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BACTERIA AND PARASITES ON THE GILLS OF PIKE *ESOX LUCIUS* L. AND TENCH *TINCA TINCA* (L.) AS INDICES OF THE TROPHIC LEVEL OF OŚWIN LAKE (NORTHEASTERN POLAND)

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ABSTRACT. The presence of bacteria and parasites on the gills of pike and tench from Oświn Lake in the Seven Islands Reserve was investigated. Parallel bacteriological analyses of the water were done. The materials were sampled in May-June, July, October 1999, six years after the water level had been increased. The bacteriological and parasitological results both indicate that this body of water is eutrophic. The increase in the incidence of infection with *Ergasilus sieboldi* and an increase in the number of heterotrophic bacteria were observed simultaneously on the gills. Based on the parasitological and microbiological analysis of gills, the prognosis for the health of pike is unfavorable.

Key words: BACTERIA, PARASITES, PIKE (*ESOX LUCIUS*), TENCH (*TINCA TINCA*), LAKE WATER

INTRODUCTION

Fish gills come into direct contact with the environment and any of the pathogens it might harbor. They provide a unique niche for many parasites which have a simple life cycle, particularly ciliates, monogenetic trematodes and crustaceans, as well as bacteria present in the water.

The eutrophication process of water basins supports the increased abundance of bacteria, which is reflected in the microflora present in fish (Zmysłowska et al. 2000). Although protected by a single-layered epithelium and a layer of mucus which neutralizes the impact of bacteria and fungi, the gills are particularly susceptible to pathogens (Jara and Chodyniecki 1999). Low oxygen and high ammonia concentrations and overcrowding cause outbreaks of Bacterial Gill Disease (BGD) caused by *Flavobacterium branchiophila* (Noga 2000), while strong algal blooms precede the occurrence of mycotic diseases caused by Branchiomyces.

Infections of parasitic protozoans and monogenetic trematodes on the gills are particularly dangerous for cultured juvenile fish. Parasitic protozoans in natural water basins do not pose as significant a problem as larger parasites do. The latter can be an immediate cause of fish death or an intermediate one by providing bacteria with an infection path through damaged gill tissues. It is also known that ectoparasites play a role in the spread of pathogenic bacteria, viruses (Cusack and Cone 1986) and fungi (Mennie et al.2000).

The aims of this study were to evaluate the current trophic state of Oświn Lake based on the occurrence of parasites and bacterial microflora on the gills of tench *Tinca tinca* (L.) and pike *Esox lucius* L. and to perform microbiological studies of the water at the sampling sites.

MATERIAL AND METHODS

STUDY AREA

Oświn Lake, located in northeastern Poland, and the area surrounding it (approximately 1000 ha in total) have been under special protection since 1983 when an internationally important nature reserve was created there to protect aquatic birds. In 1993 the water in the lake was increased by 1 m in order to return the basin to its original state after the level had been lowered significantly in the 1970s and the fertilizer pollution incident in 1983.

Currently, Oświn Lake has an area of more than 600 ha, with an average depth of 1.7 m and a maximum of 2.8 m (Wróbel and Królikowska1999). It is horseshoe shaped with differentiated areas – the eastern (E) and western (W) deeps. The lake receives inflows from lakes Węgielsztyńskie and Rydzówka in the south and from the River Ruda and a nameless stream in the east.

Based on measurements of physico-chemical parameters, Oświn Lake is classified as mezotrophic (Wiśniewski 2001a). After the water level was increased, submerged macrophytes (Wróbel and Królikowska 1999) and plankton communities, typical of macrophytic lakes, began to develop (Ejsmont-Karabin 2001), and the zoobenthos structure typical of eutrophic, polymictic lakes began to rebuild itself (Wiśniewski 2001b). The studied lake is classified as a tench-pike lake (Chybowski et al. 2001).

MICROBIOLOGICAL STUDIES

Samples for microbiological and parasitological studies were collected three times in 1999: sample 1– spring (May 26 - June 4); sample 2 – summer (July 4-7); sample 3 – fall (October 10-15).

The study materials were mucus from the gill surfaces of tench and pike and water from the surface layer (0.3 m) and the near-bottom layer (0.5 m above the bottom) at two study stations – one in the western (W) and one in the eastern (E) deeps where the fish samples were caught. The samples from the fish were collected immediately after they had been caught. Sterile swabs were used to swipe a 1 cm^2 area of the gills. Water collected with a Ruttner sampler was transferred into sterile jars.

The microbiological determinations included:

- total number of bacteria on common agar after 72 h of incubation at 20°C (TVC 20°C);
- total number of bacteria on common agar after 24 h of incubation at 37°C (TVC 37°C);
- number of *Pseudomonas fluorescens* on King B substrate after 48 h of incubation at 25°C. The presence of stains were checked under a Wood's lamp;
- number of *Pseudomonas aeruginosa* on King A substrate after 48 h of incubation at 42°C. The presence of stains were checked under a Wood's lamp (Burbianka and Pliszka 1983).

Cultures were made using covered plates. A 0.85 % NaCl solution was used for dilution. The results were calculated as Colony Forming Units (CFU) in 1 cm^3 of water.

PARASITOLOGICAL STUDIES

Fifty-five tench and 47 pike from the eastern (E) and western (W) areas of the lake were analyzed (Tables 1 and 2). The gills were examined immediately in the field laboratory and the ectoparasites were removed. A portion of the gill was fixed in a formalin and 0.7% NaCl solution so that the samples could later be analyzed in the laboratory in Olsztyn. Metazoa were isolated and stored in 70% ethanol and exposed in glycerol. Fresh smears from the gills were examined microscopically and then preserved. Staining was done with Giemsa solution and the Klein silver technique (Lom and Dykova 1992).

The prevalence of infection and the mean intensity of infection were determined

TABLE 1

Total length and weight of tench from Oświn Lake

Parameter	Samples					
	1		2		3	
	E	W	E	W	E	W
Number	10	10	10	10	6	9
Total length (cm)	27.0 – 37.0	30.0 – 40.0	27.0 – 36.0	29.5 – 32.5	30.5 – 38.0	28.0 – 38.0
Body weight (g)	275 – 750	425 – 1030	285 – 735	445 – 600	400 – 790	350 – 840

Samples: 1 – May-June 1999; 2 – July 1999; 3 – October 1999.
E – eastern part, W – western part

TABLE 2

Total length and weight of pike from Oświn Lake

Parameter	Samples					
	1		2		3	
	E	W	E	W	E	W
Number	7	10	10	4	6	10
Total length (cm)	44.0 – 64.0	40.0 – 74.0	46.0 – 61.5	34.0 – 57.0	33.0 – 41.0	33.0 – 59.5
Body weight (g)	545 – 1715	380 – 2600	660 – 1645	220 – 1185	190 – 400	200 – 720

Samples: 1 – May-June 1999; 2 – July 1999; 3 – October 1999.
E – eastern part, W – western part

and presented as the range from the minimum to the maximum intensity of infection. The relative density of infection was determined. To either confirm or exclude the impact of sample collection location (E or W) on parasitic infection dual factor variance analysis was applied (ANOVA/MANOVA). Statistical analysis was done using the Statistica program, version 6.

RESULTS

The quantitative studies of the bacterial groups TVC 20°C and TVC 37°C and the bacteria species *P. fluorescens* and *P. aeruginosa* in the waters of Oświn Lake confirmed that there were similar tendencies in occurrence at the two sampling locations - E and W (Table 3). Generally, there were higher numbers of bacteria in the water in summer and lower numbers in the spring and fall. The exceptions to this were TVC 20°C and *P. aeruginosa*, which were most numerous in spring in the near-bottom layer of the eastern part of the lake.

The number of bacteria in the water from the near-bottom layer was higher, as a rule, than at the surface. Only in summer in the W area of the lake was the number of *P. fluorescens* higher in the surface layer than it was near the bottom (Table 3).

The total number of heterotrophic bacteria from the gills of pike from the W area of the lake had a tendency to increase in summer and decrease in fall. However, in the E area of the lake an increase in these bacteria was noted (at a temperature of 20°C) from spring to fall (Table 4). The numbers of TVC 20°C bacteria from the gills of tench were higher in spring than in summer in both parts of Lake Oświn. The total number of TVC 37°C bacteria on the gills of both species from the W area of the lake were similar and exhibited a tendency to decrease in summer. However, there was an increase

TABLE 3

Comparison of the number of bacteria per 1 cm^3 in the surface and (S) and bottom (B) water layers from the eastern (E) and western (W) areas of Oświn Lake

Bacteria		Samples					
		1		2		3	
		E	W	E	W	E	W
TVC 20°C	B	5400	7800	3600	1010	250	80
	S	390	240	2160	880	250	30
TVC 37°C	B	650	750	1900	1120	280	160
	S	280	110	1320	110	260	100
P. fluorescens	B	120	35	1110	1230	25	50
	S	80	2	170	1750	10	10
P. aeruginosa	B	80	40	70	120	50	85
	S	50	30	60	40	20	25

TVC 20°C – bacteria grown on standard agar at 20°C 72 h^{-1}
TVC 37°C – bacteria grown on standard agar at 37°C 24 h^{-1}
Remaining notation as in Table 1

in these bacteria in the E area from spring to summer. The number of *P. fluorescens* and *P. aeruginosa* on the gills of pike and tench was the highest in summer in both parts of the lake. The intensity of occurrence of these bacteria was lower in spring and summer (Table 4).

TABLE 4

Comparison of the number bacteria (10^3 cm^{-2}) on the gills of pike (P) and tench (T) from the eastern (E) and western (W) areas of Oświn Lake

Bacteria		Samples					
		1		2		3	
		E	W	E	W	E	W
TVC 20°C	P	5271	17500	25520	28500	44000	7840
	T	17800	17000	192	1840	-	-
TVC 37°C	P	920	66000	8720	35000	418000	37000
	T	980	34000	60800	17000	-	-
P. fluorescens	P	22.5	8.5	32.0	14.6	1.2	1.0
	T	38.5	1.6	40.8	11.0	-	-
P. aeruginosa	P	10	40	925	111	33	28
	T	3	10600	108	86000	-	-

TVC 20°C - bacteria grown on standard agar at 20°C 72 h^{-1}
TVC 37°C - bacteria grown on standard agar at 37°C 24 h^{-1}
Remaining notation as in Table 1

The following parasites were identified on the gills of tench: *Trichodina modesta* (Lom 1970), *Trichodinella epizootica* (Raabe 1950), *T. subtilis* (Lom 1959), *Dactylogyrus*

TABLE 5

Occurrence of parasites on the gills of tench from Oświn Lake

Parasites		Samples					
		1		2		3	
		E	W	E	W	E	W
T. modesta	P	17	0	0	0	0	0
T. epizootica	P	33	0	0	0	17	0
T. subtilis	P	0	20	20	22	0	0
D. tincae	P	0	0	0	0	0	10
Sanguinicola sp.	P	10	10	30	0	20	0
E. sieboldi	P	60	80	100	100	83	100
	I	5 - 270	1 - 85	9 - 602	24 - 121	3 - 160	15 - 201
	M	75.8	24.6	159.3	70.0	56.4	98.1
	RD	45.5	19.7	159.3	70.0	47.0	98.1

E – eastern part, W – western part
P – prevalence of infection (in %), I – intensity of infection min – max
M – mean intensity of infection, RD – relative density of infection
Remaining notation as in Table 1

tincae (Gussev 1965), *Sanguinicola* sp. -ova, *Ergasilus sieboldi* (Nordmann 1832). Of the ciliate species from the genus Trichodina and Trichodinella named above, eggs of Sanguinicola trematodes and the monogenetic trematode *D. tincae* occurred at a low prevalence and intensity of infection. However, tench infestation with the crustacean *E. sieboldi* remained high at both sampling stations throughout the study period. The level of infection in tench from station E intensified in summer and then decreased in fall. The intensity of crustacean infection on the gills of tench in the W area of the lake increased steadily and maximum values were reached in fall (Table 5).

The following parasites were identified on the gills of pike: myxosporean *Henneguya lobosa* (Cohn1895); ciliates *Trichodinella epizootica* and *T. subtilis*; monogenetic trematode *Tetraonchus monenteron* (Wagener 1957); crustacean *E. sieboldi*. Ciliates occurred in a significant number of fish at both sampling stations, mainly in spring. The degree of pike infection with *T. monenteron* in the E area of the lake decreased from spring to fall to 17%, but in fish from area W this figure increased in summer and fall and reached a level of 70% (Table 6). Infection with *E. sieboldi* increased at both sampling stations; the degree of infection was statistically significantly higher in October in pike from area E than in those from area W.

TABLE 6

Occurrence of parasites on the gills of pike from Oświn Lake

Parasites		Samples					
		1		2		3	
		E	W	E	W	E	W
H. lobosa	P	0	0	0	0	17	10
T. epizootica	P	0	60	0	0	0	0
T. subtilis	P	100	20	0	0	0	10
T. monenteron	P	43	30	40	75	17	70
E. sieboldi	P	29	40	90	75	100	90
	I	4 - 5	2 - 10	2 - 21	6 - 15	33 - 158	18 - 105
	M	4.5	6.2	10.9	9.3	106.5	59.9
	RD	1.3	2.5	9.8	7.0	106.5	53.9

E - eastern part, W - western part
P - prevalence of infection (in %), I – intensity of infection min - max
M - mean intensity of infection, RD - relative density of infection
Remaining notation as in Table 1

DISCUSSION

The number, distribution and composition of heterotrophic bacteria in a water basin attest to the quantity and quality of its organic elements (Zmysłowska 1987, Zmysłowska and Lewandowska 1999). The occurrence of higher numbers of the bacteria identified in the eastern part of the lake versus those in the western part is probably connected to the morphometric and hydrological parameters of Oświn Lake. Additionally, the eastern part of the lake receives Ruda River waters which play a decisive role in the input of eutrophic elements (Wiśniewski 2001a). The organic material in the upper layer of the bottom sediments of area E play a more significant role in the nutrient cycle in the Oświn Lake ecosystem than do those in area W (Wiśniewski 2001b).

The greatest development of bacterial microflora in Oświn Lake was observed in summer and was related to the temperature of this shallow basin which warms up quickly. A large amount of trophic elements which are available to the producers are released into the waters of Oświn from the layer of sediments which are in resuspension (Wiśniewski 2001 b). This is connected to the higher number of bacteria in the near-bottom layer (Table 3). Niewolak (1974) and Lewandowska et al. (2000) obtained similar results. Increased numbers of bacteria in the near-bottom layer can be explained by the settling of detritus with adsorbed bacterial cells and the longer survival of microbes. The results obtained by the authors of the current study (TVC 20°C and TVC 37°C in the range of 10^2- 10^3), indicate that Oświn Lake is polluted to a significant degree with easily decomposed organic material. This is related to the eutrophication of the studied basin and is probably caused by repeated pollution by birds and the remains of vegetation and animals.

There was no relationship between the dynamics of the abundance of bacterial microflora on the gills of tench and pike caught in the two sampling areas of Oświn Lake. The number of TVC 20°C and TVC 37°C bacteria on the gills was higher than that in the water and was generally in the range of $10^4 – 10^7$ per 1 cm^2; this confirms the findings of other authors (Lesel and Peringer 1981, Zaleski 1985, Spanggaard et al. 1993). Zmysłowska et al. (2000) reported the number of bacteria on the surface of fish to be within the range of $10^2 – 10^7$. The fluctuation in numbers is a reflection of the fish inhabiting waters with varying degrees of pollution with heterotrophic microflora.

The bacteria *Pseudomonas fluorescens* and *P. aeruginosa* generally occurred in numbers ranging from several dozen to several thousand on 1 cm^2 of fish gill. The number of them was higher on the gills of both tench and pike in summer in both areas of Oświn Lake. This tendency was also observed in the waters of the western and eastern areas of the lake. The microflora on the fish reflected the state and developmental tendencies of the bacteria in the water and was also dependent on the impact of prevailing environmental conditions.

The current microbiological studies characterize Oświn Lake as a basin with a high degree of eutrophication. The parasitological analyses of tench and pike also confirm that this basin is eutrophic since the parasites found are characteristic of eutrophic waters, including monogenetic trematodes, Ergasilus, and ciliates. The trematode *Tetraonchus monenteron*, which infected pike, tops the list of the most common parasites in eutrophic water bodies (Dechtiar and Christie 1988). This large parasite, which is associated with pike, moves around the gills causing great damage to its surface. It is usually found at a low prevalence of infection, for example, in from 6.9 to 11.7% of the pike in Drużno Lake, or in 3.7% of them in Warniak Lake (Kozicka 1963, Grabda-Kazubska et al. 1987). This parasite was not detected in the fish of Oświn Lake in August 1958 (Grabda and Grabda 1958) or in November 1997 (Własow et al. 1998). Not until 1999 did the prevalence of *T. monenteron* in the studied lake reach 75%. It is possible that *T. monenteron* occur at a greater prevalence and intensity of infection in younger, more susceptible fish which were not the subject of this study, thus causing respiratory difficulties or death. Without the appropriate studies, it will be difficult to determine if this pike parasite, which is new to Oświn Lake, is responsible for the weak growth rates in the first years of life of this fish (Chybowski et al. 2001).

Ergasilus sieboldi also poses a serious threat to pike. In comparison with tench, the primary host of this parasite, pike exhibited lower resistance to the fall infestation of the new generation of this crustacean (Pojmańska 1984) (Table 5). Numerous ciliates were detected in pike with lowered condition in the spring. Based on the parasitological results above, the prognosis for pike health is not good, especially following the attempt to renaturalize Oświn Lake.

Although the degree of *E. sieboldi* infection of tench was high, the maximum intensity of it did not exceed the level from the 1950s (926 individuals - Grabda and Grabda 1958; 1041 individuals - Kozicka 1963). The infestation of tench gills by Trichodinidae, *D. tincae* and Sanguinicola was not great, and this might attest to a certain stability in the health of tench in Oświn Lake. This state should be correlated with the greater water volume in the lake after the water level was increased.

E. sieboldi should be considered a significant parasite in both tench and pike. Increased *E. sieboldi* infection exhibited a similar tendency to the increased numbers of TVC 20°C and TVC 37°C heterotrophic bacteria on the gills. This can be related to the greater susceptibility of gills with parasitic crustacean infections to potentially pathogenic bacteria.

CONCLUSIONS

1. The results of microbiological studies of the water indicate that there is a high degree of organic matter pollution in the waters of Oświn Lake.
2. The total number of bacteria on the gills of fish indicate that this lake has a high level of eutrophication.
3. The occurrence of the *T. monenteron*, Ergasilus, and Trichodinidae parasites indicate this lake is eutrophic.
4. The more prevalent parasitic infection of pike in the eastern area of the lake and the higher number of heterotrophic bacteria in the water indicate that this area of Oświn Lake is more fertile.
5. Based on microbiological and parasitological studies, the prognosis for the health of pike, especially in the eastern area of Oświn Lake, is not good.

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STRESZCZENIE

BAKTERIE I PASOŻYTY NA SKRZELACH SZCZUPAKA *ESOX LUCIUS* L. I LINA *TINCA TINCA* (L.) JAKO WSKAŹNIK TROFII JEZIORA OŚWIN

Badania prowadzono na jeziorze Oświn, które jest częścią rezerwatu przyrody „Siedem Wysp ",objętego specjalną ochroną (konwencja RAMSAR). Praca dotyczyła mikrobiologicznych i parazytologicznych oznaczeń w materiale pochodzącym ze skrzeli szczupaka i lina. Równolegle prowadzone były badania bakteriologiczne wody jeziornej. Materiał do badań pobierano na przełomie maja i czerwca, w lipcu i październiku 1999 r. Badania bakteriologiczne obejmowały oznaczenia ogólnej liczby bakterii na podłożu agarowym zwykłym w temperaturze 20°C i 37°C oraz bakterii *Pseudomonas fluorescens* i *P. aeruginosa*. Badania parazytologiczne dotyczyły oznaczeń występujących na skrzelach pasożytniczych pierwotniaków i Metazoa.

Duża liczebność bakterii heterotroficznych zarówno w wodzie (tab. 3), jak i w skrzelach badanych ryb (tab. 4) świadczy o znacznym stopniu trofii jeziora Oświn.

Analiza parazytologiczna ujawniając znaczny stopień zarażenia skrzeli pasożytami (tab. 5 i 6) typowymi dla wód zeutrofizowanych potwierdza wyniki badań mikrobiologicznych.

Obserwowano zbieżność występowania na skrzelach wysokiej liczebności bakterii TVC 20°C, TVC 37°C (tab. 4) i wysokiego stopnia zarażenia skorupiakiem *E. sieboldi* u szczupaka (tab. 6) we wschodniej części jeziora. Według badań bakteriologicznych ta część zbiornika charakteryzuje się wyższą trofią (tab. 3).

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