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Microbial and chemical quality assessment of the small rivers entering the South Baltic. Part II: Case study on the watercourses in the Puck Bay catchment area

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Abstract: Due to its location, Puck Bay is an area particularly vulnerable to pollution of anthropogenic origin. The aim of the study was to assess the water quality of small watercourses entering the inner part of Puck Bay. The paper presents the results of chemical and microbiological analyses of 10 rivers and canals at their estuaries located on the western shore of the internal Puck Bay. The following environmental parameters were analyzed: conductivity, pH, dissolved oxygen concentration (in situ measurements), COD (cuvette tests), concentrations of ions (ion chromatography). Microbiological analysis included assessment of sanitary condition based on the number of fecal coliforms by a cultivation method. The determination of basic microbiological parameters such as: prokaryotic cell abundance expressed as total cells number (TCN), prokaryotic cell biovolume expressed as average cell volume (ACV), the prokaryotic biomass (PB) and prokaryotic cell morphotype diversity were determined using epifluorescence microscopy method. Based on the obtained results, it was found that small watercourses may carry a notable load of anthropogenic pollution and thus affect the environment of Puck Bay. The results clearly indicate the need for quality monitoring in the rivers and canals in the Coastal Landscape Park, flowing into Puck Bay. The research showed that also smaller watercourses may have an impact on the coastal waters' state, and thus on the Baltic Sea water quality.

Introduction

Puck Bay, especially its internal part with an area of 104 km^2, limited from the east by a sandy spit, the so-called 'Seagull Rift' and often called the Puck Lagoon or 'Little Sea' (Klekot 1980b), is considered the most diversified and biologically valuable part of Polish maritime areas (Węsławski et al. 2009). The trophic state of the Baltic Sea strongly depends on the supply of biogenic substances by rivers, and this relationship is particularly clear in coastal waters which are a buffer zone for open waters (Korth et al. 2013). In 1978, as the first area of marine waters in Poland, the Bay was protected as part of the Coastal Landscape Park (CLP). Since 1992, it is also a designated Baltic Sea Protected Area (BSAP) under the Helsinki Convention and a protected area under Natura 2000 program, both under Council Directive 92/43/ EEC from May 21st, 1992 on the conservation of natural habitats and of wild fauna and flora (The Council of European Communities 1992). The legally protected areas of Puck Bay are Special Bird Protection Area (PLB220005), Special Habitat Protection Area (PLH220032) and Hel Peninsula. Two legally protected Nature Reserves were established on the west coast of Puck Bay (Krajewska and Fac-Beneda, 2016).

Nature Reserve 'Beka' (NRB) was established in pursuance of the regulation of the Ministry of the Environment (Council of Ministers, 1988), and its purpose is to preserve rich breeding and migratory avifauna, as well as rushes and meadow communities, including wet meadows. Nature Reserve "Salty Meadows", which is located in Wladyslawowo city area, was established under regulation of the Pomeranian Voivode in 1999 (Ordinance of the Governor 1999), and its purpose is to preserve halophilic meadows, rare species of salt-loving plants and habitats of valuable bird species. Therefore, on the one hand, these are key areas for the occurrence of a number of species that do not occur along the coast of the open sea, but at the same time, it has been a region under the influence of strong anthropogenic pressure for many years (Korzeniewski 1993).

Puck Bay is also an area with the oldest traditions of marine research in Poland, for which an excellent comparative material exists for observing environmental changes over almost a century. In the 1930s observations were made and the first underwater photos of the bottom of the Bay were taken. Rich underwater meadows composed of many species of macroalgae (including *Chara, Fucus vesiculosus, Furcellaria lumbricalis*) and vascular plants (*Potamogeton pectinatus, Zostera marina*) were inhabited by numerous species of macrofauna and spawning grounds of many fish species (Wojtusiak 1950). These observations were confirmed in a wider study conducted in 1969/1970 (Klekot 1980a).

Unfortunately, despite the introduction of legal protection, at the end of the 1970s a complete change of the Bay environment was noted. The complete absence of underwater meadows built of *Fueus vesiculosus and Furcellaria fastigiata* was found, as well as a clear reduction in the occurrence of *Zostera marina* meadows. It was also noted that over 70% of the bottom surface was covered by filamentous macrophytobenthos of species *Fucophyceae: Pilayella litoralis* and *Ectocarpus siliculosus*. These changes significantly affected the species composition of invertebrate fauna and, to a large extent, destruction of feeding and spawning areas for fish (Błędzki and Kruk-Dowgiallo 1983, Pliński and Florczyk 1984). All the above changes resulted from intensive acquisition of sea resources (intensive fishing, industrial macroalgae exploration and bottom sedimentation) and an increase in the number of inhabitants of Puck County. As a consequence, the amount of wastewater discharged to the Bay increased. Flowing directly into the bay or entering rivers and small watercourses, raw wastewater introduced a significant load of nutrients, causing eutrophication, and thus upsetting the biological balance in Puck Bay. The discharge of domestic wastewater caused microbiological contamination of coastal waters and the need to close bathing areas and beaches in the 1980s (Andrulewicz and Janta 1997). In 1988, the first two-stage sewage treatment plant in this area was launched in Swarzewo (Wołowicz et al. 1993). Along with the change of the economic and political system in Poland in 1989, planned activities were started in order to reduce the inflow of pollution to the bay and the sea. Notable improvement was brought by cutting off the direct discharges, and due to the construction of a collector discharging treated wastewater from the Swarzewo WWTP in 1996. A 1.5 km long collector discharges wastewater to an open sea, where the water mixing is more dynamic, providing better dilution of pollutants. At present, the state of the environment is constantly improving, however, it has been noticed that the distribution of assemblies formed by long-lived, structural species such *as Zostera marina* and other higher plants is still in the phase of regeneration and recolonization, and their current distribution does not coincide with the favorable environmental conditions for their development (Węsławski et al. 2013).

Neighboring the Bay of Puck, the Baltic Sea is one of the most eutrophic seas in the world (European Court of Auditors, 2016, Wojciechowska, Pietrzak, et al. 2019), and in consequence faces many problems including algal and cyanobacterial blooms as well as the formation of oxygen deficient zones. This is caused by anthropogenic factors, mainly nutrients that drain from fields into the rivers and then flow into the sea. The main source of biogenic loads is agriculture (45% of the total nitrogen load and 45% of the total phosphorus load) (European Court of Auditors, 2016). International agreements and laws provide a certain level of protection for the Baltic Sea. Due to the unique natural values, as well as the tourist attractiveness of the region, it is necessary to further reduce the inflow of pollution currently coming mainly from adjacent agricultural catchments located in the Puck County, being drained by numerous rivers: the Reda, the Plutnica, the Gizdepka, the Potok Bladzikowski, and smaller watercourses and canals (HELCOM 2009, Kruk-Dowgiałło L 2008, Łysiak-Pastuszak et al. 2004, Zalewska et al. 2015). Many studies addressing the quality and potential threats to the Baltic Sea waters indicate a relatively slow improvement (HELCOM, 2015, HELCOM, 2018). Puck Bay is currently monitored in scope of a wide range of studies in order to indicate the main sources of pollution and their spread. Scientists indicate that emission reduction can be achieved by optimizing fertilizer use and changing cultivation methods (Dzierzbicka-Głowacka et al. 2019, Matej-Lukowicz et al. 2020, Wojciechowska, Nawrot et al. 2019, Wojciechowska, Pietrzak, et al. 2019). What is more, in 2015, a comprehensive draft of plans for the protection of Natura 2000 areas in the Gulf of Gdańsk and Vistula Lagoon area was prepared under the Infrastructure and Environment Operational Program, financed by the European Regional Fund EU (Michałek and Kruk-Dowgiałło 2015).

The presented study is in line with these activities, focusing also on small watercourses, which similarly may have a significant impact on the condition of the aquatic ecosystem of Puck Bay and the South Baltic Sea. A comprehensive protection program for the unique CLP area should be developed as soon as possible, which would additionally correspond to the priorities of the Regional Operational Program of the Pomeranian Voivodeship for 2014–2020 (ROP WP 2014–2020) funded by the European Union (EU) under the European Regional Development Fund (ERDF) and the European Social Fund (ESF). In 2015, a preliminary assessment of threats in the CLP area was made by conducting a basic physicochemical and sanitary analysis (number of *E. coli* bacteria) at selected measurement points. Based on preliminary observations, 40 measurement points were selected and the physicochemical and microbiological tests were extended. In cooperation with CLP management, the park area was divided into two regions – catchment areas of rivers: the Piaśnica, the Karwianka and the Czarna Wda flowing directly to the Baltic Sea (part I) and rivers and smaller watercourses flowing to the Bay of Puck (part II). The obtained results confirm the urgent need of the protection intensification for this area which would help to preserve both its ecosystem and touristic values. The analyzed watercourses flow into the Bay of Puck in the near vicinity of swimming areas and beaches frequently used in the summer season, therefore improvement of their quality should be of crucial importance. In addition, during the Covid-19 pandemic tourists more often choose to travel locally, within their own country, so this area may be even more intensively visited in the future. This paper discusses the analysis of the results of research conducted in 2016 in the area of part II, research of the second area (part I) is discussed in (Bączkowska et al., 2021).

Materials & Methods

Study area

The study area is located in northern Poland, on the southern coast of the Baltic Sea. Samples were collected mainly in Puck Bay drainage area (176 km^2) within Puck commune, where the average population density is 107 people per square kilometer. This area is mainly used for agriculture. Cropland covers 60% of the area, forests 29% and urbanized areas 11% (Wielgat et al., 2021). This study is focused mainly on small rivers in Puck commune and watercourses flowing through the Nature Reserve "Beka" (NRB), because small catchments respond much faster to pollutant inflow, which is especially important when agricultural use dominates and fertilizers are washed from fields with rain (Kyllmar et al., 2014). In addition, the Reda River also flows through NRB and its catchment area is over 600 km^2 and covers 13 different communes. This catchment is characterized by significant land-use diversity: industrial and storage areas, densely built-up urban areas, areas with touristic and recreational functions, but, similarly to other rivers in the study, forests (45.9%) and agricultural areas (47.5%) constitute the largest part of the catchment area of the Reda River (Hobot et al. 2021).

In this study, water samples of 9 small watercourses located in the Coastal Landscape Park (CLP) were collected at their estuaries. Sampling sites were selected in consultation with the CLP management board. The names of the points stand for an abbreviation of the river's name. Figure 1 shows the study area and location of the sampling points on the map. Figure 2 presents all the rivers and watercourses. All the watercourses end up in the internal part of Puck Bay, but some of the estuaries are located in the NRB and flow through marshy salty meadows and wetlands (Fig. 1, watercourses MC, BC, R and ZS). The urban canal, the one located in Wladyslawowo city (Fig. 1, watercourse WC), also has its estuary in Nature Reserve "Salty Meadows". Because of the character of the rivers flowing through the marshland, and therefore lack of access to the very outlet into Puck Bay, these rivers were sampled at their outflow to the wetland area. Nevertheless, the rivers spill into the wetland only during a heavy rain (the river bed and the riverbanks have been heavily modified). Usually they flow directly into the Bay. The rivers and canals selected for this study are listed in Table 1 together with their characteristics.

Sampling, laboratory and statistical analysis

Sampling was carried out in July 2016 on the area of Coastal Landscape Park (CLP), simultaneously with the sampling described in Part I (Bączkowska et al. 2021). Sampling points were located only at the estuaries.

Ion (anions and cations) concentrations were obtained by DIONEX ICS-3000 chromatograph (DIONEX, USA). Total organic carbon (TOC) was determined on a Total Organic Carbon Analyzer TOC-VCSH/CSN (Shimadzu, Japan). Physicochemical parameters: temperature, electrical conductivity (EC), dissolved oxygen concentration (DO) and pH were measured in situ using portable multifunctional measuring device ELMETRON CX-561 (Elmetron, Poland) with a set of appropriate electrodes. Chemical oxygen demand (COD) was determined using UV-VIS spectrophotometer (Merck, Germany). Technical details are specified in Part I (Bączkowska et al. 2021).

Microbiology analyses included the cultivation of fecal indicator bacteria (fecal coliforms, including *E. coli*) and microscopic observations to determine the abundance and physical characteristics of the prokaryotic community present in the water samples. Detection and enumeration of fecal coliforms were carried out according to ISO 9308-1:2014, using membrane filtration. 1 and 10 ml samples were filtered on cellulose membrane filters (47 mm diameter, 0.45 µm pore diameter, Whatman, Germany) and incubation on mFC agar (Merck, Germany) at 44°C for 24 h. Blue colonies were counted as fecal coliforms.

Table 1. Characteristics of rivers and canals in the sampling points.

Sampling point		River/Canal	Catchment area	Flow rate	Distance between the sampling point and the coastline	References
WC		Wladyslawowo Canal	no data	no data	0.30 km	–
PI		Plutnica River	84.0 km^2	0.45–0.70 m^3/s	0.40 km	[1,6]
PC		Puck Canal	no data	no data	0.50 km	–
BS		Bladzikowski Stream	23.0 km^2	0.04 m^3/s	0.21 km	[2,6]
G		Gizdepka River	31.5 km^2	0,18–0.25 m^3/s	0.05 km	[3,6]
Rivers/canals located in the NRB	MC	Mrzezinski Canal	16.5 km^2	0.02 m^3/s	0.05 km	[4]
	BC	Beka Canal	no data	no data	0.80 km	–
	R	Reda River	485.5 km^2	4.44–5.71 m^3/s	0.90 km	[5]
	ZS	Zagorska Stream	no data	no data	0.90 km	–

[1] (Kalinowska, Wielgat, Kolerski, 2020, Wojciechowska, Nawrot, et al., 2019)
[2] (Wojciechowska, Nawrot, et al., 2019)
[3] (Kalinowska, Wielgat, Kolerski, 2020, Wojciechowska, Pietrzak, et al., 2019)
[4] (Wojciechowska, Pietrzak, et al., 2019)
[5] (Wojciechowska, Nawrot, et al., 2019)
[6] (Wielgat et al., 2021)

Microbiological parameters such as total prokaryotic cell number (TCN), average prokaryotic cell volume (ACV), prokaryotic biomass (PB) and prokaryotic cell morphotype diversity were determined using the direct epifluorescent filter technique (DEFT).

Sampling, laboratory and analytical procedures were described in detail according to the scheme (Fig. 3) in Bączkowska et al. 2021.

The PCA for this study was performed using Rstudio v.1.3.9., with the factoextra package and prcomp function. Figures were edited in CorelDRAW X7. Statistical procedures regarding image analysis in microscopic analysis were described in detail in Part I (Bączkowska et al. 2021).

Results

Chemical analysis

Temperature, DO, TOC and COD

The research was conducted in July 2016. The air temperatures were in the range 16–23°C, which is characteristic for this region – an average temperature in July for years 2009-2015 was 17.8°C (IMGW data, 2009–2015). However, during the sampling campaign, an unusually high rainfall was recorded: monthly rainfall in July was 200 mm, which was 260% of the long-term norm of precipitation for this region (IMGW data, 2016). Water temperature in the tested tributaries ranged from 15.1°C (Bladzikowski Stream, BS) to 20.2°C (the Beka Canal,

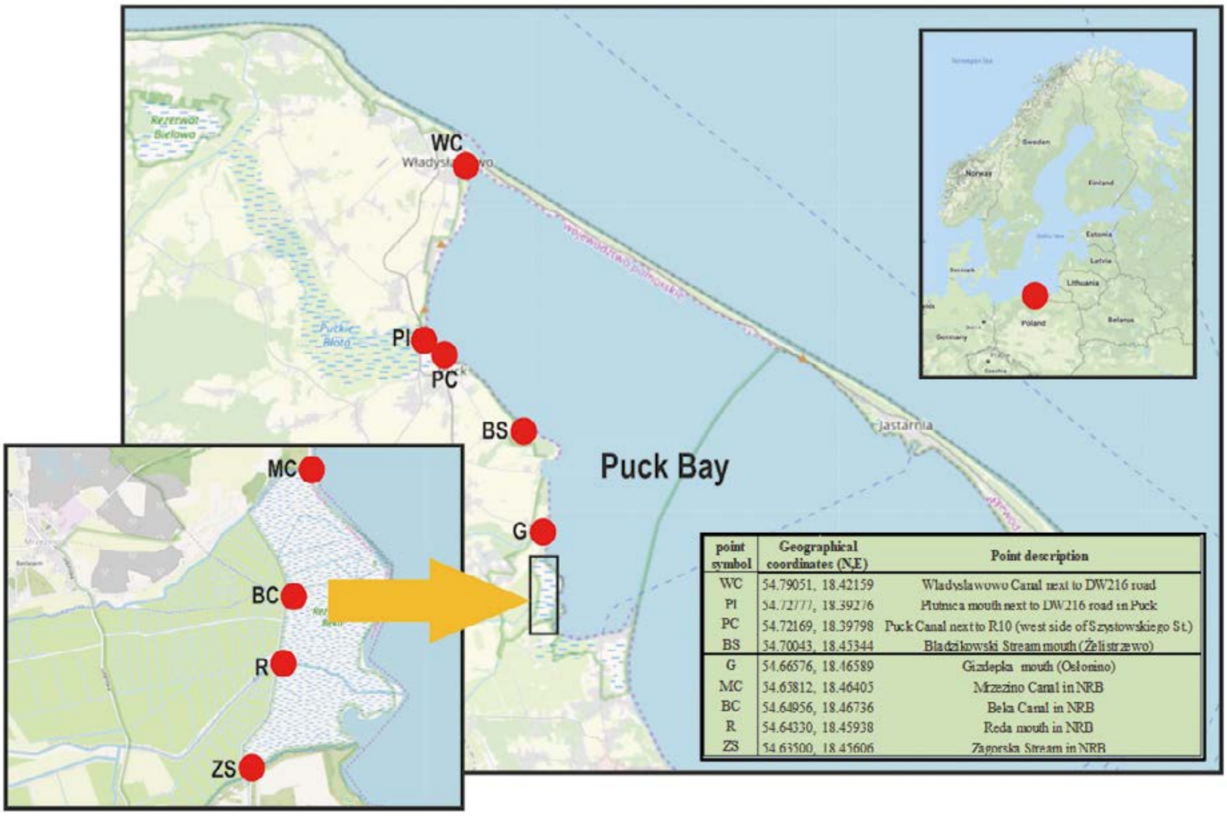

Fig. 1. Research area and distribution of measurement points on the map

Fig. 2. Rivers and canals in the sampling points: a) Wladysławowo Canal (WC), b) Plutnica River (PI), c) Puck Canal (PC), d) Bladzikowski Stream (BS), e) Gizdepka River (G), f) Mrzezinski Canal (MC), g) Beka Canal (BC), h) Reda River (R), i) Zagorska Stream (ZS).

BC) (Tab. 2). The pH values ranged from 7.0 to 7.8, with the lowest pH recorded at PC (Puck Canal) and the highest at WC (the Wladyslawowo Canal).

Dissolved oxygen concentration was highly variable depending on the stream. The highest DO value was recorded in the Władysławowo Canal (WC), which equaled 9.7 mg/L. Values above 9 mg/L were also recorded for the Plutnica River (Pl) and the Gizdepka River (G). In samples from the Bladzikowski Potok (BS), the Reda (R) and the Beka Canal (BC) oxygen concentration ranged from 7.2 to 8.6 mg/L. The lowest values were recorded in a small canal in Puck (PC, 3.0 mg/L) and in small canals in the Nature Reserve "Beka" (ZS: 3.3 mg/L and MC: 0.7 mg/L) (Tab. 2).

Total organic carbon (TOC) concentrations varied between the samples. The lowest values were recorded in samples from BS, Pl and PC and did not exceed 4 mg/L. The analysis of WC, R and G samples showed values more than two times higher. Values above 10.0 mg/L were recorded in BC samples. ZS and MC streams revealed the highest TOC values equal to 12.3 mg/L and 13.8 mg/L, respectively (Tab. 2).

Chemical oxygen demand (COD) in the analyzed samples was proportional to the TOC. Notably higher values of both parameters were observed in the samples of water taken in the NRB and its buffer zone. At all points, COD values were higher than 50.0 mg/L. The highest chemical oxygen demand was recorded in the water samples with very low values of DO: MC and ZS (84.0 mg/L and 69 mg/L COD, respectively). On the contrary, the PC water sample where dissolved oxygen concentration was equally low, COD value was one of the lowest. The lowest COD values were recorded in Pl and BS and equaled 14.0 mg/L and <10 mg/L, respectively (Tab. 2).

In the WC water sample, where the concentration of sodium, potassium, chloride and sulphate was the highest, also the highest conductivity was recorded (548 μScm^{-1}). A slightly lower EC value was reached by the water sample Pl (470 μScm^{-1}). Conductivity in PC, G and BS samples was above 350 μScm^{-1}, and among the water samples taken in the Nature Reserve "Beka" most of the results did not exceed 265 μScm^{-1} (Tab. 2).

Ions and biogenic compounds

The cations and anions analysis revealed only trace amounts of elements such as lithium and bromine in only several samples (below 0.1 mg/L (WC, PC) and 0.01–0.2 mg/L (WC, Pl, BS, MC, respectively, Tab. 3).

Potassium was detected only in WC and PC samples. Small amounts of fluorine were detected in all tested samples and its concentration ranged between 0.17–0.36 mg/L. The presence of magnesium and calcium ions was observed in WC, Pl and PC samples. Mg^{2+} ion concentration in these samples did not exceed 13.0 mg/L, while Ca^{2+} ion concentrations were in the range 25.8–42.6 mg/L. Also, in the samples collected at these points sodium ion was detected, despite the fact that chlorides were detected in each of the analyzed samples (suspected presence of NaCl due to the proximity to the marine estuary). The chloride ion concentration ranged from 6.44 mg/L in the R sample to 35.7 mg/L in the WC sample. Also, high values of chloride were observed in Pl, PC and BS samples – between 16.1–21.9 mg/L. In almost every sample, the highest concentration among all ions was presented by the sulfate ion. Its highest value was recorded in the WC sample and equaled 54.7 mg/L (Tab. 3).

Ammonia concentrations ranged from 0.07 to 2.42 mg/L among all samples except Pl, where it was not detected. The highest value was recorded in the PC water sample. Five times lower ammonia concentration was observed in the WC sample. The lowest value was recorded in the BS sample. Among the samples from the NRB the ammonia concentrations did not exceed 0.14 mg/L (Tab. 3).

Nitrites were detected only in three samples: PC, R and BC and the concentrations did not exceed 0.5 mg/L. Nitrates were

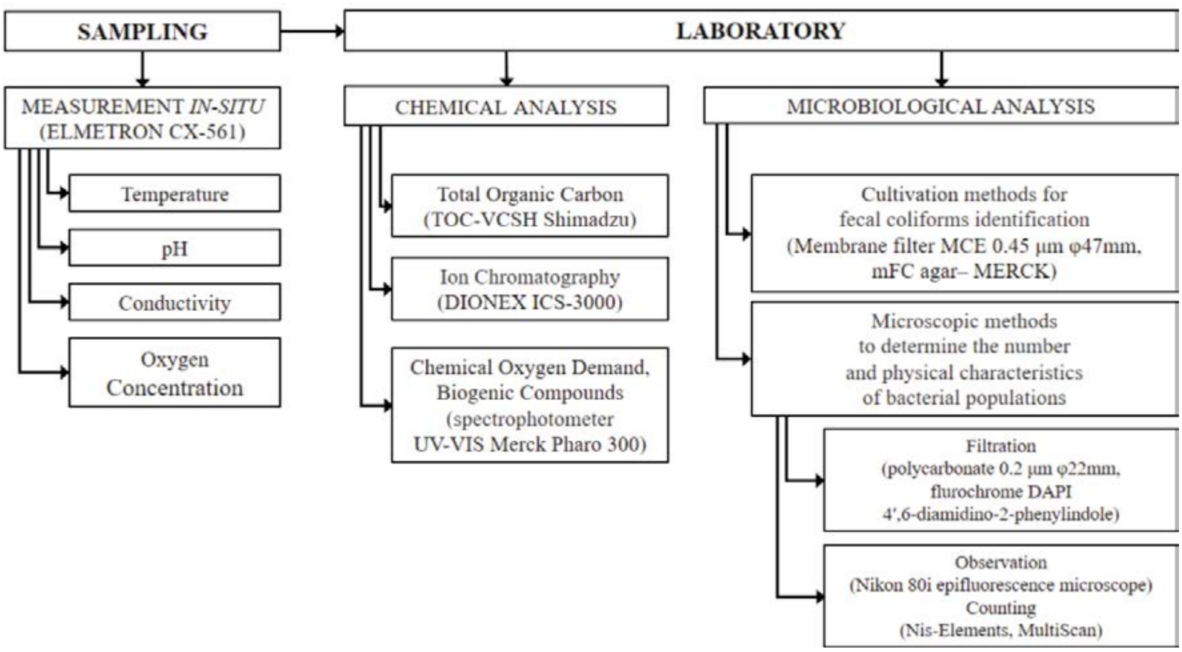

Fig. 3. Schematic diagram showing *in situ* measurements and laboratory analyses

observed in all of the collected samples. The highest N-NO_3^- concentration was recorded at point G (1.57 mg/L). In other samples from the NRB the nitrate concentrations were lower and ranged from 0.33 to 0.52 mg/L. Only nitrate in the MC sample showed lower value (0.02 mg/L). Equally low nitrate concentration was observed in point Pl (Tab. 3).

Phosphate phosphorus concentrations in water samples taken outside the Nature Reserve "Beka" did not exceed 0.41 mg/L, but these points presented higher values than the water samples collected in the NRB. In these points the value of P-PO_4^{3-} ranged from 0.18 to 0.24 mg/L. An exception was the MC sample, where the highest value was observed (0.53 mg/L, Tab. 3).

Microbiological analysis

Cultivation methods (fecal coliforms determination)

The highest average value of fecal coliforms (FC) was found at PC and BC points (11.8×10³ CFU/100 mL and 5.8×10³ CFU/100 mL, respectively). In water samples taken in the Nature Reserve "Beka" the average number of fecal coliforms ranged from 0.08×10³ CFU/100 mL in the water sample taken at point ZS to 0.6×10³ CFU/100 mL at point MC. The lowest average value of fecal coliforms counted among water samples from outside the NRB was recorded at point Pl and it was equal to 0.07×10³ CFU/100 mL. In the WC sample twice as high average value of fecal coliforms was observed. In the BS water sample this amount was equal to 0.41×10³ CFU/100 mL (Fig. 4a).

Table 2. Results of in situ measurements: average temperature (T), electrical conductivity (EC), pH and dissolved oxygen (DO), as well as total organic carbon (TOC) and chemical oxygen demand (COD). Blue, green and yellow fields indicates rivers' classification (explained in discussion).

point symbol	JCW	T	EC	pH	DO	TOC	COD
	types	[°C]	[μS/cm]	[–]	[mg/l]	[mg/l]	[mg/l]
WC	0	19.7	548	7.8	9.7	6.30	40
Pl	23	16.5	470	7.6	9.3	3.89	14
PC	0	15.9	362	7.0	3.0	3.96	27
BS	17	15.1	437	7.8	8.6	2.12	<10
G	17	15.5	380	7.6	9.2	9.81	58
MC	23	19.7	230	7.1	0.7	13.80	84
BC	17	20.2	261	7.3	7.2	10.70	64
R	22	18.2	263	7.4	7.5	8.63	54
ZS	17	19.0	336	7.2	3.3	12.3	69

Table 3. Concentrations of cations and anions determined by ion chromatograph

point symbol	JCW	N-NH_4^+	N-NO_2^-	N-NO_3	P-PO_4	Li^+	Na^+	K^+	Mg^{2+}	Ca^{2+}	F^-	Cl^-	Br^-	SO_4^{2-}
	types	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]
WC	0	0.52	<LOD	0.29	0.39	0.100	12.90	4.55	9.10	40.40	0.169	35.70	0.174	54.7
Pl	23	<LOD	<LOD	0.06	0.23	<LOD	5.43	<LOD	9.39	42.60	0.357	16.10	0.025	33.6
PC	0	2.42	0.50	0.42	0.34	0.098	5.95	2.07	5.62	25.80	0.357	21.90	<LOD	40.9
BS	17	0.07	<LOD	0.52	0.41	<LOD	<LOD	<LOD	<LOD	<LOD	0.181	19.30	0.026	48.1
G	17	0.13	<LOD	1.57	0.19	<LOD	<LOD	<LOD	<LOD	<LOD	0.159	12.60	<LOD	36.8
MC	23	0.11	<LOD	0.02	0.53	<LOD	<LOD	<LOD	<LOD	<LOD	0.215	7.18	0.011	21.8
BC	17	0.14	0.20	0.33	0.18	<LOD	<LOD	<LOD	<LOD	<LOD	0.182	12.30	<LOD	31.9
R	22	0.12	0.08	0.52	0.21	<LOD	<LOD	<LOD	<LOD	<LOD	0.168	6.44	<LOD	25.0
ZS	17	0.10	<LOD	0.40	0.25	<LOD	<LOD	<LOD	<LOD	<LOD	0.224	13.20	<LOD	45.3

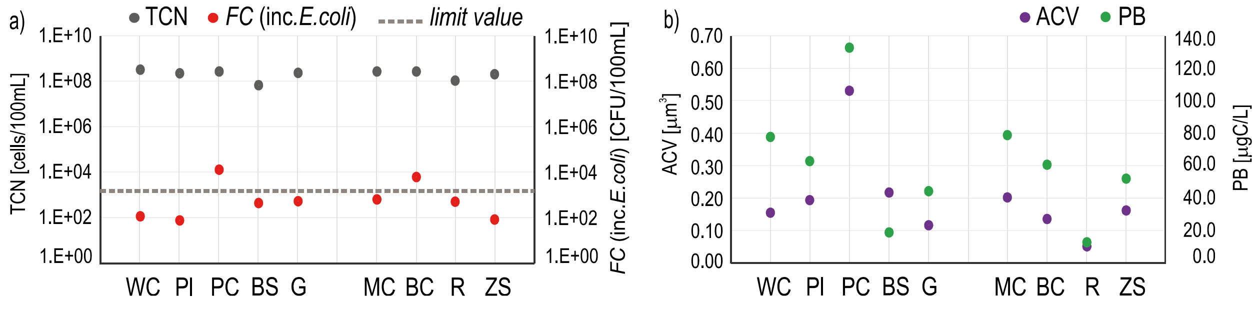

Fig. 4. Results of microbiological analysis (average value) a) total prokaryotic cell number (TCN) and abundance of fecal coliforms, b) average cell volume (ACV) and prokaryote biomass (PB).

Microscopic methods

The average value of the total number of prokaryotic organisms (TCN) was the highest at the WC point and was 3.01×10^8 cells/100 mL. Values around 2.5×10^8 cells/100 mL (±0.05) were observed at PC, MC and BC. Smaller TCN average values (2.0×10^8 cells/100mL) were recorded in water samples Pl, G and ZS. In point R the average value of TCN was equal to 1.01×10^8 cells/100 mL and the lowest total number of prokaryotic organisms was observed in the BS sample (Fig. 4a).

The largest cells were observed in the PC water sample (Fig. 4b). The average volume of prokaryotic cells in a sample of water from the Puck Canal was 0.53 μm^3. The highest prokaryotic biomass (132.4 μg C/L) was also recorded at this point. Twice smaller cells were observed at BS and MC points (0.22 and 0.20 μm^3). However, due to five times more numerous TCN in MC point, the average value of prokaryotic biomass in this sample equaled 80 μg C/L, while the PB in the BS sample was around four times lower (18.4 μg C/L). Average cell volume in the remaining samples did not exceed 0.2 μm^3. Nevertheless, the PB values for these samples ranged between 40–80 μg C/L due to the prokaryotic community size (TCN). The smallest cells were observed in water samples from R points – prokaryotic cells showed an average volume of 0.05 μm^3. The smallest average value of biomass was also recorded in sample R (12.4 μg C/L).

When analyzing the morphological structure of the prokaryotic community detected under the microscope, it could be observed that in ZS cocci and rods of various sizes were present and larger cells had a significant share in the biomass recorded for this sample (Fig. 5b), so the final value of this parameter was high (av. 51.55 μg C/L). A similar ACV value was observed at BC, WC and G points, where the smallest rods (<0.1 μm^3) were the most numerous among the morphotypes (Fig. 5a). At BC and G points, the share of individual cell sizes in the prokaryotic community size was almost identical. Prokaryotic biomass was however higher for BC because of a more numerous bacterial community. In the WC sample, where the total number of prokaryotic cells was the highest, also an increased amount of medium size and large cocci was observed, if compared to BC and G samples (Fig. 5a). Therefore, also a higher biomass was recorded at this point, because these medium and large cells had a significant impact on prokaryotic biomass (Fig. 5b). ACV for MC and Pl samples was very similar. Analyzing the share of particular cell sizes among morphological structures, it could be seen that in both samples the prokaryotic community consisted of a similar number of cells of the same size. Therefore, the higher value of biomass at MC point results from bigger TCN.

A different trend was observed for the PC sample, where the TCN value was almost identical to the MC point, whereas the largest share of large and medium (0.2–1.0 μm^3) and largest (>1.0 μm^3) cocci was observed among all the analyzed samples. Very few smallest rods (<1.0 μm^3) were found at this point, which also distinguishes this sample from the others. As in no other sample, medium to largest cocci and rods dominated among the other morphotypes and highly influenced prokaryotic biomass value in PC samples. Therefore the biomass value in the sample PC was more than two times higher than most of the remaining samples. Large abundance of large cells also influenced ACV value at this point, resulting in the highest ACV among the samples.

Discussion

Over the past century, the supply of nutrients has been particularly noticeable in the Baltic Sea basin area (Gren 2017, Elofsson 2003, Wulff et al. 2014). Eutrophication is considered to be the most serious ecological problem for surface waters in the entire Baltic Sea basin (Ducrotoy and Elliott 2008, Artioli et al. 2008, Lundberg 2013). High concentrations of nitrogen and phosphorus compounds contribute to algal blooms, which in turn decreases water quality. A particularly dangerous phenomenon are the blooms of potentially toxic cyanobacteria, which pose a health threat to the people on the beaches and swimming areas in the waters of the Baltic Sea (Diaz and Rosenberg 2008, Vahtera et al. 2007).

Nowadays, in many countries, the biggest source of water pollution is agriculture, not cities or industries (FAO, 2018).

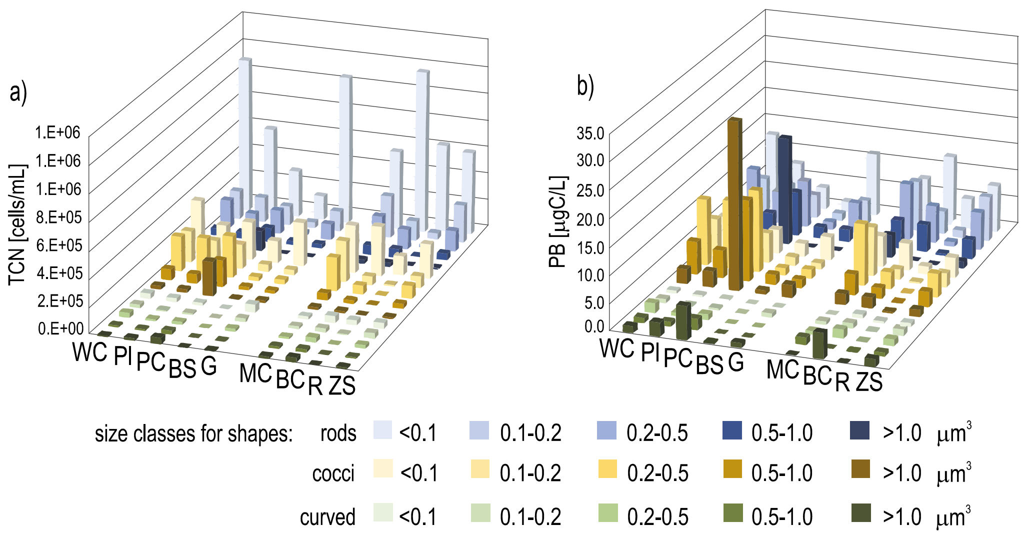

Fig. 5. Results of microbiological analysis a) total number of cells (TCN) for each size and shape class, b) prokaryotic biomass (PB) for each size and shape class.

To counteract increasing water pollution across the EU, in 2000 the European Commission adopted the Water Framework Directive (WFD). The WFD requires integrated management of water resources across the European Union and obliges Member States (including Poland) to ensure that all surface water bodies will be in ‚good' ecological status by 2027 at the latest. Since 2003 the Polish legislation has been in line with the requirements of the Water Framework Directive (2000/60/C) and in 2016, at the time of the research, a new regulation came into force under which rivers can be classified in detail and their ecological potential assessed.

Thus, it has become necessary to develop a water resources quality monitoring program. Regular monitoring and assessment of water quality is essential for proper management of water resources to improve the environment. Therefore, there is a need for constant monitoring and assessment of surface water quality. Long-term and periodic studies are an excellent source of information for water resource management and to protect waters from pollution.

This paper presents the results of chemical and microbiological analysis of nine rivers and channels flowing directly into Inner Puck Bay (the Puck Lagoon). The study was conducted in July 2016. The Polish government at that time worked intensively to improve the legal standards for assessing the status of water bodies (in Polish JCW – jednolite części wód). The assessment of the current ecological status or potential and the assessment of the risk of not achieving the environmental goals was carried out. There was also introduced an update of the Vistula River Basin Management Plan (Council of Ministers 2016a). This basin was extensively characterized and divided into 4 regions. The rivers discussed in this study are located in the fourth area defined as „Lower Vistula River Water Region covering the catchment of the Vistula River from Korabnik to the mouth and the catchment of Pomeranian rivers". The plan presents a list of surface water bodies in the area of the Vistula River basin, which was given codes and classified according to types with categories in accordance with the Regulation of the Minister of Environment on the classification of ecological status, ecological potential and chemical status of surface water bodies (Council of Ministers 2016b). The classification and assessment of the ratings for the rivers discussed in this study are presented in Table 4. The table also indicates the measurement points selected for analyses in this study.

During the sampling campaign in 2016, the previous Regulation of the Minister of Environment (Council of Ministers 2014) was in force. The existing regulation did not link the permissible values of individual parameters to the detailed classification of water bodies. However, a new regulation came into force a few days after the end of the study, which enforced such a differentiation (Council of Ministers 2016a). Therefore, this study refers to these classifications (see Table 5). In the result section, the values in the tables are referred to the limit values and marked with the same colors. Blue color indicates that the requirements for the 1st quality class are met, green color indicates that the requirements for the 2nd quality class are met, and yellow color indicates that the limit values are exceeded. Lack of color means that for a particular watercourse type the limit values of some parameters are not determined.

The monitoring of water quality is also important in modelling water resources (Massoud, 2012) and the distribution of pollutants, source location, and health hazards, in addition to protecting water resources and controlling water pollution (Hong et al., 2020). As presented by Rinke et al. (2013), the monitoring of short-term physicochemical parameters at the catchment scale is very important. The authors note that high-frequency data provide deeper insight into river-basin ecosystem dynamics. Effective surface water management

Table 4. Classification and rating of the analyzed rivers.

Typology JCW	Code JCWP	Sampling points	Status JCW	Current ecological status or potential	Assessment of the risk of not reaching the environmental goals
0	–	WC	–	–	–
23	PLRW20002347749	PI	natural	bad	threatened
0	–	PC	–	–	–
17	PLRW20001747752	BS	artificial water body	good	not threatened
17	PLRW2000174776	G	heavily modified water body	bad	threatened
23	PLRW2000234778	MC	artificial water body	bad	threatened
17	PLRW20001729888	BC	heavily modified water body	bad	threatened
22	PLRW20002247899	R	heavily modified water body	bad	threatened
17	PLRW20001747929	ZS	heavily modified water body	good	not threatened

JCW – „jednolite części wód" – water bodies
JCWP – „jednolite części wód powierzchniowych" – surface water bodies
0 – An unspecified type of watercourse, including canals
17 – A sandy lowland stream
22 – A stream or estuarine stream influenced by saltwater
23 – A stream in a valley with a high proportion of peatlands

depends primarily on a range of consistent data relating to surface water quality (Nazeer et al., 2016).

Currently some numerical models are being developed and simulations forecast an increase in nutrient supply in the future (Piniewski et al. 2014, Arheimer et al. 2012, Meier et al. 2012, Woelgat et al., 2021). Such projects are also carried out to indicate the need for changes in agriculture and farming practices, as well as in water and sewage management. It was shown long ago that, e.g., manipulation of livestock feeding improves the efficiency of nitrogen (N) use by animals, reducing N excretion and thus the risk of N losses to water bodies (Luo et al., 2008). Also, the use of cover crops can be very effective in reducing N losses (Hooker et al., 2008). It is also necessary to monitor the water quality of rivers flowing into coastal regions of the Baltic Sea. Many research studies cover both large rivers like the Vistula and the Odra (Pastuszak et al., 2018), as well as smaller, local watercourses (Kalinowska et al. 2020, Saniewska et al. 2019, Wojciechowska, Pietrzak, et al. 2019). Studies of smaller watercourses show their significant impact on the coastal waters state. However, most of the research focuses on rivers and streams, while the influence of small canals located in cities, agricultural areas or sensitive areas is usually ignored. Nevertheless, it seems that the length and size of the watercourse matters a lot for the catchment areas of the rivers flowing into the Bay of Puck. In areas of high agricultural use, small watercourses and canals may contribute large amounts of nutrients to the South Baltic waters due to the fact that the retention time of pollutants in these streams is too short and self-purification processes are probably not that effective (Wojciechowska, Pietrzak et al., 2019). Therefore, in such a particularly sensitive region as the CLP, monitoring and protection of water resources is extremely important.

Summary of chemical Water Quality Parameters and Principal Component Analysis

Water quality is determined by its physical, chemical, and biological parameters and is usually described in terms of certain criteria and standards. The water quality of a water body determines its potential uses. The factors that affect water quality are complex and are assessed on the basis of many indicators ranging from water temperature, pH and chemical constituents to biological and microbiological parameters (Novotny, 2003).

According to previous Polish regulations (Council of Ministers 2014) the water quality in the river was assessed as good if DO concentration exceeded 5.0 mg/L. According to the new regulation (Council of Ministers 2016b), this value should not be lower than 6.2–6.8 mg/L (Tab. 5). In watercourses flowing through the Nature Reserve „Beka" (group I), dissolved oxygen differed between the watercourses. In the Beka Canal (BC) and the Reda River (R) DO concentration met the standards while the other streams did not. Particularly low DO was recorded in the Mrzezinski Canal (MC, 0.7 mg/L), which did not meet any of the standards. WC and PC canals also differed notably. In the WC the oxygen concentration was high while in PC it was far below the norm for the 2nd water quality class (limit value for unclassified canals >5.0 mg O_2/L).

TOC in aquatic ecosystems includes all kinds of organic compounds of plant origin as well as waterborne bacteria and microorganisms (biomass) (Kozak et al. 2017). Nevertheless, the increase of TOC concentration in surface waters may indicate environmental pollution caused by human activities. COD also has a significant meaning in environmental studies because of its ability to indicate the degree of pollution of water (Kalenik 2014, Ngang and Agbazue 2016). TOC concentration exceed the limit value (11.8 mg/L for '17' JCW type, Tab. 5) only at one point – ZS, the watercourse assessed as good in the regulation. However, it should have been noted that notably higher TOC values (10.1–13.8 mg/L) were recorded in rivers and canals flowing through the NRB when compared to other watercourses. TOC concentrations were also higher than the average values recorded in the Vistula River (7.0 mg/L) which was considered a significant source of organic pollutants introduced into the Baltic Sea (Górniak 2017). Moreover, COD

Table 5. Limit values for each water quality class for different types of streams and rivers

Type:	17		22		23	
Class:	I	II	I	II	I	II
T	≤22.0	≤24.0	≤22.0	≤24.0	≤22.0	≤24.0
pH	7.0–7.9	7.0–7.9	7.4–8.2	7.2–8.4	7.2–8.3	7.0–8.3
EC	≤549	≤620	≤440	≤2814	≤454	≤576
COD	≤25	≤30	≤25	≤30	≤68	≤79
TOC	≤10.0	≤11.8	≤10.0	≤14.8	≤18.8	≤21.4
DO	≥7.5	≥6.8	≥7.1	≥6.5	≥7.3	≥6.2
SO_4^{2-}	≤42.0	≤57.0	≤45.9	≤114.7	≤35.2	≤64.8
Cl^-	≤26.0	≤33.7	≤37.0	≤499.0	≤10.8	≤29.4
Ca^{2+}	≤81.0	≤81.7	≤59.4	≤64.2	≤64.3	≤71.7
Mg^{2+}	≤18.4	≤22.0	≤7.3	≤40.4	≤5.8	≤10.1
N-NH_4	≤0.25	≤0.738	≤0.34	≤1.00	≤0.34	≤0.68
N-NO_2	≤0.01	≤0.03	≤0.01	≤0.03	≤0.01	≤0.03
N-NO_3	≤2.2	≤3.4	≤0.5	≤0.9	≤1.3	≤2.5
P-PO_4	≤0.065	≤0.101	≤0.065	≤0.101	≤0.065	≤0.101

values in all the NRB watercourses as well as in the Gizdepka River (G) located nearby, exceeded the acceptable standards (30 mg/L) (Council of Ministers 2016b) almost twice. Even in the case of the Mrzezinski Canal, which is a different type of stream (JCW type '23', Tab. 5) and has a lot higher limit value (79 mg/L), the results did not meet the requirements. In the watercourses from the second group, located outside NRB, COD did not exceed 15 mg/L. In the urban canals the values were much higher (PC – 27 mg/L and WC – 40 mg/L), but limits were not established for that type of water bodies. Nevertheless, COD values are indicators of organic substances availability in the water that is why in canals flowing into the Bay of Puck this parameter should result in lower values.

Cations and anions analysis did not indicate the limit values to be exceeded. The highest concentrations of most of the ions were recorded in the Władysławowo Canal (WC). The highest water conductivity was also noted there. In all the analyzed watercourses, relatively high concentrations of sulphate ions (SO_4^{2-}) but at most sampling points the water met the standards for the 1st quality class according to this parameter. Despite the coastal character of the sampling points and the estuaries being adjacent to salt marshes, chloride concentrations were relatively low.

In contrast, phosphorus concentrations exceeded the limit at all locations. An increased phosphorus and nitrogen influx to the aquatic ecosystems from point and diffuse sources is recorded worldwide (Bricker et al. 2008, Russell et al. 2008). Some watercourses located within the CLP are receivers for treated wastewater, but point sources of pollution, such as outflows from wastewater treatment plants, were not located in the studied area. These are typically agricultural areas, and agricultural production is strongly linked to nutrient emissions to rivers (Castaldelli et al. 2015, Zalidis et al. 2002). Phosphate phosphorus ($P\text{-}PO_4^{3-}$) concentrations exceeded the limit value (0.101 mg/L) at each of the studied points and did not meet the standards for the 2nd quality class. The largest $P\text{-}PO_4^{3-}$ concentrations were observed in the Mrzezinski Canal (MC), the Puck Canal (PC) and Bladzikowski Stream (BS). Similar tendencies were observed in studies carried out within the WaterPUCK project, (Wojciechowska, Nawrot, et al. 2019), where exceeded norms were noted also for the Bladzikowski Stream, the Płutnica River (Pl) and the Reda River (R). Also various forms of nitrogen in the studied watercourses were analyzed. Most rivers and canals met standards even for the 1st quality class with respect to N. The alarming value of ammonium nitrogen ($N\text{-}NH_4^+$) was recorded in the Puck Channel (2.42 mg/L). This watercourse is not classified to any type of water bodies, so it is impossible to define its quality class precisely, however, taking into account the limit values included in the regulation, it can be definitely stated that such a result significantly exceeds the standard. Our observations were also confirmed by the research conducted in the frame of WaterPUCK project (Wojciechowska, Nawrot, et al. 2019).

According to the Polish Standard for surface waters (Council of Ministers 2016b), rivers are classified as of poor quality when at least one parameter exceeds the limit values. The results from this study indicated that all the examined watercourses in their estuarial fragments did not meet the 2nd class quality requirements for surface waters and therefore might introduce significant loads of pollutants into Puck Bay, which is also confirmed by other researchers (Wojciechowska, Pietrzak, et al. 2019, Zaborska et al. 2019)

Since the wastewater management in coastal localities was upgraded, the water quality in Inner Puck Bay has improved considerably. However, data on the water status of Inner Puck Bay obtained from the State Environmental Monitoring since 2010 indicate that in 2010–2018 the ecological status and chemical status of Inner Puck Bay waters oscillated mostly between poor and poor (Michałek et al., 2021). The Puck Lagoon is a shallow water body where water exchange is difficult due to its location and characteristics, therefore the inflow of pollutants from external sources, including first of all nutrients intensively used in agriculture, should be limited. In recent years an increasing abundance and biomass of blue-green algae (Cyanobacteria) has been observed, especially in the summer season. The reason for this phenomenon can be found in nutrients flowing into the waters of the Puck Lagoon. In order to protect this area which is particularly sensitive to anthropogenic factors, all the efforts should be made to ensure that all the water bodies flowing into the Bay meet the standards for the first class of water quality. Only then the waters of the Puck Lagoon would also have a chance to be classified as a water of the highest quality, and this is not easy due to the even more restrictive standards of the first class for this water body (Council of Ministers, 2016b, Tab. 6).

The obtained results confirmed the authors' previous observations, which indicated significant improprieties in the physicochemical quality of water. In 2015 during the preparation for further research, it was found that as a result of extreme exceedances of TOC concentration (8–30 mg/L), PO_4^{3-} ions (2.9–5.3 mg/L) and NH_4^+ ions (0.5–5.0 mg/L), water in all watercourses should be classified far below the requirements for the 2nd class of water quality. Therefore, the study was extended in 2016, and showed that the rivers and canals were still under strong anthropogenic influence and monitoring should be continued in order to achieve the environmental goals.

Table 6. Limit values for surface water quality classes of selected indicators of surface water quality for water bodies of transitional surface water types in the Puck Lagoon

Parameters \ Quality class		I	II
pH	–	7.0–8.0	8.0–8.8
DO	[mg/l]	>6.0	>4.2
TOC	[mg/l]	<5	<10
$N\text{-}NO_3$	[mg/l]	<0.007	<0.011
$P\text{-}PO_4$	[mg/l]	<0.02	<0.03

According to Ling et al. (2017) a monitoring of surface water quality in large river catchments can generate a huge data set. Therefore, a form of multivariate statistical analysis such as principal component analysis (PCA) is useful for assessing variability in river water quality. PCA provides information on the most significant parameters that describe the entire data set, allowing data reduction with minimal loss of original information (Helena et al., 2000, Shrestha & Kazama, 2007). It is a powerful pattern recognition technique that attempts to explain the variance of a large set of interdependent variables and transform them into a smaller set of independent variables (Kumar et al., 2014).

According to principal component analysis (PCA, Fig. 6a,b), over 60% of the dataset variability was explained by two principal components. Dim1, explaining 39.3% of samples' variability, was mostly shaped by chemical parameters as well as microbiological parameters. TOC and COD showed common trend and were opposed to electrical conductivity and presence of the related ions. Dim1 mainly separated two groups of points located within and outside the Nature Reserve "Beka" (Fig. 2, Fig. 5 and Fig. 6). Dim2, explaining approximately 25.8% of the total variance, was also defined by pH and DO values, which were defined as vectors acting almost opposite to fecal coliforms. Nutrients (N-NO_3^- and P-PO_4^{3-}) played a less significant role in explaining the total variance among all the samples. All the microbiological parameters correlated relatively closely and positively with NH_4^+, and were the highest in the case of the Puck Canal. Another urban canal, located in Wladyslawowo municipality (WC) was characterized by the highest concentrations of most of the ions, especially Na^+, Cl^-, Br^- and SO_4^{2-}, and thus the highest conductivity.

The obtained results allowed to divide tested watercourses into three groups according to their location, which was confirmed by the PCA. In particular watercourses located in the Nature Reserve "Beka" (MC, BC, R, ZS) revealed similar characteristics and they were likely to be grouped together (Fig. 6a, samples marked with yellow font, group I). It was worth noting that points BC, R and ZS were not directly located on the seashore but several hundred meters upstream, at the boundary of salty meadows of the NRB. Therefore, the chemical composition of the water actually discharged to Puck Bay could be a bit different from that presented in this study as wetland can significantly change the water quality, e.g., in terms of humic substances presence. This can consequently cause anaerobic conditions in rivers and canals (Duan et al. 2017), which was confirmed in this study. DO concentrations reported at sampling points in the NRB were among the smallest reported in our study. This has been also reflected on the PCA plot (Fig. 6).

In order to prevent reed overgrowth, cattle grazing in the NRB is carried out which might be the source of both organic matter and fecal contamination. This could be reflected by the high values of fecal coliforms at point BC. All the samples from the NRB were also characterized by the highest TOC and COD values. The Mrzezino Canal (MC) combined the lowest DO with the highest TOC, COD and P-PO_4^{3-} concentrations among all the samples, however this can also mirror the stagnant water conditions and the drainage character of the watercourse.

The relation between COD and TOC is usually proportional, however COD reached values higher than TOC. This could be explained by the fact that TOC corresponded to the amount of carbon bound to organic compounds (Serajuddin et al. 2018), whereas chemical oxygen demand referred to the amount of oxygen consumed for the organic and inorganic compounds oxidation.

All the rivers (the Plutnica – Pl, the Bladzikowski Stream – BS, the Gizdepka – G and the Reda – R) showed somewhat similar characteristics to the first group, but were also likely to be grouped together (group II, Fig. 6a) due to their greater water flow and larger cross-section in the rivers. The Reda River (R), flowing through the Nature Reserve "Beka", could be influenced by similar factors as streams from the first group, and therefore it showed the highest resemblance to them (Fig. 6a). The same applied to the Gizdepka River, as it is located in the NRB vicinity. PCA analysis revealed similarities between the Gizdepka, the Zagorska Struga and the Beka Canal, which was in line with their status – all of them are heavily modified water bodies (Council of Ministers 2016a). Therefore the right part of the PCA plot (group I and II) reflected combination of geographical proximity and the similar hydrological nature of the watercourses.

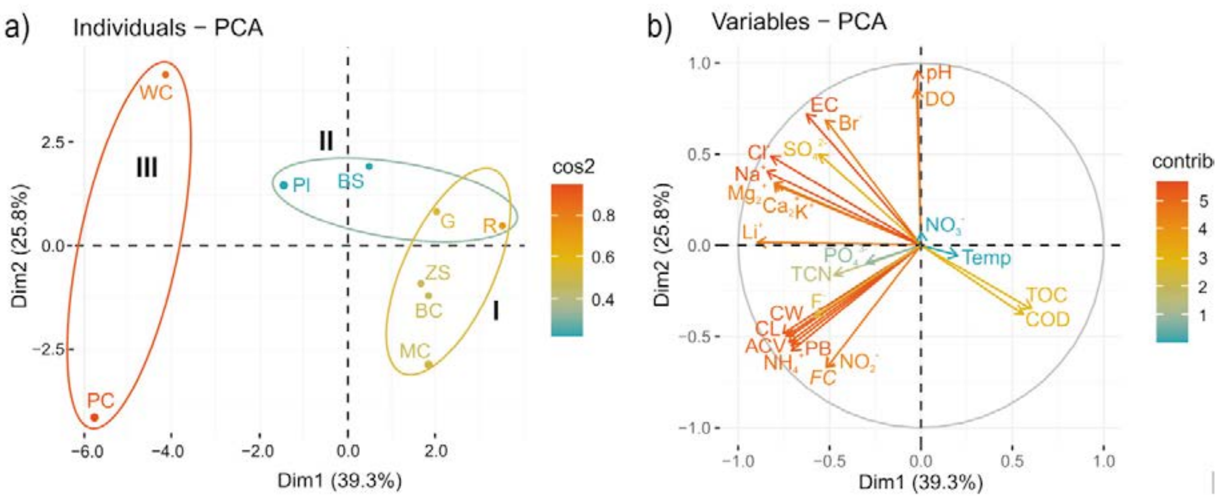

Fig. 6. Principal component analysis of the dataset: a) samples b) variables, plotted in space defined by principal components Dim1 and Dim2. CW refers to cell width and CL to cell length.

The Plutnica (Pl) and the Bladzikowski Stream (BS) (Fig. 6a, blue font color) were slightly separated due to the higher concentration of biogenic compounds, which could be connected to their location in the agricultural areas. These two sampling points seemed to differ mostly because of nitrogen compounds. Concentrations of PO_4^{3-} were most likely too low to affect bacterial growth. Studies showed that phosphorus was usually a limiting factor (Gillor et al. 2010). The Puck (PC) and the Wladyslawowo canals (WC) (Fig. 6a, red font) were distinct from the previous groups, probably due to the urban character of their catchment. Moreover, they also differed from each other – WC showed the highest ion concentration and conductivity which reflects its location on the salty reedbed and the potential impact of the nearby road. PC was characterized by the largest values of microbiological parameters. The largest amount of fecal coliforms and high $N\text{-}NH_4^+$ concentration might indicate wastewater-related contamination. In addition these two canals were not classified in the regulation.

Microbiological analysis

Apart from physicochemical analysis, microbiological parameters were also tested in order to assess the sanitary condition of the watercourses. Aquatic microorganisms possess high metabolic and growth rates and they are highly responsive to pressures and are directly influenced by changes in physico-chemical characteristics, including inputs of organic and inorganic compounds and pollutants (Caruso et al., 2016, Pernthaler, 2017, Šimek et al., 2014).

Including microorganisms as bioindicators in continuous monitoring can provide improved water quality assessments, with both general and specific diagnostics of stressors (Sagova-Mareckova et al., 2021). This is particularly important when considering the possibility of fecal contamination and potentially pathogenic organisms introduction to the coastal waters, intensively used for recreational purposes.

Basic microbiological parameters such as total prokaryotic cell number (TCN), average cell volume (ACV), prokaryotic biomass (PB), and cell morphological structure were analyzed using the direct epifluorescent filter technique (DEFT). It is believed that the analysis of morphological shapes of prokaryotic cells may be a sensitive marker of changes in aquatic and terrestrial ecosystems. In the framework of the EU Marine Strategy Framework Directive (MSFD) on the assessment of „good environmental status" of marine waters, it is recommended to include prokaryotes in biodiversity monitoring programs (Cochrane et al. 2010).

Microscopic analysis of single prokaryotic cells showed differences in the composition of prokaryotic community of microorganisms in the studied watercourses. The average value of the total prokaryotic cell number (TCN) varied within the 1.5 order of magnitude. Earlier studies indicate that cell size was an indicator of activity and development dynamics of the prokaryotic community (Norland S 1993, Šimek et al. 1994). Cells of small size were more active and determine the prokaryotic cell size increase (Baath 1994, Giovannoni 2017). The most active cells were those with the volume of ~ 0.12 μm^3 (Gasoll et al. 1995). Based on the morphological structure analysis, the smallest cells, mostly rods and cocci, were dominant. Specific morphological types might show specific physiological activities (Cottrell and Kirchman 2004, Posch et al. 2009). Trophic conditions might also cause morphometric and morphological changes of prokaryotic organisms. It was found that in the aquatic environment the volume of cells shows a clear connection with their biomass (La Ferla et al. 2010, La Ferla et al. 2014). Moreover, research on the link between bacterial cell size, metabolism and the genetic diversity of bacteria showed that small cells were more diverse than larger cells, and a few genotypes might be dominant among the largest and most productive cells (Bernard et al. 2000).

The largest cells (ACV) and highest biomass (PB) were found in the Puck Canal (PC). The amount of the smallest cocci was comparable to other sampling points, but the smallest rods were the least abundant, compared to all the other samples. However, large cocci were the most numerous there, and their biomass was several times higher than in other watercourses. Taking into account also the physicochemical parameters: lowest DO concentration, highest ammonia concentration and *E. coli* bacteria permissible level exceeded ten times, it can be assumed that this canal was affected by the greatest contamination, most probably of domestic sewage origin.

The DEFT analysis was supported by a sanitary quality assessment based on the abundance of *E.coli* indicator bacteria. Microbiological quality of the water environment is one of the priorities of the World Health Organization (WHO), American Environmental Protection Agency (US EPA) and European Commission (EC) (Bartram and Rees 2002). For more than two decades risk assessment methods and recommendations have been developed (Noble et al. 2003, Council of Ministers 2015, Council of Ministers 2019, Cochrane et al. 2010). According to the recreational areas' monitoring regulations, bacteria from the fecal enterococci and enteric rods (*E. coli*) are regarded as sensitive and accurate indicators of the sanitary condition of the environment.

It should be noted that, unlike for chemical parameters, in Poland there are no regulations regarding the microbiological quality of surface waters or wastewater discharged to the environment. Therefore, in this paper, the water sanitary quality assessment was based on the law regulating water quality of swimming areas. This is legitimate, as all the examined watercourses are entering coastal waters of Baltic Sea, being intensively used for recreational purposes. In our study the average value of fecal coliforms was tested. As there are no quality standards referring to the abundance of fecal coliforms, we assumed that 80% of the fecal coliforms detected on mFC agar are *E. coli* bacteria (Hachich et al. 2012). According to current Polish Minister of Health Regulations on the supervision of the water quality in the swimming areas and the place occasionally used for bathing (Council of Ministers 2019), the maximum allowed average values are 4.0×10^2 CFU/100 mL for enterococci and 10.0×10^2 CFU/100mL for *E. coli*. In 2016, during the sampling campaign, some other law was in force (Council of Ministers 2015), however the allowable *E. coli* limits did not change.

Estimated value of *E. coli* exceeded the limit value only at two points: the Beka Canal (BC) and the Puck Canal (PC). In PC particularly high estimated *E. coli* abundance was noted – it exceeded the acceptable standard as much as ten times. Notably, high fecal contamination has been recorded at that point for several years (Bączkowska, unpublished data). In this point, also very low DO concentration (3.0 mg/L) and the highest ammonia concentration (2.42 mg/L) among all the samples were recorded. All the above suggest potential unregulated wastewater discharge.

In the Beka Canal (BC), the estimated *E. coli* abundance exceeded limit value five times and P-PO_4^{3-} and COD concentrations were exceeded around two times. All the above suggest pollution inflow, possibly of domestic sewage origin, and such fecal contamination may pose a significant risk to people using Puck Bay waters for recreational purposes.

The obtained results clearly indicate the necessity of introducing more extensive monitoring based on analysis of prokaryotic communities, including small watercourses flowing into such sensitive areas as the Bay of Puck. Molecular methods based on the extraction of nucleic acids or proteins from environmental samples may serve as a particularly noteworthy approach. These methods provide a comprehensive view of microbial communities. As a result, their composition, structure, and diversity may be linked to environmental features and stressors (Newton & McLellan, 2015, Tanentzap et al., 2019). Furthermore, metagenomics and metatranscriptomics may predict community function, even if the function is rare (Achermann et al., 2020). New sequencing techniques (Illumina NGS) were used in further studies of the region described in this paper (Bączkowska et al., 2022, currently in progress)

Conclusion

In this study, the water quality of nine small rivers flowing into Puck Bay was analyzed. Changes in microbiological parameters such as the total number of prokaryotic cells, prokaryotic cells biomass and the presence of fecal coliforms were tested in relation to chemical parameters. The results did not show any heavy contamination, but some physicochemical and microbiological parameters were exceeded. Unambiguous indication of the most polluted watercourse is not possible, as in each of the studied rivers other pollution indicators were exceeded. Despite the fact that the watercourses flowing into Puck Bay described in this study do not receive treated wastewater and therefore are under smaller anthropopressure, they are characterized by reduced water quality.

Based on the obtained results' analysis, three areas differing in terms of physicochemical and microbiological parameters were identified, which was confirmed by PCA. The parameters of urban canals (WC and PC) were different from other analyzed watercourses, as, e.g., they receive rainwater from city streets. Therefore it may be worthwhile to consider their purification before discharge into Puck Bay. The second group are watercourses flowing through agricultural areas (Pl, BS). Chemical analysis showed increased concentrations of nutrients, which is in agreement with the other observations described in the literature. Additionally, this study also described channels flowing through protected areas, their parameters differed both from urban channels and streams flowing through agricultural areas. The canals in the NRB were adjacent to heavily drained farmland and although they were not influenced by the water from drainage ditches, the increased nutrient concentrations and the highest COD values were observed there.

The results clearly indicated the need for more quality research and extending monitoring in the rivers and canals in the Coastal Landscape Park, flowing into Puck Bay. According to the latest knowledge, the use of bio-indicators at the genetic level is also highly recommended. The research showed that smaller watercourses may have an impact on the coastal waters' state, and thus on the Baltic Sea water quality. Unfortunately, most of the research does not take into account the urban canals and small watercourses located in the NRB, which also can shape the water environment of Puck Bay, as was confirmed in this study.

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Mikrobiologiczna i chemiczna ocena jakości małych rzek uchodzących do Południowego Bałtyku. Część II: Analiza cieków wodnych w zlewni Zatoki Puckiej

Streszczenie: Zatoka Pucka, ze względu na swoje położenie, jest obszarem szczególnie narażonym na zanieczyszczenia pochodzenia antropogenicznego. Celem pracy była ocena jakości wód małych cieków wodnych uchodzących do wewnętrznej części Zatoki Puckiej. W pracy przedstawiono wyniki analiz chemicznych i mikrobiologicznych 10 rzek i kanałów w ujściach zlokalizowanych na zachodnim brzegu wewnętrznej Zatoki Puckiej. Analizie poddano następujące parametry środowiskowe: przewodność, pH, stężenie tlenu rozpuszczonego (pomiary in situ), ChZT (testy kuwetowe), stężenie jonów (chromatografia jonowa). Analiza mikrobiologiczna obejmowała ocenę stanu sanitarnego na podstawie liczby bakterii grupy coli typu kałowego metodą hodowlaną. Oznaczenia parametrów mikrobiologicznych takich jak: liczebność komórek prokariotycznych, średnia objętość komórek, biomasa oraz zróżnicowanie morfotypowe komórek prokariotycznych określono metodą mikroskopii epifluorescencyjnej. Na podstawie uzyskanych wyników stwierdzono, że małe cieki mogą przenosić znaczny ładunek zanieczyszczeń antropogenicznych i tym samym wpływać na środowisko Zatoki Puckiej. Uzyskane wyniki jednoznacznie wskazują na potrzebę prowadzenia monitoringu jakości rzek i kanałów w Nadmorskim Parku Krajobrazowym uchodzących do Zatoki Puckiej. Z badań wynika, że również mniejsze cieki mogą mieć wpływ na stan wód przybrzeżnych, a tym samym na jakość wód Morza Bałtyckiego.