations and at the shallow, inner stations an additional temperature gradient was noted at 5–10 m as a result of salinity changes due to the river outflow. The surface water temperature lay between 12.5 and 18.9°C, with the highest values noted in the southern part of the gulf in the vicinity of the Vistula mouth (Table 1).

3.2. Nutrients

Dissolved inorganic nitrogen (DIN) concentrations (Table 1), in both the surface 0–5 m layer and the 0–25 m layer, were negatively correlated with salinity (Table 2, Figure 2); these concentrations indicated dilution of river water along the salinity gradient. NH_4 concentrations in the 0–25 m layer were much lower than nitrate levels; the highest values were measured below 5 m depth and not in the river waters. PO_4 was not correlated with salinity and its concentrations were very low except in the river water. Property-salinity plots indicated depletion of PO_4 relative to conservative mixing (Figure 2), that is, the utilization of this nutrient was probably greater than its discharge from river waters and/or remineralization. The molar DIN to PO_4 ratio yielded high values in the mixing zone close to the river mouth because PO_4 was depleted; in the open waters both nutrients became depleted.

High APA activities are an indicator of phosphorus deficiency for bacterial and algal growth because APA activity is believed to be regulated by ambient PO_4 concentrations (Chróst 1991). With the exception of the river water (where the PO_4 concentration was 1.65 μmol dm^{-3}), PO_4 concentrations in the surface layer (0–10 m) were always below 0.23 μmol dm^{-3}, the threshold level below which APA was always negatively correlated with phosphate concentrations in all the marine ecosystems studied by Agatova et al. (2001). In this study APA_{sp} was negatively correlated with PO_4 (Table 2). A high APA_{sp} activity (normalized to particulate protein) was measured only at low PO_4 concentrations, as shown

Table 1. Parameters measured in the 0–25 m layer (phytoplankton biomass measured in the 0–5 m layer)

Parameter	Unit	No. of samples	River water	Mean Gulf of Gdańsk	Max Gulf of Gdańsk
salinity	PSU	123	0.00	6.76	7.34
temp.	$^\circ$C	120	18.90	12.80	17.70
N_{tot}	μmol dm^{-3}	106	59.90	27.70	73.80
P_{tot}	μmol dm^{-3}	108	5.62	0.79	2.55
NH_4	μmol dm^{-3}	109	0.14	0.29	2.45
NO_3	μmol dm^{-3}	109	27.90	1.03	18.00
DIN	μmol dm^{-3}	109	28.60	1.35	18.30
PO_4	μmol dm^{-3}	109	1.65	0.08	0.42
N_{tot}/P_{tot}	molar	105	11.70	37.70	65.80
DIN/PO_4	molar	108	17.30	18.30	311.00
APA	μmol P h^{-1} dm^{-3}	67	0.07	0.04	0.10
APA_{sp}	μmol P h^{-1} mg prot.$^{-1}$	67	0.01	0.05	0.12
ETS	μl O$_2$ h^{-1} dm^{-3}	76	34.70	8.80	38.00
ETS_{sp}	μl O$_2$ h^{-1} mg prot.$^{-1}$	76	6.85	9.97	17.70
Chl a	mg Chl a m^{-3}	61	62.40	9.45	68.20
phyt. biomass*	mg C m^{-3}	14	1418.00	509.00	1200.00
C/Chl a*	g/g	14	22.00	56.00	98.00
Chl a/Biomass*	g/g	14	0.45	0.30	0.44
GPP	mg C m^{-2} d^{-1}	13	4570.00	1522.00	3370.00
doubl. phytopl.	day	12	6.20	4.00	4.90
TBN	cells ml^{-1} $\times 10^6$	56	6.87	1.82	3.00
BBM	mg C m^{-3}	56	89.00	28.40	52.80
BP	mg C m^{-3} d^{-1}	56	18.50	10.60	34.60
BP cell spec.	cell spec. fg C cell^{-1} d^{-1}	56	2.69	5.51	13.70
doubl. bact.	day	14	4.80	3.30	5.60

*In the 0–5 m layer.

in Figure 2, whereas in the mixing area near the river values were close to the APA activity in the river water, which was 0.013 μmol P h^{-1} mg prot.$^{-1}$.

3.3. Phytoplankton

Autotrophic phytoplankton was observed in integrated samples (0–5 m). The highest phytoplankton biomass, Chl a (Figure 3) and POC concentrations were measured in the Vistula and gradually decreased with increasing salinity. Phytoplankton biomass and Chl a (0–5 m) were correlated (Table 2). In the gulf, Chl a made up from 0.17% to 0.44% of the autotrophic phytoplankton biomass calculated from the biovolume 1 ml = 1 g (Table 1), and this ratio did not depend significantly

Table 2. Partial correlation matrix of salinity and environmental and biological variables; values integrated over the 0–20 m layer (Phyt. Biom. values integrated over the 0–5 m layer); $n = 10$, $p < 0.05$, nc = no statistically significant correlation
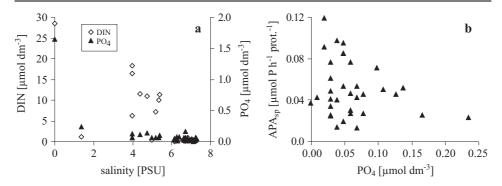
	Salinity	BP	Temp.	PAR	Eu. depth	Chl a	BBM	APA$_{sp}$	PO$_4$	DIN	Phyt. biom.	POC	ETS$_{sp}$
GPP	−0.68	0.79	nc	nc	nc	nc	nc	nc	nc	nc	0.79	nc	nc
BP	−0.76		nc	nc	nc	0.72	0.75	nc	nc	nc	0.67	nc	nc
Temp.	−0.56			nc	nc	nc	nc	0.69	−0.73	nc	nc	nc	nc
PAR	nc				nc	nc	nc	nc	−0.80	nc	nc	nc	nc
Eu. depth	0.91					nc	nc	nc	nc	nc	nc	nc	nc
Chl a	−0.95						nc	nc	nc	nc	0.77	nc	nc
BBM	nc							nc	nc	nc	nc	nc	nc
APA$_{sp}$	nc								−0.68	nc	nc	nc	nc
PO$_4$	−0.78									nc	nc	nc	nc
DIN	−0.65										nc	nc	nc
Phyt. biom.	−0.87											nc	nc
POC	−0.80												nc
ETS$_{sp}$	nc												nc

Figure 2. a) Concentration of dissolved nutrients [μmol dm^{-3}] in the 0–5 m layer as a function of salinity. b) Specific alkaline phosphatase activities APA$_{sp}$ [μmol P h^{-1} mg prot.$^{-1}$] in the 0–5 m layer as a function of PO$_4$ concentrations
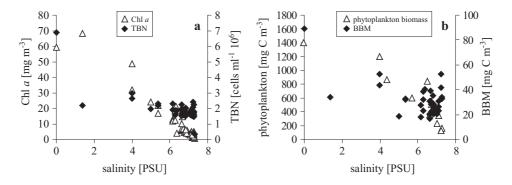

Figure 3. a) Volumetric Chl a [mg Chl a m^{-3}] concentrations and TBN [cells ml^{-1} $\times$ 10^6] – data from the 0–20 m layer plotted as a function of salinity. b) Phytoplankton biomass [mg C m^{-3}] and average data volumetric BBM [mg C m^{-3}] from the 0–5 m layer plotted as a function of salinity; values in the river were sampled only at the surface

on salinity. The carbon to Chl a ratio (g/g) (for the carbon conversion method, see Material and Methods) ranged from 26 to 98 and increased with distance from the river mouth (Table 1).

140 phytoplankton taxa were identified in the samples. They belonged to 8 main groups, whose contribution to the whole phytoplankton biomass is shown in Figure 4. In the river and at station E52a the phytoplankton was dominated by centric diatoms (78–80%), mainly from the genus *Cyclotella*, and green algae (15–24%), principally *Acutodesmus* spp., *Coelastrum astroideum*, *Crucigenia quadrata*, *Pediastrum boryanum*, *P. duplex* and *Oocystis* spp. At the marine stations the mixotrophic dinoflagellate *Heterocapsa triquetra* was abundant, as were the mixotrophic *Dinophysis norvegica* and *Glenodinium* spp. Cyanobacteria also constituted a significant
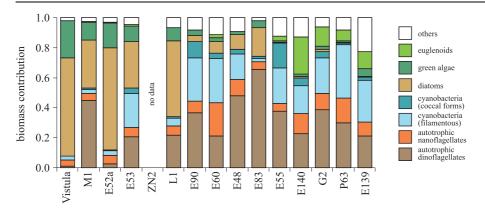
Figure 4. Phytoplankton biomass composition at each station – stations segregated according to salinity in the 0–5 m layer

fraction of the biomass (26–36%), largely *Aphanizomenon flos-aquae*. In open waters *Eutreptiella gymnastica* was quite frequent, and so were nanoflagellates (among them Prymnesiales). Phytoplankton composition analyses indicated the gradual replacement of freshwater species by species typical of the Baltic sea water within 10–20 km of the river mouth, e.g. *Skeletonema subsalsum* (coastal waters) being replaced by *Skeletonema marinoi* (open waters).

The composition of the phytoplankton community was typical of the southern coast of the Baltic Proper in the second half of June, i.e. a typical early summer situation. The final phase of the dinoflagellate bloom was noted, which followed diatom bloom and preceded the occurrence of cyanobacteria in summer (Bralewska 1992, Witek et al. 1993, Gromisz & Witek 2001). In the coastal region massive growth of *Heterocapsa triquetra* characteristic for the beginning of this phase was observed and a multispecies community of relatively low biomass was noted in the open sea (Bralewska 1992, Witek et al. 1993, Gromisz & Witek 2001). Another typical feature of the phytoplankton community in its summer stage is the appearance of *Aphanizomenon flos-aquae*, a blue-green algae. Similar patterns of phytoplankton succession were observed in other parts of the Baltic Sea (Gasiūnaitė et al. 2005).

The highest integrated gross primary production (GPP) was measured in the Vistula; it was found to decrease gradually with increasing salinity (Figure 5a). The phytoplankton doubling time was calculated from the measured carbon to Chl *a* ratio (Figure 5d) as the ratio of areal GPP to the sum of Chl *a* in the 0–20 m layer. The doubling time ranged from 6.2 days (the Vistula) to 2.6 days (station E53). A similar doubling time was calculated by Renk (2000).
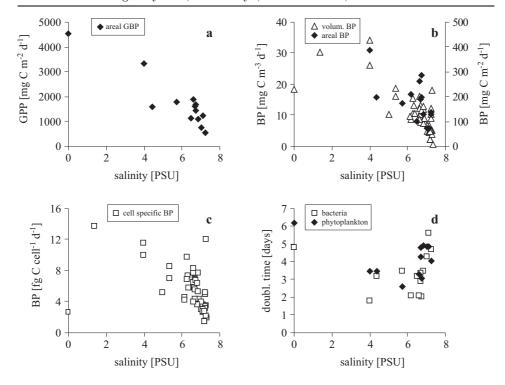
Figure 5. a) Areal GPP [mg C m^{-2} d^{-1}]; b) daily volumetric BP [mg C m^{-3} d^{-1}] and areal BP [mg C m^{-2} d^{-1}], both integrated over the 0–20 m layer; c) daily volumetric cell-specific BP [fg C cell^{-1} d^{-1}]; d) doubling time of phytoplankton and bacteria (days) in the 0–20 m layer as a function of salinity. Except for GPP (see Material and methods), measurements in the river are not presented for areal values because the river water was sampled only at the surface

The highest measured light-saturated specific rate of photosynthesis (the assimilation number AN, i.e. the maximum volumetric production rate normalized to Chl a) ranged from 2.3 to 4.2 mg C mg Chl a^{-1} h^{-1}. AN values were within the range given by Ochocki et al. (1995) and the spring values measured by Witek et al. (1997).

3.4. Bacteria

The maximum total bacterial number (TBN) and bacterial biomass (BBM) were recorded in the river waters. TBN and BBM dropped between the river and the first stations in the Gulf of Gdańsk and did not change much along the salinity gradient in the gulf waters (Figure 3a). TBN and BBM dropped with depth, especially in the deeper parts of the euphotic zone; at 20 m the average BBM was 30% less than at the surface.

Bacterial production (BP) in the inner Gulf of Gdańsk was much higher than in Vistula water (at station M1 it was twice as high as in the river water; Table 1, Figure 5), where it was at the same level as the highest values measured in the open sea water, despite the much higher riverine TBN and BBM (Figure 3). The bacterial doubling time ranged from 5.6 days (station G2) to 1.8 days (station M1). The average BP decreased with depth, by about 20% at 5 m and by 55% at 20 m.

The average bacterial respiration (BR) and cell-specific BR were measured at nine stations only (see Material and methods), in samples integrated over 0–5 m. Higher BR values were measured at stations in the inner zone of the gulf, with one exceedingly high value at station ZN2 (Table 3). Neither parameter was correlated with salinity; it is important to note, however, that the BP values measured at these nine stations were not correlated to salinity either. In the river water, volumetric BR was high, but cell-specific BR was the lowest of all the values measured. The bacterial growth efficiency (BGE calculated as $BP/(BP+BR) \times 100\%$) was similarly low in the river water and in the outermost open sea waters (Table 3) but was higher in the inner parts of the Gulf of Gdańsk (with the exception of station ZN2). According to data reviewed by del Giorgio & Cole (1998), the median BGE in estuarine systems was about 34%, which is similar to our measurements. In Chesapeake Bay, BGE ranged from 20.4 to 41.3% (Smith & Kemp 2003).

Table 3. Bacterial respiration (BR), and bacterial production (BP), bacterial growth efficiency (BGE), and bacterial carbon demand (BCD) integrated over the 0–5 m layer

Station	Salinity [PSU]	BR [fg C cell^{-1} d^{-1}]	BR [mg C m^{-3} d^{-1}]	BP [mg C m^{-3} d^{-1}]	BCD [mg C m^{-3} d^{-1}]	BGE [%]
E139	7.25	7.7	60	13	73	18
E140	7.02	4.7	57	7.9	65	12
E55	6.85	4.4	53	8.4	62	14
E83	6.73	8.9	17	15	33	47
E60	6.62	6.2	26	11	37	29
E90	6.72	6.0	22	13	35	38
ZN2	6.20	7.5	98	14	113	13
M1	3.98	9.7	50	28	77	36
Mean	–	6.9	48	14	62	26
Vistula	0.00	2.7	70	19	89	21

Two bacterial groups characteristic of fresh and marine waters were studied at station ZN2 and E139 (Figure 1). The percentage of hybridized

Betaproteobacteria decreased from 4.3% of DAPI stained cells at station ZN2 to 2.3% at station E139, and the fraction of *Gammaproteobacteria* increased from 4.2% at ZN2 to 10.6% at E139. Similar patterns were also observed in other estuaries such as Chesapeake Bay (Bouvier & del Giorgio 2002) and Delaware Bay (Cottrell & Kirchman 2003). Despite differences in the methods used (CARD-FISH and 454 pyrosequencing), the fractions of these bacterial groups resembled the values obtained by Herlemann et al. (2011) for the same salinity range in the Baltic Sea.

3.5. ETS

Measurement of the electron transport system (ETS) gives an estimate of the metabolic potential of the community and may be used as an indication of oxidation rates under natural conditions (Packard & Williams 1981). The highest volumetric ETS activity was measured in the Vistula and gradually decreased with increasing salinity (Figure 6). ETS activities amounted to 33.8–35.5 μl O_2 h^{-1} dm^{-3} in the river water and decreased to 2.9–9.7 μl O_2 h^{-1} dm^{-3} in the photic layer of the open sea water. Specific ETS (ETS_{sp}), normalized to particulate protein, did not reveal any trend along the salinity gradient.
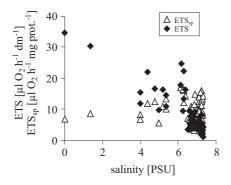

Figure 6. ETS [μl O_2 h^{-1} dm^{-3}] and ETS_{sp} [μl O_2 h^{-1} mg prot.$^{-1}$] as a function of salinity in the 0–20 m layer

4. Discussion

In an area of mixing, both freshwater and marine species encounter osmotic stress. As a result the species composition changes: its diversity may be reduced or new communities established that are specific to mixing zones (Findlay et al. 1991, Chin-Leo & Benner 1992, Cauwet 2002, Troussellier et al. 2002, Crump et al. 2004, Kirchman et al. 2005, Lee et al. 2012). Oceanic patterns are not always fully represented in the

brackish environment of Baltic Sea waters (Jost & Pollehne 1998, Wasmund et al. 1999, 2001, Piwosz et al. 2013). However, river impact is also important in the transition zone between fresh and Baltic Sea waters, and the final community structure and condition is the result of multi-factor control and reverse-acting processes. For example, trophic conditions improve for marine species but deteriorate for freshwater ones in the vicinity of the river, whereas the reverse situation obtains with respect to light conditions. The ability of new communities to establish themselves is also controlled by the water retention time. In the present study environmental parameters and production/respiration processes along the salinity gradient were measured in order to observe changes in plankton dynamics along the Vistula river plume. To analyse these changes salinity was used as a proxy of fresh water mixing with sea water.

The study was carried out during the second half of June. The water stratification, i.e. the summer location and strength of the thermocline, was indicative of typical summer conditions. The composition of the phytoplankton community was characteristic of the southern coast of the Baltic Proper in the second half of June and indicated an early summer situation. Although during the growing season the central part of the Baltic Sea is believed to be mostly N-limited, phosphate concentrations typically reach minimum values in July, even below detection limits (Renk 2000, Nausch et al. 2008); this was the situation that we found in the present study. Because nutrient availability vs. light and temperature conditions changes seasonally, the patterns observed in this study may be representative of the summer but not necessarily of the other seasons.

4.1. Comparison between phytoplankton and bacterial biomasses

Phytoplankton and bacterial biomass varied differently along the salinity gradient. Chl a and phytoplankton biomass were correlated with salinity (Table 2); the phytoplankton biomass appeared to be the result of conservative mixing (dilution) of the river water in the sea water (Figure 3), whereas bacterial abundance and biomass dropped abruptly between the river station and first stations in the Gulf of Gdańsk, which cannot be explained by the dilution effect (Figure 3). BBM in the Gulf of Gdańsk did not change so much along the salinity gradient and BBM was not correlated with salinity (Table 2). The drop in BBM (and TBN) observed between the station at Kiezmark located 11.6 km upriver and the first stations in the Gulf of Gdańsk (Figure 3) indicated that a major change in bacterial community took place very close to the river mouth (where no measurements were carried out), and not in the plume extending into the Gulf of Gdańsk. As a result, BBM in the Gulf of Gdańsk was at least twice as low as the values

measured at the river station. Division of POC into non-living (detritus) and living fractions (Figure 7) revealed that a drop in detritus-POC occurred between the river station and the stations in the gulf. This could partly explain the decrease in the number of particle-attached fresh water bacteria, which in turn resulted in lower overall TBNs and BBMs measured in the Gulf of Gdańsk. Such a pattern was also found in other marine regions (e.g. Cauwet 2002, Troussellier et al. 2002, Crump et al. 2004, Kirchman et al. 2005).
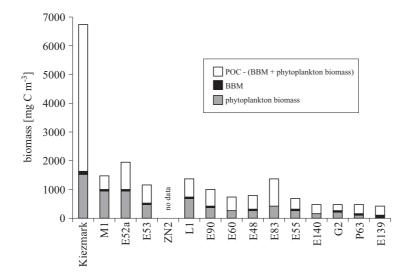

Figure 7. Average bacterial and phytoplankton biomass as POC components [mg C m^{-3}] in the 0–5 m layer, at stations along the salinity gradient. Vistula water was sampled only at the surface

The BBM (and TBN) gradient between the first stations in the gulf and those in the outer gulf waters was much weaker than the phytoplankton gradient (Figure 3). At the outermost station P63, the phytoplankton biomass was only 9% of that in the river, and BBM in the same layer was 48% of the river BBM. As a result, the BBM/Phytopl. biomass ratio increased with increasing salinity (Figure 7). BBM made up 6% of the phytoplankton biomass in the river (both expressed in carbon units) but ca 32% of this biomass at the outermost station P63. These changes in proportion form a gradient typically observed between eutrophic and oligotrophic waters: the plankton biomass is dominated by heterotrophs in oligotrophic waters (here, open sea waters) and by autotrophs in eutrophic waters (here, the river water) (e.g. Cole et al. 1998).

4.2. Environmental factors governing phytoplankton and bacterial production

4.2.1. Environmental factors governing phytoplankton production

Areal GPP along the salinity gradient was high (Figure 5); it was highest in the river water and lowest in the open sea waters and displayed a strong negative correlation with salinity (Table 2). Despite the low PO_4 concentration and high APA_{sp} activity (Figure 2b), the correlation with APA_{sp} activity did not indicate that phytoplankton production was phosphate-limited (Table 2). Partial correlation analysis, excluding the influence of salinity, showed that GPP was correlated with phytoplankton biomass; hence, its gradient appeared to be a consequence mainly of phytoplankton biomass dilution. Nutrient- or temperature-dependence were not controlling factors, as inter-seasonal studies of primary production usually show. Analysis of AN values did not reveal a gradient in the photosynthesis rate. Similar AN values were noted across the entire salinity gradient, as also indicated in earlier studies on primary production (e.g. Ochocki et al. 1995), with some lower values only at the outermost, open sea stations (data not shown). However, the overall phytoplankton biomass turnover (calculated as the doubling time) was shorter at the inner stations in the gulf compared to the outermost stations (a minimum of 2.6 days compared to the maximum of 4.9 days). The lower rate of reproduction calculated in both the river water and the open sea water (Figure 5) indicated stimulation of phytoplankton growth in the area impacted by river water. The resultant GPP rate was most probably a result of reverse-acting factors: improved light conditions for freshwater phytoplankton as compared to river water and improved nutrient availability for marine species as compared to the open sea waters. Because there was no measurable increase in Chl a concentration or phytoplankton biomass at the inner stations (as mentioned above, the phytoplankton biomass seemed to be due to river water being diluted in the sea water), faster growth must have been accompanied by a greater loss of algae (greater mortality or sedimentation). However, in the vicinity of the Vistula mouth, the residence time of water masses (the average salinity of a river water lens is 2–3 PSU) within the area bounded by the hydrological front (salinity at the front = 4–5 PSU) is a matter of hours (up to 20 hours) (Nowacki & Matciak 1996), which is rather short for the reconstruction of the phytoplankton community, assuming a doubling time of 3–5 days. The approximate residence time of the entire upper water layer (which comprises 75–84% of the total volume of the Gulf of Gdańsk) is only

about 15 days (Witek et al. 2003). Therefore, conservative mixing of fresh water and sea water probably had a more pronounced impact on the phytoplankton community than its enhanced growth rate. Moreover, the phytoplankton composition did not indicate changes other than conservative mixing (Figure 4). Although this pattern arises from observations made during one particular measurement time, similar observations with respect to phytoplankton composition were also noted in earlier studies (Witek et al. 1999, Wasmund et al. 2001, Wasmund & Siegel 2008).

4.2.2. Environmental factors governing bacterial production

The level of BP was similar at the river and the open sea stations (Figure 5). In the Gulf of Gdańsk BP displayed a strong negative correlation with salinity (Table 2). The highest values were noted close to the river mouth; cell-specific BP at the inner stations was 3–4 times higher than at the outer stations (Figure 5), indicating a faster reproduction rate of bacteria. Such a pattern suggested that bacterioplankton growth was being stimulated in the area impacted by river water. The higher BGE at the inner stations (Table 3) also gave an indirect indication of better growth conditions resulting from a better quality food supply (e.g. Zweifel et al. 1995). This is usually attributed either to the admixture of fresh water rich in nutrients and organic matter (e.g. Findlay et al. 1991, Zweifel et al. 1995, Smith 1998) or to higher phytoplankton production (e.g. Chin-Leo & Benner 1992), which is a source of organic substances (exudates) used by heterotrophic bacteria. Because there is a significant positive correlation only between BP and GPP and Chl a (Table 2), primary production most probably contributed to this enhanced growth in this measurement season. Environmental factors such as temperature or nutrients were not important, as is usually the case in inter-seasonal studies (e.g. Ameryk et al. 2005). For phosphate limitation of BP, correlation between BP and APA activity would be expected, but this did not occur (Table 2). Although on smaller temporal and spatial scales there might be different patterns (e.g. Shiah & Ducklow 1994), bacterial production and phytoplankton production usually correlate well (Cole et al. 1988).

Stimulation of bacterioplankton growth in the area of river water impact was also indicated by the faster bacterial doubling time there (Figure 5d) (a minimum of 1.8 days as compared to a maximum of 5.6 days). The exact impact of the intensified growth on bacterial biomass in the river plume versus conservative mixing of fresh and sea water cannot be estimated precisely because of the losses in BBM and TBN between the river water and the first sea water stations in the gulf (Figure 3). It should be emphasized that the bacterial doubling time would be substantially shorter if its

calculation were based not on the overall bacterial number but only on its active fraction (Vaqué et al. 2001). Hence, this pattern along the salinity gradient could change if we knew the bacterial cells' activity. This was not identified in this study, however.

The bacterioplankton composition was not studied in detail either. We only noted the changes in bacterioplankton composition between station ZN2 (inner station – Figure 1) and station E139 (outer station). The percentage of hybridized *Betaproteobacteria* typical of fresh waters (Glöckner et al. 1999, Kirchman 2002) decreased from 4.3% (ZN2) to 2.3% (E139) of DAPI stained cells, whereas the fraction of *Gammaproteobacteria*, which are typically marine bacteria, increased from 4.2% to 10.6% respectively. Although in this study we measured an increase in marine bacteria at higher salinities and a decrease in fresh water bacteria, other investigations into the seasonal dynamics and activity of bacteria in the Gulf of Gdańsk have shown that fresh water bacteria can also be active members of the brackish water communities in the Gulf of Gdańsk (Piwosz et al. 2013).

4.3. Comparison between primary and bacterial production

Ameryk et al. (2005), who estimated the areal BP to GPP ratio (0–20 m) on the basis of data from different seasons, found that it was higher in the inner zone of the Gulf of Gdańsk (on average 22%) and lower in the outer zone (on average 8%), and concluded that allochthonous organic matter had an influence on BP. Witek et al. (1999) indicated that in the vicinity of the Vistula this ratio was more variable but, on average, also higher than in the open waters. There was no clear trend along the salinity gradient in our results and the BP/GPP ratio (0–20 m) was 8% in Vistula waters and from 6 to 25% at the outermost stations (Figure 8).
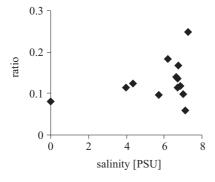

Figure 8. Average BP/GPP ratio along the salinity gradient in the 0–20 m layer

In the surface 0–5 m layer the BP/GPP ratio clearly had the opposite pattern to the one observed by Ameryk et al. (2005). In the surface layer

BP/GPP was ca 4% at the inner stations and ca 10% at the outermost ones. A similar trend can be calculated for the overall bacterial consumption of organic carbon (BCD). In our study BCD made up ca 12% of GPP at stations near the river mouth and 47–74% at the outermost ones. Some of these values are high: Witek et al. (1997), for example, measured BP (but not bacterial respiration) in the Gulf of Gdańsk and calculated the average bacterial food demand at 20–36% of GPP in the 0–20 m layer, assuming an average BGE of 0.25. However, it should be stressed that in the surface layer (0–5 m) maximum GPP rates were measured closer to the surface at the inner stations than at the outer ones; high (maximum) GPP rates fell with depth towards the outer zone of the gulf following an increase in water transparency. As a result, such a gradient indicates that the GPP gradient was steeper than the BP gradient; but this is true only for the surface layer, and the proportion of BP increases if deeper layers (or the entire euphotic depth) are analysed.

4.4. Impact of the River Vistula

The overall estimate of the plankton community activity – measured as the respiration rate (ETS) – decreased linearly with increasing salinity (Figure 6), following changes in POC and with Chl a as a proxy for the phytoplankton biomass – the dominant component of the living fraction of POC (Figure 7). The phytoplankton biomass in the gulf was the dominant component of POC with the exception of the river station, where the non-living fraction of POC was exceedingly high. In contrast to oceanic waters, no distinct front indicating the boundary of the river plume was noted in the brackish water of the southern Baltic, nor was the reduction in respiration activity measured as ETS_{sp} (Figure 6). The same observations were made in the Vistula river plume and also the plumes of other rivers entering the southern Baltic Sea with respect to phytoplankton biomass and Chl a (the Oder, Nemunas and Daugava) in studies carried out in different seasons (Jost & Pollehne 1998, Wasmund et al. 1999, 2001).

Although not apparent in terms of overall specific planktonic community respiration (ETS_{sp}, Figure 6), the impact of Vistula river waters on the plankton community other than conservative mixing of water masses with different concentrations of constituents was reflected in an increase in production rate, i.e. the biomass turnover time of phytoplankton and heterotrophic bacteria. However, the final impact of these increases was limited owing to the short water retention time. The GPP and BP pattern off the Vistula mouth appeared to differ from that described by Jost & Pollehne (1998) in the Pomeranian Bight, where those authors did not measure changes in the turnover time along the salinity gradient and concluded that

the river was not a direct source of substrates for growth. This could be viewed as a difference between the Vistula river plume, which makes direct entry to the sea waters in the Gulf of Gdańsk and the River Oder, whose waters are first transformed in the Szczecin Lagoon before entering the sea waters of the Pomeranian Bight.

5. Conclusions

The impact of the River Vistula other than conservative mixing of water masses with different concentrations of constituents was manifested in an increase in production rate, i.e. the biomass turnover time of phytoplankton and heterotrophic bacteria. This was the most visible impact of the river water on the pelagic community within the Gulf of Gdańsk and could be interpreted as a difference between the Gulf of Gdańsk, which is the direct recipient of the Vistula river waters and the Pomeranian Bight, into which the waters of the River Oder flow after having being initially transformed in the Szczecin Lagoon.

The biomass turnover time of phytoplankton and bacteria calculated as the doubling time was faster in the river water impact area than in the river waters themselves and at the open sea stations. This stimulation did not result in a measurable increase in their biomasses, however, probably because of the short retention time of the water in the river plume.

The phytoplankton biomass appeared to be the result of the dilution of the river water in the sea water, whereas bacterial abundance and biomass dropped between the river station and first sea water stations, a decrease not explainable by the dilution effect. The phytoplankton composition appeared to be an effect of conservative mixing, whereas the bacterial community probably changed between the river and first station in the gulf, but the composition of the bacterial community was not studied in detail.

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