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Impact of the Słupia River waters on microbial communities in the port of Ustka and adjacent Baltic Sea waters

by

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Abstract

The distribution of bacterial and ciliate abundance, ciliate community composition and other parameters were studied during summer along a transect from the mouth of the Słupia River to offshore waters (southern Baltic Sea). Bacteria were examined under an epifluorescence microscope and ciliates were observed under an inverted microscope. Two water masses were identified along the transect. Less saline waters in the river mouth and in the surface layer in the port of Ustka were characterized by high bacterial abundance ($5.51–6.16 \times 10^6$ ml^{-1}) and low ciliate abundance (0.34–0.90 cells ml^{-1}). More saline waters in the near-bottom zone in the port of Ustka and in the surface layer outside the port contained smaller numbers of bacteria ($0.99–2.14 \times 10^6$ ml^{-1}) and larger numbers of ciliates (2.65–5.40 cells ml^{-1}). The differences were statistically significant. The separation of the two water masses indicated that the Słupia River exerted a minor impact on the marine waters. The ciliate community composition changed along the transect studied. The main statistically significant difference observed was the low contribution of oligotrichs and choreotrichs to ciliate biomass (3–4%) in less saline waters and their dominance (45–80% of ciliate biomass) in more saline waters.

Key words: ciliates, bacteria, chlorophyll, estuary, microbial food web, *Mesodinium rubrum*

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Introduction

Estuaries are crucial areas for the assessment of eutrophication (HELCOM 1998; Golubkov et al. 2017), because they supply marine waters with nutrients and organic matter, which, in turn, fuel phytoplankton and bacterial productions (Jost & Pollehne 1998; Urrutxurtu et al. 2003; Mironova et al. 2013; Wielgat-Rychert et al. 2013; Rychert et al. 2014; Golubkov et al. 2017). Both phytoplankton and bacteria are food sources for protozoa (heterotrophic protists) that transfer a considerable part of phytoplankton primary production and bacterial secondary production to higher trophic levels, i.e. zooplankton and larval fish (Sherr & Sherr 2002; Rychert et al. 2016). This microbial food web is equally important in freshwater and marine environments (Sherr & Sherr 2002; Weisse 2017).

Gradients of water salinity and concentrations of nutrients and organic matter obviously drive the distribution of organisms. The abundance of protists (algae and protozoa) is high in the inner estuaries, but it drops significantly toward the open sea (Urrutxurtu et al. 2003; Christaki et al. 2009; Mironova et al. 2013; Wielgat-Rychert et al. 2013; Rychert et al. 2014). At the same time, a decrease in the abundance of bacteria is observed, but it is usually less conspicuous (Christaki et al. 2009; Wielgat-Rychert et al. 2013). To date, studies on the abundance of microorganisms along salinity gradients have generally been limited to large estuaries, while gradients along small estuaries remain understudied.

In this work, we studied the estuary of a medium-sized coastal river – the Słupia River, which discharges its waters into the Baltic Sea. Its catchment area covers 1623 km^2, its length is 138.6 km, and mean annual discharge is 18 m^3 s^{-1} (HELCOM 1998). Thus, it is much smaller than the largest inflow to the southern Baltic, i.e. the Vistula River, the average discharge of which is 1081 m^3 s^{-1} (HELCOM 1998). The mouth of the Słupia River enters the port in the town of Ustka, which is mainly used by fishing cutters. The length of the port is about 1.1 km. The objective of this study was to assess the distribution of environmental parameters, chlorophyll *a* concentration (a proxy of phytoplankton abundance), and the abundance of bacteria and ciliates along the salinity gradient between the mouth of the Słupia River and offshore waters. The study was performed in summer. Special emphasis was placed on ciliates, and their communities were studied taxonomically. The discharge of the Słupia River is rather low (18 m^3 s^{-1}, HELCOM 1998) and previous studies (Rychert et al. 2013) report only irregular effects of fresh water on environmental parameters observed in coastal waters located 1.3 km to the west of the port of Ustka. Thus, we hypothesized that freshwater input affects microbial communities within the port of Ustka, but it does not exert a significant impact on waters outside the port.

Materials and methods

The study was conducted on 18 July 2014. During the study, the weather was good with cloud cover of 2–3/8, and the sea state was 3–4°B. No precipitation was observed. Samples were collected from the Słupia River, the port of Ustka, and the marine waters outside the port (Fig. 1). Sampling was performed between 11:00 and 17:00. Samples 1–8 were collected from the surface zone. At sampling site 3, an additional sample (3D) was collected from the near-bottom zone (5.4 m). This sample was collected with a bathometer. Sampling at sites 1–3 was performed from bridges, while at sites 4–8 (outside the port) – from aboard a small cutter. The water depth at sampling site 1 (at the mouth of the Słupia River) was 0.2 m and it ranged from 4.0 to 5.4 m at sites 2–3 (the port). Outside the port, the depth gradually increased from 5.6 m (sampling site 4) to 26.5 m (sampling site 8). At each sampling site, 200 ml of water was fixed with formalin and another 250 ml of water was fixed with acid Lugol's solution. Additionally, unfixed water was collected for measurements of chlorophyll and total suspended matter concentrations. These measurements were performed a few hours after sampling. Chlorophyll *a* was extracted with acetone and measured spectrophotometrically according to Jeffrey & Humphrey (1975). The total suspended matter was determined gravimetrically. Each sampling event was also accompanied by temperature, salinity, and Secchi depth measurements.

Bacterial numbers were studied in samples fixed with formalin. Analysis was performed under an epifluorescence microscope after concentrating bacteria onto nucleopore filters and staining them with acridine orange (Hobbie et al. 1977). Ciliates were analyzed in samples fixed with acid Lugol's solution under an inverted microscope, i.e. using the Utermöhl method (HELCOM 2001). Taxonomic identification was performed according to Marshall (1969), Maeda & Carey (1985), Foissner & Berger (1996), a web-based guide (Strüder-Kypke & Montagnes 2002), and other keys. Generic and species names were verified in accordance with the World Register of Marine Species (WoRMS 2017). Each ciliate cell was measured using a camera and image analysis software to estimate its volume. Cell volume (CV, μm^3) was recalculated into biomass (CC, carbon content, pg C) according to the

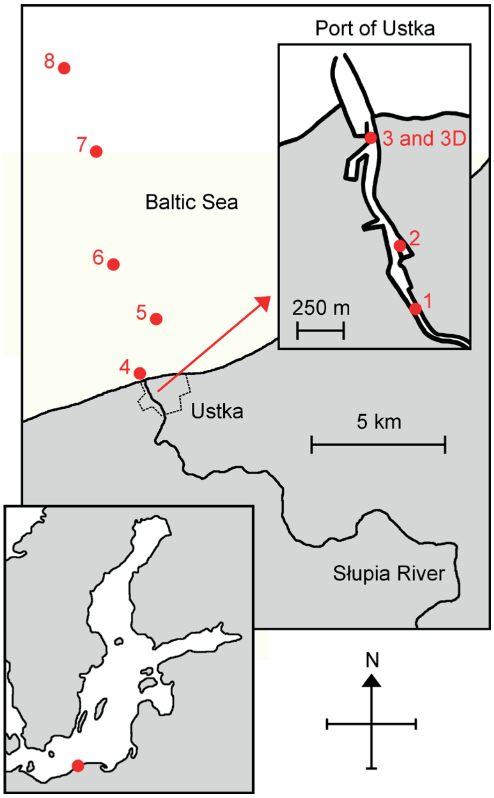

Figure 1

Location of sampling sites near the town of Ustka. Samples were collected on 18 July 2014 from the Słupia River (sample 1), the port of Ustka (samples 2, 3, and 3D) and from Baltic Sea waters (samples 4–8). Samples 1–8 were collected from the surface zone. Sample 3D was collected from the near-bottom zone.

following formula published by Menden-Deuer & Lessard (2000):

$$CC = 0.216 \times CV^{0.939} \tag{1}$$

The biomass of loricate ciliates was calculated from the volume of loricae (LV, µm^3) with the following formula by Verity & Langdon (1984):

$$CC = 0.053 \times LV + 444.5 \tag{2}$$

Freshwater residence time for the port of Ustka was calculated as the quotient of water volume and mean annual discharge of the Słupia River. The area of the port is 11.5 ha, but that of the inner port (the area between stations 1 and 3; Fig. 1) is only 5.39 ha (Maritime Office in Słupsk 2012). The depth of the canal and basins in the port ranges from 4.5 to 6.5 m (Konkol & Wickland 2016). The mean annual discharge of the Słupia River is 18 m^3 s^{-1} (HELCOM 1998). Based on the mean depth of the port and the mean discharge of the river, the freshwater residence time computed for the inner port was 4.6 h. This residence time for the whole area of the port (including the outer harbor) was estimated at 9.8 h. Both values are annual means.

Statistical analyses were performed with PAST 3.16 statistical software (Hammer et al. 2001).

Results

The temperature of the surface waters ranged from 19.4 to 20.4°C at all sampling sites. In the near-bottom zone at station 3 (sample 3D, depth 5.4 m), the temperature was lower at 18.1°C. At the mouth of the Słupia River (station 1; Fig. 1), water salinity was 0.21 PSU, and in the port of Ustka (stations 2–3) it ranged from 0.77 to 0.87 PSU. The salinity at the near-bottom zone at site 3 (sample 3D) was much higher at 6.81 PSU, which corresponded to the salinity of the surface waters outside the port (6.61–7.80 PSU, stations 4–8). Two different water masses were identified based on the salinity: (i) less saline water in the river and in the surface layer in the port (samples 1–3), and (ii) more saline water in the near-bottom zone in the port (sample 3D) and in the surface layer outside the port (samples 4–8; Table 1). Within the port, more saline and dense water was below the less saline surface water. The two water masses were also indicated by different water transparencies measured as Secchi depth: 2.5–3.0 m in the port (sites 2–3) and 5.0–7.5 m outside the port (stations 4–8).

Bacterial abundance in less saline waters (samples 1–3) ranged from 5.51 to 6.16 × 10^6 ml^{-1}, whereas in more saline water (samples 3D, 4–8) it was lower and ranged from 0.99 to 2.14 × 10^6 ml^{-1}. The difference between the bacterial abundance in the two separate water masses was statistically significant (p = 0.03; Table 1). The abundance and biomass of ciliates in less saline waters were, respectively, 0.34–0.90 cells ml^{-1} and 0.27–0.75 ng C ml^{-1}, whereas in more saline waters they were higher at 2.65–5.40 cells ml^{-1} and 1.46–4.40 ng C ml^{-1} (Table 1). The ciliate abundance and biomass in the two separate water masses were also significantly different (both p = 0.03, Mann-Whitney *U* test; Table 1).

The separation of the water into two masses was demonstrated to be statistically significant with respect to most of the parameters studied (Table 1). However, the pattern of distribution of chlorophyll *a* and total suspended matter was slightly different. The

Table 1

Parameters measured in two water masses separated along the transect between the Słupia River mouth and offshore waters. Less saline water was observed in the river mouth and in the surface layer in the port (samples 1–3), whereas more saline water was observed in the near-bottom zone in the port (sample 3D) and outside the port (samples 4–8). Obviously, Secchi depth was not determined at near-bottom sampling site 3D. Statistically significant differences between the two separate water masses were demonstrated for each parameter.

Parameter	Samples 1–3	Samples 3D, 4–8	Statistical significance according to Mann-Whitney *U* test
Salinity (PSU)	0.21–0.87	6.61–7.80	$p = 0.03$
Secchi depth (m)	2.5–3.0	5.0–7.5	$p = 0.03$
Bacterial abundance (10^6 ml^{-1})	5.51–6.16	0.99–2.14	$p = 0.03$
Ciliate abundance (cells ml^{-1})	0.34–0.90	2.65–5.40	$p = 0.03$
Ciliate biomass (ng C ml^{-1})	0.27–0.75	1.46–4.40	$p = 0.03$

highest chlorophyll *a* (4.82 μg l^{-1}) and total suspended matter (11.5 mg l^{-1}) concentrations were recorded in the sample collected from the near-bottom zone in the port of Ustka (3D; Fig. 1), which was clearly the result of the sedimentation of phytoplankton and matter from the upper part of the water column. Consequently, the distribution of these two parameters along the distance from the river mouth was analyzed for surface waters only (stations 1–8, without station 3D). In surface waters, the chlorophyll *a* concentration gradually decreased between the Słupia River mouth (2.73 μg l^{-1}) and offshore station 8 (0.85 μg l^{-1}; Fig. 2). The correlation was statistically significant ($p = 0.02$). The concentration of total suspended matter in surface waters ranged from 0.63 to 3.14 mg l^{-1} (Fig. 2) and the correlation between the distance and total suspended matter was non-significant.

It was observed (Fig. 3) that the ciliate community composition reconstructed itself along the transect from the river mouth to offshore waters. In the river (sample 1), the ciliate community consisted of hypotrichs (40% of ciliate biomass), prostomatids (26%), and scuticociliates (14%). At this sampling site, many ciliates classified into the group "other and unidentified" (Fig. 3) were benthic migrants, e.g. hymenostomatids. In the surface waters in the port (samples 2–3), the most important ciliates were prostomatids, which contributed 50–90% of the ciliate biomass. Both in the river mouth and in the surface waters in the port (samples 1–3), oligotrichs and choreotrichs contributed only 3–4% of ciliate biomass (Fig. 3). In the near-bottom zone in the port and in the surface waters outside the port (samples 3D and 4–8, more saline waters), the most important were oligotrichs and choreotrichs, the contribution of which to ciliate biomass ranged from 45 to 80%. The remaining biomass (Fig. 3) consisted mainly of prostomatids (5–37%) and haptorids (up to 14%). The difference between the contribution of oligotrichs and choreotrichs to ciliate biomass observed in (i) less saline waters (3–4% of ciliate biomass) and in (ii) more saline waters (45–80% of ciliate biomass) was statistically significant (Mann-Whitney *U* test, $p = 0.03$).

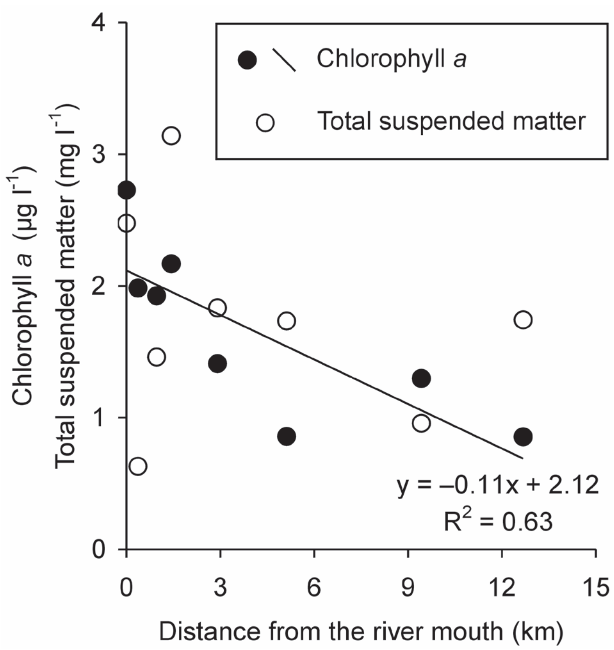

Figure 2

Changes in chlorophyll *a* (black dots) and total suspended matter (circles) concentrations in surface waters (excluding sample 3D) along the transect between the Słupia River mouth and offshore waters. The correlation between the distance and chlorophyll *a* is statistically significant (trend line, $R^2 = 0.63$, $p = 0.02$). The highest concentration of total suspended matter was encountered close to the entrance to the port of Ustka (station 4, 1.43 km from the river mouth, value 3.14 mg l^{-1}; Fig. 1).

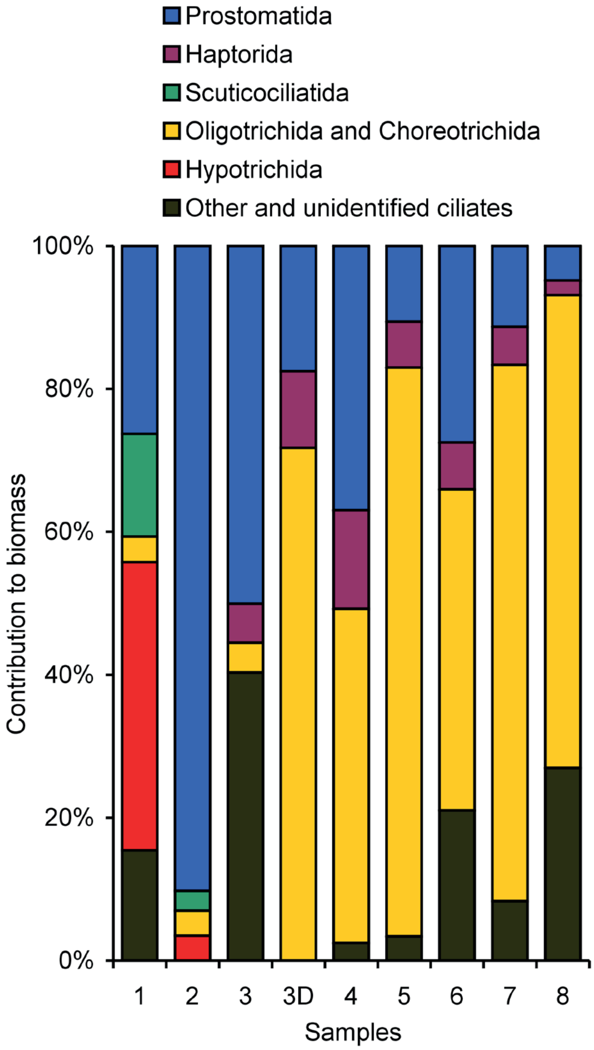

Figure 3

Taxonomic composition of ciliate communities observed along the transect between the Słupia River mouth and offshore waters. Samples 1–8 were collected from surface waters and sample 3D was collected from the near-bottom zone at sampling site 3. The main difference between less saline waters (samples 1–3) and more saline waters (samples 3D and 4–8) was the occurrence of ciliates from the orders Oligotrichida and Choreotrichida, which constituted 3–4% of the ciliate biomass in less saline waters and 45–80% of its biomass in more saline waters. The difference is statistically significant (*U* test, *p* = 0.03).

Among the prostomatids observed in less saline waters (samples 1–3), the most frequent were those from the genera *Urotricha*, *Holophrya* and *Prorodon*. In more saline waters (samples 3D, 4–8), *Urotricha* spp. and *Balanion comatum* Wulff 1922 were observed. The latter is a typical marine organism (WoRMS 2017). Among oligotrichs and choreotrichs encountered in less saline waters, we observed mainly small *Rimostrombidium* spp. In more saline waters, larger oligotrichs and choreotrichs were recorded, e.g. *Rimostrombidium sphaericum* (Lynn & Montagnes 1988) Petz & Foissner 1992 and *Pelagostrobilidium spirale* (Leegaard 1915) Petz, Song & Wilbert 1995. Both are typical marine organisms (WoRMS 2017). It is worth mentioning that most of the *R. sphaericum* specimens at sampling sites 6–8 were found in conjugation. At the most offshore site (8), a few specimens of *Tintinnopsis* sp. were observed. Haptorids were represented by *Askenasia* spp., heterotrophic *Mesodinium* spp., and autotrophic *Mesodinium rubrum* (Lohmann 1908), which has recently been reported to be a *M. major/rubrum* species complex (Garcia-Cuetos et al. 2012; Johnson et al. 2016). The latter was observed in more saline waters only (samples 3D, 4–8). At the most inner stations (1–2), some scuticociliates were observed, the most frequent being *Cyclidium glaucoma* Möller. At the most offshore stations (6–8), on the other hand, few peritrichs (*Vaginicola* sp. and *Vorticella* sp.) were recorded.

Detailed data on abundance, biomass, and composition of ciliate communities were provided in the supplementary material.

Discussion

Outside the port of Ustka, water salinity and Secchi depths corresponded to typical values observed in the southern Baltic Sea (Matthäus et al. 2008; Fleming-Lehtinen & Laamanen 2012). Water transparency in the port was lower because chlorophyll concentrations were higher (Fig. 2). The concentrations of chlorophyll *a* in the Słupia River and in the surface waters of the port were rather low compared to values observed in the Słupia River during summer 2006 (2.1–14.0 μg l^{-1}; Gorzeń & Załupka 2007). Even the higher chlorophyll *a* concentration observed in the near-bottom zone of the port (4.82 μg l^{-1}, station 3D) was rather low. The chlorophyll *a* concentration outside the port of Ustka corresponded to the mean chlorophyll *a* concentration of 2.09 μg l^{-1} averaged for July for the Słupsk Furrow (waters off Ustka) based on long-term studies performed between 1965 and 1998 (Renk 2000). The concentrations of total suspended matter observed in this study were comparable to summer values observed previously in the Słupia River (2.0–5.8 mg l^{-1}; Gorzeń & Załupka 2007) and in the surface waters of the Oder River plume (0.51–5.98 mg l^{-1}, the Baltic Sea; Ferrari et al. 2003).

The bacterial abundance reported in this study was lower than that in the eutrophic Warnow River (southwestern Baltic Sea, July, 10–20 × 10^6 ml^{-1}; Freese et al. 2006), but it corresponded well to abundance observed during summer in estuaries and coastal waters of the Baltic Sea (Mironova et al. 2012; Wielgat-Rychert et al. 2013; Rychert et al. 2013; Rychert et al. 2015) and other waters (Christaki et al. 2009). For example, in the early summer the bacterial abundance in the Vistula River was 6.87 × 10^6 ml^{-1} and in the offshore waters of the Gulf of Gdańsk (Baltic Sea) it was on average 1.82 × 10^6 ml^{-1} (Wielgat-Rychert et al. 2013).

The ciliate abundance in the river mouth and in the surface waters in the port was low compared to the abundance observed previously in July in the Słupia River (mean 4.00 cells ml^{-1}, 4.35 ng C ml^{-1}; Rychert 2009). The ciliate abundance observed in this study in more saline waters was also lower than that reported in July 2006 and 2007 in coastal waters located 1.3 km to the west of the port of Ustka at 6.01–46.5 cells ml^{-1} and 3.69–71.87 ng C ml^{-1} (Rychert et al. 2016). The ciliate abundance observed in this study was comparable to values observed in the Neva River estuary (Baltic Sea) in July 2008 (about 3 cells ml^{-1} and 5 ng C ml^{-1}; Mironova et al. 2012), and it was higher than that observed there during summer 2010 (0.03–1.9 cells ml^{-1}; Mironova et al. 2013).

In large estuaries, e.g. the Vistula River estuary (Baltic Sea; Wielgat-Rychert et al. 2013) and lagoon and coastal waters of the Pomeranian Bight (Baltic Sea; Jost & Pollehne 1998), gradual decreases in chlorophyll *a* concentration were determined along transects from the river mouths to offshore waters. It is also reported that the amount of suspended matter transported by rivers, which is re-suspended from the bottom, gradually decreases along estuaries (Naudin et al. 2001; Emeis et al. 2002). In this study, a significant correlation between the concentration of chlorophyll *a* and the distance from the river mouth was demonstrated for the Słupia River estuary (surface waters, $p = 0.02$; Fig. 2). A similar correlation for the total suspended matter in the surface waters was not statistically significant. Bacterial abundance, similarly to chlorophyll *a* concentration, decreased along the transect from the river to offshore waters (Table 1). This is typical, because fresh water, which is enriched with nutrients and organic matter and contains more phytoplankton and bacterioplankton, is diluted in sea water (Wielgat-Rychert et al. 2013).

In contrast to phytoplankton (chlorophyll *a*) and bacteria, ciliate abundance and biomass in the sea were higher than in the river, which is unusual (see e.g. Christaki et al. 2009). We suspect that the rapid water flow in the Słupia River offers unfavorable conditions for ciliates. In the port, the ciliate community underwent substantial reconstruction that led to the dominance of prostomatids and the elimination of many freshwater ciliates (Fig. 3). In the port, where water flow is slower, the ciliate community could increase its abundance. However, the mean annual freshwater residence time computed for the inner port (the area between stations 1 and 3; Fig. 1) is only 4.6 h, which is shorter than ciliate doubling time in temperate waters in July (about 11 h; Rychert et al. 2016). Thus, it was not surprising that their abundance remained lower than that in marine waters. The mean annual freshwater residence time for the entire port area is about 9.8 h (including the outer harbor; Fig. 1; see Materials and Methods for data used in the calculations), which is also short. The surface waters at sampling site 4, the first site located outside the port (Fig. 1), did not differ from the offshore stations with respect to the parameters presented in Table 1. This indicated the minor impact of fresh water on this station. It is worth mentioning that elevated concentrations of chlorophyll *a* and total suspended matter were observed at sampling station 4 (Fig. 2), which resulted from the resuspension of bottom sediments at this shallow station (5.6 m) located in the coastal zone of the sea.

Ciliates observed in less saline waters (the river mouth, surface waters in the port) were previously reported in the Słupia River (Rychert 2009) and other Baltic rivers (e.g. Grinienė et al. 2011), whereas ciliates encountered in more saline waters (near-bottom waters in the port, surface waters outside the port) were reported in the Baltic Sea (Grinienė et al. 2011; Mironova et al. 2012; Mironova et al. 2013; Rychert et al. 2013; Mironova et al. 2014). The reconstruction of the ciliate community observed in this study generally resembled the changes in the ciliate community composition previously described along large Baltic estuaries, e.g. the Neva estuary (the Gulf of Finland; Mironova et al. 2012), and the Vistula River estuary (the Gulf of Gdańsk; Rychert et al. 2014). Ciliate communities in the inner part of these estuaries contain some freshwater species and benthic migrants. In the outer parts of the Neva and the Vistula estuaries, ciliate communities are dominated by oligotrichs and choreotrichs (including tintinnids) and are accompanied by haptorids (Mironova et al. 2012; Rychert et al. 2014). The same dominants in the ciliate community, but with prostomatids, were observed in this study in more saline waters (samples 3D, 4–8). We observed that the importance of prostomatids was considerable since they contributed 26–90%

of the ciliate biomass in less saline waters (stations 1–3) and 5–37% of the ciliate biomass in more saline waters (samples 3D, 4–8). This corresponds well to the previously demonstrated high importance of prostomatids in the Słupia River, where they contribute 41% of the ciliate biomass annually (Rychert 2009), and the lesser importance of prostomatids in the coastal waters near Ustka, where they contribute only 13% of the ciliate biomass annually (Rychert et al. 2013, unpubl. details). In some estuaries, e.g. that of the Nervión River (the Bay of Biscay, Atlantic Ocean; Urrutxurtu et al. 2003), ciliate communities were dominated by scuticociliates, which was explained by the high availability of bacteria that are their food resources (Urrutxurtu et al. 2003). In this study, bacterial abundance was lower and scuticociliates were observed only in small quantities. Mironova et al. (2012) reported that in the Neva estuary (Baltic Sea) in summer, 28–67% of ciliates were mixotrophs. A significant presence of mixotrophs was also reported in estuaries located in other seas, e.g. in the Rhone River estuary (Mediterranean Sea; Christaki et al. 2009). In this study, ciliates were analyzed in samples fixed with Lugol's solution, which made it impossible to observe the chloroplasts inside the cells. Thus, we did not estimate the fraction of mixotrophs in the ciliate community. Among easily identifiable marine mixotrophs (*Mesodinium major/rubrum* species complex, *Laboea strobila*, *Tontonia* spp.; Christaki et al. 2009; Stoecker et al. 2009), we observed only the *Mesodinium major/rubrum* complex. It was absent in samples collected from less saline water (samples 1–3), but it was present in all samples collected from more saline waters (samples 3D, 4–8). Its abundance was low at 0.08–0.50 cells ml^{-1} (0.16–0.23 ng C ml^{-1}), whereas in July 2006 and 2007 the abundance reported from a sampling site located 1.3 km westward from the port of Ustka ranged from 0.34 to 12.7 cells ml^{-1}, which corresponded to a biomass of up to 5.06 ng C ml^{-1} (Rychert & Pączkowska 2012). In conclusion, the abundance of the *M. major/rubrum* complex was low in this study and specimens observed were rather small with diameters ranging from 10.5 to 25.5 µm. These small specimens were certainly *M. rubrum*. Single larger specimens were observed only at sampling sites 5 and 7 (28.2 µm and 31.5 µm) and they could be *M. major*.

Conclusions

Two different water masses were identified based on the majority of parameters studied (water salinity, Secchi depth, bacterial abundance, ciliate abundance, ciliate biomass): (i) less saline water in the river and in the surface layer in the port, and (ii) more saline water in the near-bottom zone in the port and in surface layer outside the port. The distinct separation of the two water masses, i.e. the absence of gradual gradients of these parameters, indicated that the Słupia River exerted a minor impact on the marine waters. Thus, the hypothesis presented in the introduction was generally confirmed. Only in the case of chlorophyll *a* was a gradual decrease of its concentration in surface waters demonstrated toward offshore waters. Changes in the ciliate community composition generally resembled those reported in other estuaries. The low importance of oligotrichs and choreotrichs was observed in less saline waters. On the other hand, these groups dominated in more saline waters.

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References

Christaki, U., Courties, C., Joux, F., Jeffrey, W.H., Neveux, J. et al. (2009). Community structure and trophic role of ciliates and heterotrophic nanoflagellates in Rhone River diluted mesoscale structures (NW Mediterranean Sea). *Aquat. Microb. Ecol.* 57: 263–277. DOI: 10.3354/ame01339.

Emeis, K., Christiansen, C., Edelvang, K., Jähmlich, S., Kozuch, J. et al. (2002). Material transport from the near shore to the basinal environment in the southern Baltic Sea II: Synthesis of data on origin and properties of material. *J. Mar. Syst.* 35: 151–168.

Ferrari, G.M., Bo, F.G. & Babin, M. (2003). Geo-chemical and optical characterizations of suspended matter in European coastal waters. *Est. Coast. Shelf Sci.* 57: 17–24. DOI: 10.1016/S0272-7714(02)00314-1.

Fleming-Lehtinen, V. & Laamanen, M. (2012). Long-term changes in Secchi depth and the role of phytoplankton in explaining light attenuation in the Baltic Sea. *Est. Coast. Shelf Sci.* 102–103: 1–10. DOI: 10.1016/j.ecss.2012.02.015.

Foissner, W. & Berger, H. (1996). A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. *Freshwater Biol.* 35: 375–482.

Freese, H.M., Karsten, U. & Schumann, R. (2006). Bacterial abundance, activity, and viability in the eutrophic River Warnow, northeast Germany. *Microb. Ecol.* 51: 117–127. DOI: 10.1007/s00248-005-0091-5.

Garcia-Cuetos, L., Moestrup, Ø. & Hansen, P.J. (2012). Studies on the genus *Mesodinium* II. Ultrastructural and molecular investigations of five marine species help clarifying the taxonomy. *J. Eukaryot. Microbiol.* 59: 374–400. DOI: 10.1111/j.1550-7408.2012.00630.x.

Golubkov, S., Golubkov, M., Tiunov, A. & Nikulina, V. (2017). Long-term changes in primary production and mineralization of organic matter in the Neva Estuary (Baltic Sea). *J. Mar. Syst.* 171: 73–80. DOI: 10.1016/j.jmarsys.2016.12.009.

Gorzeń, A. & Załupka, A. (2007). *Water quality of the Słupia River at Charnowo, Łupawa at Smołdzino, Łeba at Cecynowo in 2006*. Słupsk: Provincial Inspectorate for Environmental Protection in Gdańsk branch in Słupsk. (In Polish).

Grinienė, E., Mažeikaitė, S. & Gasiūnaitė, Z.R. (2011). Inventory of the taxonomical composition of the plankton ciliates in the Curonian Lagoon (SE Baltic Sea). *Oceanol. Hydrobiol. Stud.* 40: 86–95. DOI: 10.2478/s13545-011-0045-0.

Hammer, Ø., Harper, D.A.T. & Ryan, P.D. (2001). Past: paleontological statistics software package for education and data analysis. *Palaeontol. Electronica* 4(1), 4: 1–9.

HELCOM. (1998). *The third Baltic Sea pollution load compilation*. Helsinki: Balt. Sea Environ. Proc. (No. 70).

HELCOM. (2001). *Manual for marine monitoring in the COMBINE programme of HELCOM*. Retrieved September 17, 2017, from http://www.helcom.fi/groups/monas/CombineManual/AnnexesC/en_GB/annex6/.

Hobbie, J.E., Daley, R.J. & Jasper, S. (1977). Use of nucleopore filters for counting bacteria by epifluorescence microscopy. *Appl. Environ. Microbiol.* 33: 1225–1228.

Jeffrey, S.W. & Humphrey, G.F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pfl.* 167: 191–194.

Johnson, M.D., Beaudoin, D.J., Laza-Martinez, A., Dyhrman, S.T., Fensin, E. et al. (2016). The genetic diversity of *Mesodinium* and associated cryptophytes. *Front. Microbiol.* 7, 2017: 1–16. DOI: 10.3389/fmicb.2016.02017.

Jost, G. & Pollehne, F. (1998). Coupling of autotrophic and heterotrophic processes in a Baltic estuarine mixing gradient (Pomeranian Bight). *Hydrobiologia* 363: 107–115.

Konkol, H. & Wickland, W.M. (2016). *Expansion of the port in Ustka. Simulation of wave conditions.* Master Thesis, Norwegian University of Science and Technology, Trondheim, Norway.

Lindholm, T. (1978). Autumnal mass development of the "red water" ciliate *Mesodinium rubrum* in the Åland Archipelago. *Memoranda Soc. Fauna Flora Fennica* 54: 1–5.

Maeda, M. & Carey, P.G. (1985). An illustrated guide to the species of the family Strombidiidae (Oligotrichida, Ciliophora), free swimming protozoa common in the aquatic environment. *Bull. Ocean Res. Inst. Univ. Tokyo*, 19: 1–68.

Maritime Office in Słupsk. (2012). *Regulation No. 1 of the Director of the Maritime Office in Słupsk regarding determining the port areas and public objects, facilities and installations in the port infrastructure.* Gdańsk: Official Journal of Law of the Pomeranian Voivodeship from 2012, item. 2103. (In Polish).

Marshall, S.M. (1969). Protozoa, order Tintinnida. In J.H. Fraser & V.K. Hansen (Eds.), *Fiches d'Identification du Zooplankton* (pp. 117–127). Copenhagen: Conseil International pour l'Exploration de la Mer.

Matthäus, W., Nehring, D., Feistel, R., Nausch, G., Mohrholz, V. et al. (2008). The inflow of highly saline water in the Baltic Sea. In R. Feistel, G. Nausch & N. Wasmund (Eds.), *State and Evolution of the Baltic Sea* (pp. 265–309). Hoboken: Wiley & Sons.

Menden-Deuer, S. & Lessard, E. (2000). Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol. Oceanogr.* 45: 569–579.

Mironova, E., Telesh, I. & Skarlato, S. (2012). Diversity and seasonality in structure of ciliate communities in the Neva Estuary (Baltic Sea). *J. Plankton Res.* 34: 208–220. DOI: 10.1093/plankt/fbr095.

Mironova, E., Telesh, I. & Skarlato, S. (2013). Planktonic ciliates of the Neva Estuary (Baltic Sea): Community structure and spatial distribution. *Acta Protozool.* 52: 13–23. DOI: 10.4467/16890027AP.13.002.0830.

Mironova, E.I., Telesh, I.V. & Skarlato, S.O. (2014). Ciliates in plankton of the Baltic Sea. *Protistology* 8: 81–124.

Naudin, J.-J., Cauwet, G., Fajon, C., Oriol, L., Terzić S. et al. (2001). Effect of mixing on microbial communities in the Rhone River plume. *J. Mar. Syst.* 28: 203–227.

Renk, H. (2000). *Primary production in the southern Baltic.* Gdynia: Sea Fisheries Institute Publ. (In Polish).

Rychert, K. (2009). Planktonic ciliates in the coastal medium-size river: diversity and productivity. *Pol. J. Ecol.* 57: 503–512.

Rychert, K. & Pączkowska, M. (2012). Ciliate *Mesodinium rubrum* in the coastal zone of the southern Baltic Sea (Central Pomerania). *Balt. Coast. Zone* 16: 97–102.

Rychert, K., Kownacka, J., Wielgat-Rychert, M. & Pluto-Prądzyńska A. (2014). Protozoan communities in the Vistula River estuary (Baltic Sea). *Balt. Coast. Zone* 18: 39–53.

Rychert, K., Spich, K., Laskus, K., Pączkowska, M., Wielgat-Rychert, M. et al. (2013). Composition of protozoan communities at two stations in the coastal zone of the southern Baltic Sea. *Oceanol. Hydrobiol. Stud.* 42: 268–276. DOI: 10.2478/s13545-013-0083-x.

Rychert, K., Wielgat-Rychert, M., Wołoszynek, M. & Sojda, G. (2015). Pelagic respiration in the coastal zone of the southern Baltic Sea. *Ecohydrol. Hydrobiol.* 15: 215–219. DOI: 10.1016/j.ecohyd.2015.06.001.

Rychert, K., Kozłowska, J., Krawiec, K., Czychewicz, N., Pączkowska, M. et al. (2016). Annual production to biomass (P/B) ratios of pelagic ciliates in different temperate waters. *Oceanol. Hydrobiol. Stud.* 45: 388–404. DOI: 10.1515/ohs-2016-0035.

Sherr, E.B. & Sherr, B.F. (2002). Significance of predation by protists in aquatic microbial food webs. *Antonie Leeuwenhoek* 81: 293–308.

Stoecker, D.K., Johnson, M.D., de Vargas, C. & Not, F. (2009). Acquired phototrophy in aquatic protists. *Aquat. Microb. Ecol.* 57: 279–310. DOI: 10.3354/ame01340.

Strüder-Kypke, M.C. & Montagnes, D.J.S. (2002). Development of web-based guides to planktonic protists. *Aquat. Microb. Ecol.* 27: 203–207.

Urrutxurtu, I., Orive, E. & de la Sota, A. (2003). Seasonal dynamics of ciliated protozoa and their potential food in an eutrophic estuary (Bay of Biscay). *Est. Coast. Shelf Sci.* 57: 1169–1182. DOI: 10.1016/S0272-7714(03)00057-X.

Verity, P.G. & Langdon, C. (1984). Relationships between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. *J. Plankton Res.* 6: 859–867.

Weisse, T. (2017). Functional diversity of aquatic ciliates. *Eur. J. Protistol.* 61: 331–358. DOI: 10.1016/j.ejop.2017.04.001.

Wielgat-Rychert, M., Ameryk, A., Jarosiewicz, A., Kownacka, J., Rychert, K. et al. (2013). Impact of the inflow of the Vistula River waters on the pelagic zone in the Gulf of Gdańsk. *Oceanologia* 55: 859–886. DOI: 10.5697/oc.55-4.859.

WoRMS. (2017). *World Register of Marine Species*. Retrieved September 18, 2017, from http://www.marinespecies.org. DOI: 10.14284/170.

Supplementary material

Abundance, biomass and composition of ciliate communities studied on 18 July 2014

Group	Abundance (cells ml⁻¹)	Biomass (ng C ml⁻¹)	Composition
Sample 1 – the Słupia River mouth			
Prostomatida	0.24	0.07	*Urotricha* sp., *Prorodon* sp.
Scuticociliatida	0.24	0.04	*Cyclidium glaucoma*
Oligotrichida and Choreotrichida	0.06	0.01	*Rimostrombidium* sp.
Hypotrichida	0.18	0.11	Unidentified
Other and unidentified ciliates	0.18	0.04	Hymenostomatid ciliates, unidentified
Total	0.90	0.27	
Sample 2 – surface waters in the Port of Ustka			
Prostomatida	0.33	0.32	*Urotricha* spp., *Prorodon* sp., *Holophrya* sp.
Scuticociliatida	0.03	0.01	*Cyclidium* sp.
Oligotrichida and Choreotrichida	0.07	0.01	*Rimostrombidium* sp.
Hypotrichida	0.03	0.01	Unidentified
Total	0.47	0.35	
Sample 3 – surface waters in the Port of Ustka			
Prostomatida	0.14	0.37	*Urotricha* sp., *Holophrya* spp.
Haptorida	0.05	0.04	*Askenasia* sp., *Mesodinium* sp. (heterotrophic)
Oligotrichida and Choreotrichida	0.05	0.03	*Rimostrombidium* sp.
Unidentified	0.10	0.30	Unidentified
Total	0.34	0.75	
Sample 3D – near-bottom waters in the Port of Ustka			
Prostomatida	2.60	0.52	*Balanion comatum*, *Urotricha* sp.
Haptorida	0.70	0.32	*Mesodinium rubrum*, *Askenasia* sp., *Mesodinium* sp. (heterotrophic)
Oligotrichida and Choreotrichida	1.10	2.14	*Rimostrombidium sphaericum*, *Strombidium* spp.
Total	4.40	2.98	
Sample 4 – surface waters outside the port of Ustka			
Prostomatida	3.30	0.54	*Balanion comatum*, *Urotricha* spp., *Holophrya* sp.
Haptorida	0.80	0.20	*Mesodinium rubrum*, *Mesodinium* sp. (heterotrophic), *Askenasia* sp.
Oligotrichida and Choreotrichida	1.10	0.68	*Rimostrombidium sphaericum*, *Strombidium* spp., *Strobilidium* sp.
Unidentified	0.20	0.04	Unidentified
Total	5.40	1.46	
Sample 5 – surface offshore waters			
Prostomatida	1.84	0.35	*Balanion comatum*, *Urotricha* sp.
Haptorida	0.32	0.22	*Mesodinium rubrum/major*, *Mesodinium* sp. (heterotrophic), *Askenasia* sp.
Oligotrichida and Choreotrichida	1.28	2.67	*Rimostrombidium sphaericum*, *Pelagostrobilidium spirale*, *Strombidium* sp.
Unidentified	0.56	0.11	Unidentified
Total	4.00	3.35	
Sample 6 – surface offshore waters			
Prostomatida	2.64	0.57	*Balanion comatum*, *Urotricha* sp.
Haptorida	0.40	0.14	*Mesodinium rubrum*, *Askenasia* sp.
Oligotrichida and Choreotrichida	0.88	0.93	*Rimostrombidium sphaericum*, *Strombidium* spp., *Lohmanniella oviformis*
Peritrichida	0.24	0.35	*Vaginicola* sp.
Unidentified	0.56	0.08	Unidentified
Total	4.72	2.06	
Sample 7 – surface offshore waters			
Prostomatida	2.12	0.37	*Balanion comatum*, *Urotricha* sp.
Haptorida	0.52	0.18	*Mesodinium* sp. (heterotrophic), *Mesodinium rubrum/major*, *Askenasia* sp.
Oligotrichida and Choreotrichida	1.00	2.45	*Rimostrombidium sphaericum*, *Pelagostrobilidium spirale*, *Strombidium* spp., others
Peritrichida	0.12	0.18	*Vaginicola* sp.
Unidentified	0.56	0.09	Unidentified
Total	4.32	3.27	
Sample 8 – surface offshore waters			
Prostomatida	0.88	0.21	*Urotricha* sp., *Balanion comatum*, *Holophrya* sp.
Haptorida	0.19	0.09	*Mesodinium rubrum*
Oligotrichida and Choreotrichida	1.45	2.91	*Rimostrombidium sphaericum*, *Tintinnopsis* sp., *Lohmanniella oviformis*
Peritrichida	0.06	1.17	*Vorticella* sp.
Unidentified	0.06	0.02	Unidentified
Total	2.65	4.40	

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