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INFLUENCE OF FISHERY MANAGEMENT AND ENVIRONMENTAL FACTORS ON OCCURRENCE OF HETEROTROPHIC, HEMOLYTIC AND MESOPHILIC BACTERIA AND *AEROMONAS HYDROPHILA* IN WATERS OF THE DRWĘCA RIVER, POLAND

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Keywords: *Aeromonas hydrophila*, hemolytic bacteria, psychrophilic, psychrotolerant and mesophilic heterotrophic bacteria, water.

Abstract: The research covered the determination: of the numbers of heterotrophic bacteria: psychrophilic, psychrotolerant, mesophilic and percentage participation of hemolytic bacteria and *Aeromonas hydrophila* (with aerolysine and hemolysine genes) in the waters of the Drwęca River depending on environmental factors and fishery management. The mean quantities of heterotrophic bacteria (HPC) at 4, 14 and 28°C ranged: $0.78–7.57 \cdot 10^3$, $1.40–6.65 \cdot 10^3$ and $1.93–16.23 \cdot 10^3$ cfu·cm^{-3}, respectively. The percentage participation of hemolytic heterotrophic bacteria (HemPC) and *A. hydrophila* among psychrophilic, psychrotolerant, mesophilic microorganisms determined at 4, 14, 28°C, ranged: 7.9–10.4, 6.8–12.2, 8.6–22.0 and 1.1–6.4%, respectively. Statistically significant correlation between examined bacteria and temperature values, flows and O_2 saturations confirm that the occurrence of those microorganisms depends on the degree of microbiological contamination of that ecosystem, resulting from the fishery management and environmental factors.

INTRODUCTION

A river is a system comprising both the main reach and the tributaries, carrying on one-way flow a significant load of matter in dissolved and particulate phases from both natural and anthropogenic sources [2]. Those water ecosystems are characterized by a variety of heterotrophic microflora. Its quantitative and qualitative composition changes depending on climatic, morphometric and environmental conditions (temperature, pH, oxygen saturation) as well as anthropogenic factors (sewage inflow, recreation, fishery management) [16, 19, 26, 27, 40]. Climatic zones, the seasons of the year and inflow of various contaminants have an impact on periodic dominance of different types and species of bacteria belonging to psychrophilic, psychrotolerant or mesophilic microorganisms [7,

13, 29, 32, 45]. Due to their enzymatic properties, they perform the most important role, as by decomposing autochtonic and allochtonic organic matter [11, 16, 27], they take part in natural processes of self-purification of flowing waters [38]. However, an increase in the number of bacteria, especially mesophilic ones, in surface waters may pose a direct or indirect sanitary and epidemiological threats to aquatic organisms, people and animals [6, 30, 44]. This applies particularly to bacteria species with an ability to produce hemolysin which is considered one of the most important pathogenic factors [12, 34, 44]. It is especially adverse in the case of protected waters, one of which being the upper reach of the Drwęca River – since 1961 an aquatic and ichthyologic nature reserve. Previous research over the waters of the Drwęca River, conducted in the years 2001–2004, showed a quantitative and qualitative variety of heterotrophic microflora related to physicochemical parameters of the water and human activity [18, 19] and dominance of mesophilic *A. hydrophila* [26] potentially pathogenic to people and fish [4, 25, 34]. Therefore, this study was aimed at establishing the changes in: quantitative occurrence of heterotrophic bacteria (psychrophilic, psychrotolerant and mesophilic ones), the number and percentage participation of hemolytic microorganisms and mesophilic strains of *Aeromonas hydrophila* (with aerolysine and hemolysine genes) in the waters of the Drwęca River depending on environmental and anthropogenic factors.

MATERIAL AND METHODS

Study area

The Drwęca River, which is a right tributary of the Vistula River, flows through a lake district. The river is 207.2 km long and drains a catchment basin of 534 350 ha in surface area. The section of the river flowing within the boundaries of the Province of Warmia and Mazury is about 95 km long. In its upper reach the river flows through a small lake known as Ostrowin and a typical ribbon lake called Drwęckie [39] (Fig. 1). In 1961 the whole length of the Drwęca River was turned into a nature reserve. This aquatic nature reserve covers 1888.4 ha from the river sources to its outflow to the Vistula. The reserve, called the "River Drwęca Nature Reserve", was established to protect the river's water habitats as well as the fish living in the Drwęca such as trout, salmon, brown trout and vimba. The Drwęca River Nature Reserve is the longest ichthyological reserve in Poland, comprising 444.38 ha of protected area. Owing to large differences in elevation between the Drwęca and its tributaries, at several sections the river appears submontane in character. This favors the occurrence of rare fish and lamprey species, the species which prefer waters high in oxygen saturation [35–37].

In its upper reach, the valley of the Drwęca River forms a gorge 20–30 m deep and 8 km long. Known as Czarci Jar (Devil's Gorge), the gorge comprises a Polish Angling Association fish hatchery. The major sources of point pollution reaching the Drwęca include household and industrial sewage and wastewater as well as post-production water from three fish farms (in the villages of Czarci Jar and Rychnowska Wola) [39].

Sampling sites

The microbiological assays covered a 15-km long section of the upper reach of the Drwęca. Water samples were collected at 8 sampling sites designated in certain characteristic places along the river from its sources to the inflow into Lake Ostrowin (Fig. 1):

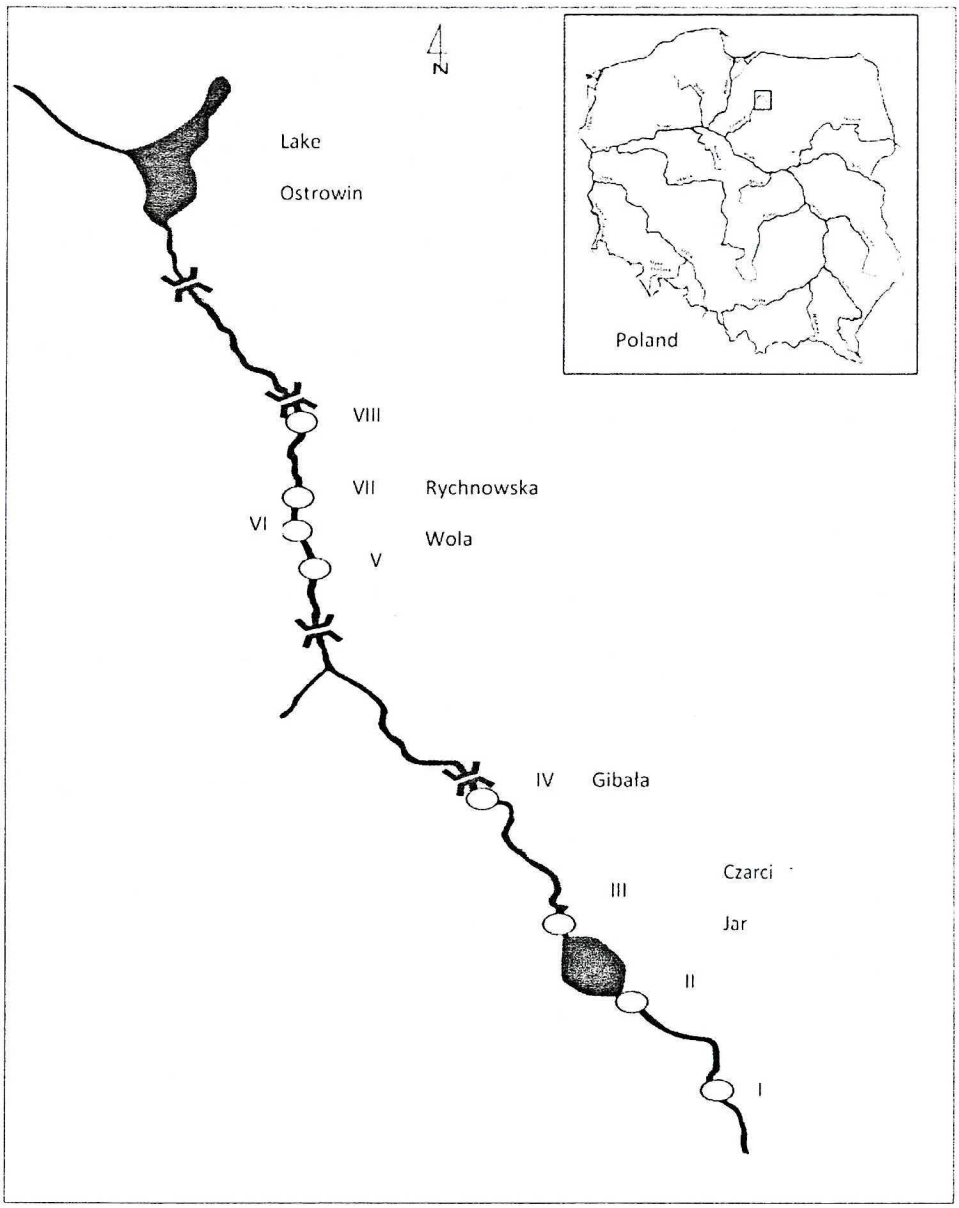

Fig. 1. Location sketch of sampling sites (I, II... VIII) in the River Drwęca; map of Poland showing the studied River Drwęca

- site I – 2 km away from the river sources, as the control site (the least of all the sampling sites exposed to contamination);
- site II – the outflow from the 'trout section' of the fish farm no. 1 (which produced 6.5 Mg of trout fry in 2005 and 5 Mg in 2006) located in the village of Czarci Jar;
- site III – the outflow from the ground fish farming ponds at the fish farm no. 1 in Czarci Jar;

- site IV – 2 km away from the fish farm no. 1, in the village of Gibała;
- site V – in the village of Rychnowska Wola, before the fish farms no. 2 and 3;
- site VI – the outflow from the fish farm no. 2 (which produced 54 Mg of commercial trout in 2005 and 55 Mg in 2006) located in Rychnowska Wola;
- site VII – the outflow from the fish farm no. 3 (which produced 40 Mg of commercial trout in 2005 and 42 Mg in 2006) located in Rychnowska Wola;
- site VIII – 2.5 km from the fish farms no. 2 and 3 in Rychnowska Wola;

Sampling

Water samples were collected from the Drwęca River at 0.3–0.5 m depth every 4–6 weeks from January 2005 to December 2006. Water was collected directly into sterile bottles according to the Standard Methods [20]. The time which elapsed between each sampling event and assays never exceeded 6 hours. In 2005–2006, 30 water samples were collected and analyzed per each site. Generally, the microbiological and physicochemical tests were performed on 240 water samples from the Drwęca River.

Microbiological studies

Microbiological analyses of all water samples from the river were determined by spread plate procedure on tryptone soy agar (TSA) (Oxoid) containing 5% (v/v) of sheep blood [10]. The studies included:

- the numbers of the psychrophilic heterotrophic bacteria as HPC 4°C and percentage estimation of psychrophilic hemolytic heterotrophic bacteria as HemPC 4°C after 10 day incubation at 4°C [13] among HPC 4°C,
- the numbers of psychrotolerant heterotrophic bacteria as HPC 14°C and percentage estimation of the psychrotolerant hemolytic heterotrophic bacteria as HemPC 14°C after 7 day incubation at 14°C [45] among HPC 14°C,
- the numbers of mesophilic heterotrophic bacteria as HPC 28°C and percentage estimation of the mesophilic hemolytic heterotrophic bacteria as HemPC 28°C and mesophilic *A. hydrophila* after 72 hour incubation at 28°C [38] among HPC 28°C.

The microbiological analyses were run in three parallel repetitions following general microbiological standards. The mean and range were calculated.

The results obtained for HPC 4°C, HPC 14°C and HPC 28°C were assumed and recalculated in colony forming units (cfu) per 1 cm^3 of water according to the methodology described by Standard Methods [20]. To investigate HemPC 4°C, HemPC 14°C and HemPC 28°C the strains, producing the transparent circular zones around the colonies, on TSA containing 5% of sheep blood, were assumed and recalculated in colony forming units (cfu) per 1 cm^3 of water. Next, percentage estimation of those hemolytic bacteria were calculated among HPC 4°C, HPC 14°C and HPC 28°C, respectively. For determination of quantitative occurrence of the mesophilic strains of *A. hydrophila*, the total colonies HemPC 28°C were preliminarily screened using the following tests: gram stain, oxidase, susceptibility to O/129 vibriostatic disk (10 and 150 μg), motility, glucose and trehalose fermentation and nitrate reduction. Only the strains found to be gram negative, oxidase positive, negative for O/129 vibriostatic, motile, glucose and trehalose fermenting and nitrate reducing were identified with API 20 NE strips (bioMérieux).

Molecular analysis

From the 152 strains preliminarily identified, with API 20 NE strips (bioMérieux), as belonging to *A. hydrophila*, nucleic acids were isolated by CTAB method [15] with own modifications (personal communications, Korzekwa, 2008). Quality and quantity of isolated DNA were determined photometrically at 260 nm (OD_{260}) and adjusted to a final template PCR concentration of about 20 $\mu g \cdot cm^{-3}$ in TE buffer. Multiplex PCR was realized for 16S rDNA, hemolysin and aerolysin sequences present in *A. hydrophila* tested genomes. Primers A16S based on the *A. hydrophila* ATCC 7966 16S rRNA sequence (GenBank accession no. X74677) were applied to confirm presence of the 16S rRNA specific gene as an internal control [43]. The AHH1 primer set was designed to amplify a 130-bp fragment of *A. hydrophila* extracellular hemolysin gene *ahh1* [24]. The AH-aerA primer set amplified a 309-bp fra gment of the *A. hydrophila* aerolysin gene *aerA* (GenBank accession no. M16495) [43]. DNA samples (10 ng per reaction mixture) were amplified in a $20 \cdot 10^{-6}$ dm^3 reaction mixture consisting of 1.25 mM magnesium chloride; 200 µM (dNTP), 2.0 µM AHH1 primers; 1.5 µM AH-aerA, 0.05 µM A16S primers (Integrated DNA Technologies, Coralville, USA), and 1.25 U of Tfl DNA polymerase (Epicentre Biotechnologies, Madison, USA). DNA templates were amplified by thermalcycler model 2400 (Mastercycler gradient, Eppendorf, Germany) with thermal profile according to Wang *et al.* [43]. Amplification parameters for all primer sets included an initial denaturation at 95°C for 5 min, followed by 50 cycles of denaturation at 95°C for 0.5 min, annealing of the primers at 59°C for 0.5 min, and primer extension at 72°C for 0.5 min. A final extension at 72°C for 7 min was used. Amplicons were electrophoretically separated in 1.5% ethidium bromide stained agarose at 5 $V \cdot cm^{-1}$, and then visualized with UV (Kucharczyk, TE, Poland). The obtained patterns (Fig. 2) were compared with superladder-MID1 mass molecular marker (GenSura Laboratories). Among the 152 studied strains 83 were finally confirmed as *A. hydrophila* with aerolysine and hemolysine genes. Next, percentage estimation of *A. hydrophila* were calculated among HPC 28°C.

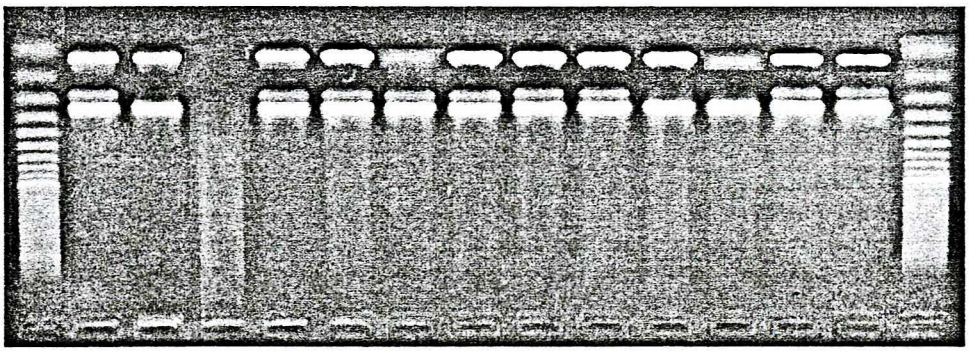

Fig. 2. Example of patterns obtained after multiplex-PCR of *Aeromonas hydrophila* realized for presence confirmation of: species-specific 16S rDNA (~ 356 bp; bottom band), aerolisine gene (~ 306 bp, middle band) and hemolysine gene (~ 150 bp; upper band); MM – molecular marker, 1 – *A. hydrophila* LMG 7864, 3 – blind sample (PCR mixture plus DNA template without primers)

Physicochemical tests

In the experimental period, the river water was additionally subjected to physicochemical determinations of the following parameters: temperature (°C), flow ($dm^3 \cdot s^{-1}$), pH and

oxygen saturation (mg $O_2 \cdot dm^{-3}$). All microbiological and physicochemical determinations were carried out on the same (common) water samples. The physicochemical determinations: temperature, flow, pH, oxygen saturation, were conducted by multimeasurement apparatus Hydrolab Multi 12 (Schott, Germany) with the precision of measurements.: ± 0.1°C, ± 1.0 $dm^3 \cdot s^{-1}$, ± 0.01 pH, ± 0.01 mg $O_2 \cdot dm^{-3}$ respectively.

Statistical evaluation

The results of microbiological and physicochemical examinations were subjected to statistical evaluation by determining the correlation (estimation by Spearman' correlation) between a given set of parameters with simple correlation coefficients [42]. The Spearman's correlation coefficient was calculated with the use of the STATISTICA PL 7.0 computer software.

RESULTS

Microbiological studies

The numbers (means and ranges) of the HPC 4°C, HPC 14°C and HPC 28°C and percentage participation of occurrence of the studied HemPC 4°C, HemPC 14°C, HemPC 28°C and *A. hydrophila* among HPC 4°C, HPC 14°C and HPC 28°C occurring in the waters of the Drwęca River over the years 2005–2006 are presented in Table 1. Their quantitative occurrence fluctuated within the range of a few orders of magnitude depending on the bacteria group assayed (psychrophilic, psychrotolerant, mesophilic) as well as the sampling site and period of study. Regardless of temperature requirements, the smallest mean quantities of HPC 4°C, HPC 14°C and HPC 28°C (respectively: $0.78 \cdot 10^3$, $1.40 \cdot 10^3$ and $1.93 \cdot 10^3$ cfu·cm^{-3}) were found at site I (2 km away from the source of the river), while the largest quantities (respectively: $7.57 \cdot 10^3$, $6.65 \cdot 10^3$ and $16.23 \cdot 10^3$ cfu·cm^{-3}) – at site VI (the outflow from fish farm no. 2 located in Rychnowska Wola). Similar regularities were also recorded for the minimum and maximum numbers thereof. Throughout the study period, the smallest quantitative occurrence of HPC 4°C and the highest numbers of HPC 14°C and HPC 28°C found in summer months, corresponded to the maximum values of water temperature (19.9–20.8°C) and the minimum concentrations of oxygen dissolved in the water (6.40–6.88 mg $O_2 \cdot dm^{-3}$). Regardless of the temperature preferences, the smallest mean percentage participation of HemPC 4°C, HemPC 14°C and HemPC 28°C was found at sites I and VIII. It amounted to, respectively: 7.9, 6.8 and 8.6% for the former, and 7.0, 8.9 and 10.1% for the latter, among HPC 4°C, HPC 14°C and HPC 28°C. The highest contamination with HemPC 4°C, HemPC 14°C and HemPC 28°C was characteristic of site VI, where on average the percentage participation of hemolytic bacteria among HPC 4°C, HPC 14°C and HPC 28°C, was recorded at: 10.4, 12.2 and 22.0%. Taking into account temperature requirements, the mean percentage participation of HemPC 4°C and HemPC 14°C fluctuated slightly in the water samples at most of the same sampling sites. It ranged from 6.8 to 12.2% depending on the place of sampling. In turn, the highest percentage participation of hemolytic bacteria at all the sampling sites was found for HemPC 28°C. It ranged from 8.6% at site I to 22.0% at site VI. The mesophilic hemolytic bacteria *A. hydrophila* (with aerolysine gene) constituted from 0 to 25.6% among HPC 28°C depending on the sampling site, the period of study and certain physicochemical parameters assayed (temperature, flows, pH and dissolved O_2 satura-

Table 1. The occurrence of HPC 4°C, HPC 14°C and HPC 28°C and the percentage participation of HemPC 4°C, HemPC 14°C, HemPC 28°C and mesophilic strains of *A. hydrophila* (among of the HPC 4°C, HPC 14°C and HPC 28°C, respectively) in the water of the Drwęca River in 2005–2006

Group of bacteria		Sampling sites							
		$I_{(30)}$	$II_{(30)}$	$III_{(30)}$	$IV_{(30)}$	$V_{(30)}$	$VI_{(30)}$	$VII_{(30)}$	$VIII_{(30)}$
HPC 4°C	[× 10^3 cfu·cm^{-3}]	0.78 [1] 0.1–1.9 [2]	1.91 0.2–11.1	5.04 0.1–36.5	3.92 0.1–28.4	3.63 0.1–24.4	7.57 0.1– 45.3	3.59 0.1–8.2	1.70 0.2–4.7
HPC 14°C		1.40 0.3–3.3	1.66 0.1–6.0	2.33 0.1–7.8	3.18 0.1–11.3	1.99 0.2–6.0	6.65 0.2–33.1	4.94 0.2–13.3	1.99 0.2–8.9
HPC 28°C		1.93 0.4–5.9	3.84 0.1–12.7	3.7 0.4–11.6	3.4 0.5–10.7	3.3 0.1–15.7	16.23 0.8–63.6	4.03 0.6–18.8	3.84 0.1 –20.0
HemPC 4°C	[%]	7.9 2.8–18.7	7.6 1.3–17.9	8.0 1.7–14.3	9.7 1.6–23.1	7.8 0.0–22.2	10.4 3.3–16.4	9.8 2.7–15.8	7.0 1.0–25.6
HemPC 14°C		6.8 0.9–11.5	9.3 3.6–33.3	10.2 1.1–27.3	10.5 0.5–29.8	8.5 2.1–16.7	12.2 1.4–31.3	10.7 1.0–38.1	8.9 0.0–25.1
HemPC 28°C		8.6 1.3–18.2	15.5 5.3–36.8	15.0 0.9–38.1	15.3 1.6–29.4	13.2 0.7–36.4	22.0 4.8–95.0	18.1 6.8–31.1	10.1 3.7–18.3
A. hydrophila		1.1 0.0–4.8	1.6 0.0–6.5	2.1 0.0–9.8	1.6 0.0–8.0	2.4 0.5–11.0	5.8 1.3–18.9	6.4 2.5–25.6	3.0 1.1–16.8

[1] – mean, [2] – range, ( ) – number of samples

tion). On average, they were found to be the fewest at site I (1.1% among HPC 28°C), and the most numerous at sites VI and VII (5.8 and 6.4% respectively). Throughout the period of study, this species was not found to occur at sites I, II, III, and IV and only in winter months when the water temperature ranged from 0.2 to 1.0°C. In turn, the highest percentage participation of *A. hydrophila* (18.9 and 25.6% HPC 28°C) was recorded in July 2005 at sites VI and VII respectively, when the water temperature fluctuated within the range of 18.9 to 19.9°C.

Physicochemical studies

The ranges of the physicochemical parameters (temperature, flow, pH and oxygen) measured in the waters of the Drwęca River in 2005–2006 are presented in Table 2. Their values changed within a few orders of magnitude, depending on the sampling site, period of study and kind of parameter. In the study period, the temperature of the water varied from 0.2°C (in January 2005) to 20.8°C (in July 2006). The smallest mean value of this parameter (6.4°C) was noticed at site I, whereas the highest one (9.8°C) occurred at site III. The values of flows Drwęca River ranged from 82.0 to 832.0 $dm^3 \cdot s^{-1}$ The smallest mean flow of the river water (99.0 $dm^3 \cdot s^{-1}$) was detected at site I, and the highest one (688.0 $dm^3 \cdot s^{-1}$) observed at site VIII. The value of water reaction (pH) measured for the water samples collected from the Drwęca River varied from 7.16 to 8.32. The smallest mean value of this parameter (7.47) was noticed at site VIII, whereas the highest one (7.96) occurred at site III. The concentrations of oxygen dissolved in water of the Drwęca River ranged from 6.40 to 12.96 mg $O_2 \cdot dm^{-3}$. The smallest mean concentration of this index was observed at site II (8.46 mg $O_2 \cdot dm^{-3}$), the highest ones were noticed at sites VI and VII (11.48 and 11.14 mg $O_2 \cdot dm^{-3}$, respectively).

Statistical evaluation

The results of the statistical analysis of the correlation between the numbers of the studied bacteria (HPC 4°C, HPC 14°C, HPC 28°C, HemPC 4°C, HemPC 14°C, HemPC 28°C, *A. hydrophila*) recovered from the water of Drwęca River during the whole time of the study and the values of some physicochemical compounds (temperature, flow, pH, O_2) in the analyzed water samples are shown in Table 3. The Spearman's test proved that there were both positive and negative statistically significant ($p < 0.05$) correlations:

- the counts of all the assayed groups of microorganisms versus values of temperature and oxygen saturation of the water samples collected from the Drwęca River;
- the quantitative occurrence of HemPC 28°C and mesophilic strains *A. hydrophila* versus water flow values;
- the quantitative occurrence of HPC 4°C with HemPC 4°C, HPC 14°C and HPC 28°C;
- HemPC 4°C with HemPC 14°C, HemPC 28°C and mesophilic *A. hydrophila*;
- HPC 28°C with HemPC 28°C and mesophilic *A. hydrophila*;
- HemPC 28°C with the quantitative occurrence of mesophilic *A. hydrophila*.

Table 2. The values of some physicochemical parameters of the waters of the Drwęca River in 2005–2006

Sampling sites	Number of samples	Physicochemical parameters			
		Temperature [°C]	Flow [$dm^3 \cdot s^{-1}$]	pH	Oxygen [mg $O_2 \cdot dm^{-3}$]
I	30	6.4 [1] 0.2–11.4 [2]	99.0 82.0–121.0	7.75 7.50–8.10	8.92 6.40–10.40
II	30	8.5 0.5–18.3	118.0 91.0–145.0	7.75 7.37–8.20	8.46 6.88–10.56
III	30	9.8 0.8–20.8	197.0 180.0–218.0	7.96 7.56–8.32	9.82 7.84–11.04
IV	30	8.4 1.0–15.2	265.0 227.0–306.0	7.73 7.50–8.10	8.88 6.40–10.56
V	30	8.0 1.0–14.9	309.0 250.0–365.0	7.80 7.65–8.10	10.12 8.00–11.36
VI	30	8.0 1.0–18.9	308.0 250.0–365.0	7.64 7.20–8.04	11.48 9.12–12.96
VII	30	8.1 1.0–19.9	308.0 250.0–365.0	7.59 7.34–7.82	11.14 9.12–12.00
VIII	30	8.0 0.4–16.1	688.0 529.0–832.0	7.47 7.16–7.80	9.48 8.16–10.72

[1] – mean, [2] – range

Table 3. Statistic estimation by Spearman's correlation ($p < 0.05$, $N = 240$) between the numbers [cfu·cm^{-3}] of studied groups of microorganisms recovered from the water of the Drwęca River during whole time of study and some physicochemical parameters in water; BD eliminated in couple

	HPC 4°C	HemPC 4°C	HPC 14°C	HemPC 14°C	HPC 28°C	HemPC 28°C	*A. hydrophila*
Temperature [°C]	-0.06*	-0.26*	0.22*	0.30*	0.27*	0.17*	0.44*
Flow [dm^3·s^{-1}]	0.08	0.12	-0.08	-0.041	-0.037	-0.16*	-0.17*
Reaction [pH]	-0.033	0.056	0.01	-0.05	0.01	0.01	0.06
Oxygen [mg O_2·dm^{-3}]	0.36*	0.27*	-0.39*	-0.11*	-0.23*	-0.13*	-0.34*
HPC 4°C	1.00	0.87*	-0.57*	-0.14	-0.27*	-0.05	-0.11
HemPC 4°C	0.87*	1.00	0.17	-0.59*	0.07	-0.26*	-0.18*
HPC 14°C	-0.57*	0.17	1.00	0.17	0.21	0.01	-0.01
HemPC 14°C	-0.14	-0.59*	0.17	1.00	0.06	0.15	-0.05
HPC 28°C	-0.27*	0.07	0.21	0.06	1.00	0.47*	0.23*
HemPC 28°C	-0.05	-0.26*	0.01	0.15	0.47*	1.00	0.29*
A. hydrophila	-0.11	-0.18*	-0.01	-0.05	0.23*	0.29*	1.00

HPC– heterotrophic plate count, HemPC – hemolytic plate count, * – statistically important differences ($p < 0.05$)

DISCUSSION

The research results obtained, concerning the number of heterotrophic bacteria, the numbers and percentage participation of the potentially pathogenic hemolytic and mesophilic bacteria *A. hydrophila* in the Drwęca water, demonstrated differences in their occurrence ranging from one to a few orders of magnitude, depending on the group of microorganisms assayed (HPC or HemPC), their temperature requirements, the sampling site and the time of sampling. Directly proportional relations between the water temperature and HPC 14 and 28°C as well as the minimum and maximum HPC 4°C corresponding to the highest and lowest values of that parameter in the samples of the water from the Drwęca River indicated dominance of the studied psychrotolerant bacteria (HPC 14°C) and/or the mesophilic ones (HPC 28°C) over the summer months, as well as the psychrophilic bacteria (HPC 4°C) in the winter periods. It was confirmed by statistical analysis, which demonstrated statistically significant (positive or negative) relations between the water temperature and oxygen saturation, respectively: HPC 14°C, HPC 28°C and HPC 4°C. In the waters of the Drwęca River, significant negative correlations between HPC 4°C on the one side and HPC 14°C and HPC 28°C on the other indicate a seasonal nature of the occurrence of particular groups of bacteria under examination, whose numbers are also conditioned by the content of oxygen dissolved in water, which changes depending on the water temperature [1]. It is confirmed by the maximum numbers of HPC 4°C recorded in the winter months at 10.40–12.96 mg $O_2 \cdot dm^{-3}$ as well as HPC 14 and 28°C in the summer months at 6.40–6.88 mg $O_2 \cdot dm^{-3}$. Similar trends and relations between the water temperature and the content of oxygen dissolved in it on the one side and the numbers of heterotrophic bacteria on the other were found in the waters of two Canadian rivers: the Meduxnekeang and the Dunbar by Bell *et al.* [7] as well as in the samples of water from the Portrero de los Funes River in Argentina Almeida *et al.* [3]. The smallest and the largest mean ranges of the occurrence of all the HPC groups assayed, regardless of their temperature requirements, observed respectively at site I (control site) and sites VI and VII (constituting outflows from fish farms no. 2 and 3) indicate a local and/or periodic influence of intensive fishery management on the microbiological quality of the Drwęca River waters. It results from the fact that organic substances supplied to the water in the form of unused fish feed and fish excrement create favorable conditions for the growth and development of heterotrophic microorganisms [9, 17, 31]. This is also confirmed by similar trends in the minimum and maximum numbers of heterotrophic bacteria (proteolytic, ammonifying and denitrifying ones) at appropriate sites recorded in previous research over the Drwęca River waters [18, 19]. In aquatic environments, the presences of hemolytic bacteria may pose an epidemiological threat to people and organisms inhabiting them [5, 12]. In the Drwęca River waters, the changing numbers of HemPC 4°C, 14°C and 28°C depending on the period of study, temperature and O_2 saturation were confirmed by statistical analysis. Their lowest percentage participation found at sites I and VIII confirms the fact that those microorganisms, represented by numerous bacteria species, belonging, among others, to the genera: *Escherichia* spp., *Yersinia* spp., *Vibrio* spp. or *Aeromonas* spp., commonly occur in aquatic environments where they often get from the catchment area [14, 33, 41]. In turn, the highest contamination with all the hemolytic microorganisms under examination recorded in the summer months at site VI suggests an adverse local and/or periodic impact of the fishery management. In the circumstances of intensive

fattening, fish excrete large numbers of bacteria belonging to various genera [8, 21, 22, 46, 47], which, despite their potential pathogenicity, constitute saprophytic microflora of the fish [10, 23]. In the studied samples of the Drwęca River waters, the determined percentage diversity of the identified mesophilic bacteria *A. hydrophila* among HPC 28°C and the statistically significant differences in their numbers depending on the time and place of sampling confirm the thesis that the occurrence of those strains changes under the influence of various environmental factors and depending on the level of contamination of water reservoirs [4, 26, 28, 30]. It is also proved by the statistically significant correlations ($p < 0.05$) between the numbers of the bacteria of that species on the one side and HPC 28°C and HemPC 28°C on the other, as well as the values of physicochemical parameters (temperature, flow and O_2).

CONCLUSION

In the waters of the Drwęca River, the quantitative diversification of the bacteria under examination, particularly between the periods of study, indicates a significant impact of environmental and/or fishery management. It was confirmed by the statistical analysis which demonstrated correlation between their quantitative occurrence and the temperature values and O_2 concentration in the water. The highest contamination with HPC 4°C, HPC 14°C and HPC 28°C as well as the highest percentage participation of all the studied HemPC and identified mesophilic strains of *A. hydrophila* (with aerolysine and hemolysine genes) found in water samples from sites VI and/or VII (constituting outflows from fish farms no. 2 and 3) in the summer months suggest a local and/or periodic adverse impact of fishery management. Statistically significant correlations ($p < 0.05$) between the quantitative occurrence of mesophilic bacteria *A. hydrophila* on the one side and HPC 28°C and HemPC 28°C on the other, as well as the values of physicochemical parameters (temperature, flow and O_2) confirm the thesis that the occurrence of those microorganisms in the waters of the Drwęca River depends on the degree of microbiological contamination and environmental factors. Along with the changes in physicochemical parameters of the Drwęca River waters, related to the seasons of the year and/or intensification of the fishery management, there occur a lot of reciprocal processes between particular physiological groups, genera or species of microorganisms, manifesting themselves in dominance of different groups of microorganisms [7], including also potentially pathogenic strains or pathogens [34].

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WPŁYW GOSPODARKI RYBACKIEJ I CZYNNIKÓW ŚRODOWISKOWYCH NA WYSTĘPOWANIE BAKTERII HETEROTROFICZNYCH, HEMOLIZUJĄCYCH I MEZOFILNYCH ORAZ *AEROMONAS HYDROPHILA* W WODACH RZEKI DRWĘCY, POLSKA

Badania obejmowały oznaczenia liczebności bakterii heterotroficznych: psychrofilnych, psychrotolerancyjnych i mezofilnych oraz procentowego udziału bakterii hemolizujących i *Aeromonas hydrophila* (posiadających geny aerolizyny i hemolizyny) w wodach rzeki Drwęcy w zależności od czynników środowiskowych i gospodarki rybackiej. Średnie liczebności bakterii heterotroficznych (HPC) oznaczanych w 4, 14 i 28°C były odpowiednio w zakresach: $0{,}78–7{,}57 \cdot 10^3$, $1{,}40–6{,}65 \cdot 10^3$, $1{,}93–16{,}23 \cdot 10^3$ jtk·cm^{-3}. Procentowy udział bakterii hemolizujących (HemPC) i *A. hydrophila* wśród drobnoustrojów heterotroficznych: psychrofilnych, psychrotolerancyjnych i mezofilnych oznaczanych w 4, 14, 28°C wynosił odpowiednio: 7,9–10,4, 6,8–12,2, 8,6–22,0 i 1,1–6,4%. Statystycznie istotne zależności pomiędzy liczebnością badanych grup bakterii a wartościami temperatury, przepływu i stężeniami rozpuszczonego w wodzie tlenu wskazują, że występowanie tych drobnoustrojów jest związane ze stopniem mikrobiologicznego zanieczyszczenia tego ekosystemu, wynikającym z gospodarki rybackiej i czynników środowiskowych.