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MICROBIOLOGICAL STUDIES OF TENCH (*Tinca tinca* L.) AND WATER OF DGAŁ WIELKI LAKE

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ABSTRACT. Microbiological analyses were carried out of the digestive tract contents of tench and of water from Lake Dga ł Wielki. Qualitative and quantitative parameters were evaluated. Numbers of bacteria indicatory of water production (TVC 20°C and TVC 37°C) and sanitary state (TC, FS, FS), *Clostridium perfringens*, and *Pseudomonas aeruginosa* were calculated. Qualitative analyses involved classification of the genera of bacteria isolated on agar in 20°C and on Endo medium in 37°C from the lake water and tench digestive tract contents.

Keywords: BACTERIA, TENCH (*Tinca tinca* L.), DIGESTIVE TRACT CONTENTS, LAKE WATER

INTRODUCTION

Tench is a valuable freshwater fish, fairly resistant to adverse environmental conditions. Due to considerable ecological and feeding plasticity (Galinat 1978) tench occurs in most Polish fresh waters. In eutrophic lakes populations of other valuable fish species, more susceptible to unfavorable environmental conditions, become reduced or extinct, but tench is present in quite high densities even in strongly eutrophic reservoirs (Prejs 1978). Tench is an indicatory species of shallow, productive lakes of wide littoral zone. It occurs in the reservoirs where temporary DO depletion and considerable temperature fluctuations take place. Deterioration of water quality causes changes in qualitative and quantitative composition of microflora living in fish and in water. In fish, most numerous micro-organisms occur on the skin surface (Gillespie and Macrae 1975, Spanggaard et al. 1993) and in the digestive tract (Lesel 1979 a, Zaleski 1985, Sugita et al. 1991). Differences of their density result from environmental conditions, mainly contamination with heterotrophic bacteria (Zmysłowska et al. 2000).

Water pollution and the related occurence of bacteria indicatory of sanitary state and contamination are reflected in bacteriological state of the fish. This was confirmed, among others, by Zmysłowska et al. (2000), and Niewolak and Tucholski (1995b). The results by Geldreich and Clarke (1966) indicate that 30.8% of coliform strains and 31% of fecal coliform strains from Little Miami River sampled at the site of

fish harvest were identical with bacteria strains present in fish digestive tracts. Presence of coliforms and fecal Streptococci in fish guts strongly depends on water quality, while fish are only their carriers (Trust and Sparrow 1974).

Many authors observed that micro-organisms present in water, and other environmental conditions affect qualitative composition of microflora in fish (Lesel 1979 b, Esteve and Garay 1991, Sugita et al. 1983). Austin and Allen-Austin (1985) reported that in aquatic ecosystems such genera as Acinetobacter, Bacillus, Corynebacterium, Cytophaga, Flavobacterium, Micrococcus, Moraxella and Pseudomonas usually predominated. These authors found mainly Aeromonas, Acinetobacter, Pseudomonas in digetive tracts of freshwater fish, and Enterobacter, while Vibrio, Aeromonas, and Pseudomonas were most frequent in marine fish.

The present study was undertaken to evaluate composition and density of bacterial microflora in Dgał Wielki lake water and in tench digestive tracts. Quantitative analyses involved determination of the numbers of indicatory bacteria of pollution and sanitary state (micro-organisms grown on common agar at 20 ^{o}C and 37°C, coliforms, fecal coliforms), *Clostridium perfringens* and *Pseudomonas aeruginosa*. Qualitative analyses involved identification of genera of bacteria grown on standard agar at 20 ^{o}C, and on Endo medium at 37 ^{o}C.

MATERIAL AND METHODS

1. **Material.** Water from Dgał Wielki Lake and digestive tract contents of tench (*Tinca tinca* L.) from this lake were analysed.
2. **Study site.** Lake Dgał Wielki is situated in the Warmia and Masuria Voivodship. According to the fishery classification it is a bream lake. Detailed data are shown in Tab. 1
3. **Sampling.**
   a) Lake water. Samples of water for bacteriological analyses were taken three times – in May, October and November 1994, simultaneously with fish sampling. Water was collected in 100 ml sterile flasks.
   b) Tench digestive tract contents. The fish were harvested using a tow net. Three individuals were randomly selected for the analyses, placed in a tank filled with lake water, and transported to the laboratory. The fish were dissected under sterile conditions and digestive tracts were isolated to sterile vessels and weighed.

TABLE 1

Limnological and geographic data of Dgał Wielki Lake

Area	water	93.92 ha
	islands	0.01 ha
Depth	maximum	18.8 m
	average	5.75 m
Depth index		0.3
Water volume		$5388.9 \cdot 10^3$ m^3
Dimensions	maximum length	1275 m
	maximum width	1110 m
Shoreline development index		1.3
Geographic position	latitude	54°06.5′
	longitude	21°47.7′

4. Microbiological studies.

a) Quantitative analyses. Digestive tract contents of tench and water of Dgał Wielki Lake were analysed for:

1. total number of bacteria grown on standard agar for 48 h in 20°C (TVC 20 °C) in 1 cm^3 of water and 1 g of gut content.
2. total number of bacteria grown on standard agar for 24 h in 37°C (TVC 37 °C) in 1 cm^3 of water and 1 g of gut content.
3. total number of coliform bacteria (TC) incubated for 48 h in 37°C on Eijkman medium as most probable number (MPN) per 100 cm^3 of water, and on Endo medium as colony-forming units (CFU) in 1 g of fish gut content.
4. number of fecal coliforms (FC) incubated for 24 h in 44.5°C on Eijkman medium as most probable number (MPN) per 100 cm^3 of water, and on Endo medium as colony-forming units (CFU) in 1 g of fish gut content.
5. number of fecal Streptococci (FS) incubated for 72 h in 37°C on broth medium with sodium azide and dextrose, as most probable number (MPN) per 100 cm^3 of water, and on Enterococci plus agar medium as colony-forming units (CFU) in 1 g of fish gut content.
6. number of sporaceous anaerobes (*Clostridium perfringens*) incubated for 18 h at 37 °C on Wilson-Blair medium after pasteurization of water for 10 min. in 80°C (Przesmycki 1953), per 1 cm^3 of water or 1 g of fish gut content.
7. number of *Pseudomonas aeruginosa* incubated for 48 h in 37°C on Kinga A

medium (Burbianka and Pliszka 1983), per 1 cm^3 of water or 1 g of fish gut content.

The colonies obtained on Kinga A were subjected to further confirmation test (Dutka and Kwan 1977).

All analyses were performed in 3 replicates. The samples were diluted with physiological solution (NaCl 0.85%).

Water. The results obtained using plate method were recalculated per colony-forming units (CFU), per 1 cm^3 of water. Most probable number (MPN) of bacteria was evaluated using three tube sets per each dilution. MPN values were read from McCrady's tables, and recalculated per 100 cm^3 of water (Paluch 1973).

Fish. Samples of 1 g of tench digestive tract content were diluted 10 times with physiological NaCl solution (0.85%) and homogenized in sterile mortars with sea sand. Further dilutions were then introduced into appropriate media, each in three replicates. Cultured colonies were counted, and recalculated for colony-forming units (CFU) per 1 g of gut content.

b) Qualitative analyses. All types of colonies were isolated from the cultures of bacteria from tench digestive tract content and from water of Dgał Wielki lake, grown on common agar in 20°C for 72 h, and in 37°C for 48 h. The colonies were then inoculated into the same media to obtain single strains. Each monoculture was subjected to morphological analyses: Gram-staining, cell shape determination, motility test, sporulation test, and cytochrome oxidase test. The strains were identified to genus according to Shewan et al. (1960 a, b). Biochemical properties of *Enterobacteriaceae* were determined using multi-test media kits „Enteroplast" made by Plastomed.

Totally 400 bacterial strains isolated from tench digestive tract contents (290 strains) and from Dgał Wielki Lake water (110 strains) were studied.

RESULTS

The results of quantitative microbiological analyses of Dgał Wielki Lake water and tench digestive tract contents are shown in Tables 2 and 3.

Numbers of bacteria cultured on standard agar in 20°C (TVC 20°C), and in 37°C (TVC 37°C), and coliforms (TC) in water and fish gut contents were higher in May than in October and November. Numbers of TVC 20°C in May were 4.2×10^3 cm^{-3} in lake

TABLE 2

Numbers of bacteria per 1 cm^3, or MPN per 100 cm^3 in the water of Dgał Wielki lake

Bacteria	Month		
	May	October	November
TVC 20 °C	4.2×10^3	5.3×10^2	2.1×10^2
TVC 37°C	6.4×10^2	2×10^1	9×10^1
TC*	2.4×10^2	1.5×10^1	4.3×10^1
FC*	0	0.7×10^1	0
FS*	0.4×10^1	2.3×10^1	2.3×10^1
Pseudomonas aeruginosa	0	1	-

TVC 20°C – bacteria grown on standard agar in 20°C for 48 h.
TVC 37°C – bacteria grown on standard agar in 37°C for 24 h.
**MPN – most probable number*
TC – coliforms cultured on Eijkman's medium in 37°C for 48 h.
FC – fecal coliforms cultured on Eijkman's medium in 44.5°C for 24 h.
FS – fecal Streptococci grown on broth medium with sodium azide and dextrose in 37°C for 72 h.

TABELA 3

Numbers of bacteria per 1 g of tench digestive tract contents

Bacteria	Month		
	May	October	November
TVC 20 °C	3.9×10^5	9.7×10^3	3.3×10^4
TVC 37 °C	1.6×10^3	1.7×10^3	6.2×10^3
TC	9×10^2	1.3×10^1	2.3×10^1
FC	0	0	0
FS	0	0.6×10^1	1.3×10^1
Pseudomonas aeruginosa	0	1.3×10^1	-

TVC 20°C – bacteria grown on standard agar in 20°C for 48 h.
TVC 37°C – bacteria grown on standard agar in 37°C for 24 h.
TC – coliforms cultured on Endo medium in 37°C for 48 h.
FC – fecal coliforms cultured on Endo medium in 44.5°C for 24 h.
FS – fecal Streptococci grown on Enterococci plus agar medium in 37°C for 72 h.

water and 3.9×10^5 g^{-1} of fish gut contents. In October the values of TVC 20°C were 530 per cm^3 and 9.7×10^3 g^{-1}, and in November 210 per cm^3 and 3.3×10^4 g^{-1} respectively.

Numbers of TVC 37°C in water were 640 in May, 20 in October, and 90 cells per cm^3 in November. In tench digestive tract contents the numbers were: 1.6×10^3, 1.7×10^3 and 6.2×10^3 per 1 g respectively.

Total number of coliforms (TC) in water, expressed as MPN per 100 cm^3 ranged

from 15 to 240 cells. In fish gut contents this number was expressed as CFU and it ranged from 13 to 900 cells per 1 g.

Fecal coliforms (FC) were present in water only in October, and their MPN was 7 cells per 100 cm^3. The remaining water samples (May and November) and all fish digestive tract content samples were free from these bacteria.

Fecal Streptococci (FS) were scarce in water. Their MPN was 4 in May, while in October and November there were 23 cells per 100 cm^3. No fecal Streptococci were found in fish gut contents in May; in October their number was 6, and in November there were 13 cells per 1 g.

Pseudomonas aeruginosa were absent from water and fish digestive tract contents in May and November. In October 1 cell per cm^3 was observed in water, and 13 cells per 1 g of tench gut contents.

No *Clostridium perfringens* was found either in the water or in fish digestive tract contents at any time, so the results are not shown in the tables.

The results of qualitative microbiological studies of lake water and tench gut contents are shown in Tables 4 and 5. Among the bacteria grown on standard agar in 20°C (TVC 20°C) (Tab. 4) 60 strains were isolated from water, and 180 strains from the fish gut contents. Bacteria of Aeromonas genus were most common in water and fish gut contents, comprising 30% and 40.6%, respectively.

TABLE 4

Composition of bacterial communities isolated on standard agar in 20°C from water of Dgał Wielki Lake (W) and from tench digestive tract contents (T).

Bacteria	W		T	
	N	%	N	%
Aeromonas	18	30.0	73	40.6
Pseudomonas	15	25.0	0	0.0
Acinetobacter	7	11.7	26	14.4
Vibrio	6	10.0	24	13.3
Plesiomonas	3	5.0	24	13.3
Bacillus	3	5.0	0	0.0
Sarcina	2	3.3	10	5.6
Niesklasyfikowane	6	10.0	23	12.8
Totally	60	100.0	180	100.0

N – number of isolated bacterial strains

TABLE 5

Composition of Enterobacteriaceae isolated on Endo medium in 37°C from water of Dgał Wielki Lake (W) and from tench digestive tract contents (T)

Bacteria	W		T	
	N	%	N	%
Yersinia	17	34.0	42	38.1
Enterobacter	15	30.0	22	20.0
Citrobacter	9	18.0	26	23.6
Proteus	6	12.0	10	9.1
Escherichia	2	4.0	-	-
Serratia	1	2.0	7	6.6
Edwardsiella	-	-	3	2.6
Totally	50	100.0	110	100.0

N – number of isolated bacterial strains

Sarcina genus was the least frequent – 2% in water and 5.6% in tench gut contents. Such genera as Pseudomonas, Acinetobacter, Vibrio, Plesiomonas, and Bacillus were also found in lake water. In tench digestive tract contents no Pseudomonas or Bacillus were observed.

Among bacteria grown on Endo medium in 37°C, 50 strains were isolated from water, and 100 strains from the fish gut contents (Tab. 5). In this group Yersinia predominated, comprising 34% in water and 38.1% in tench digestive tracts. The bacteria of Serratia genus were least common in water – 2%, and Edwardsiella in the tench intestine contents – 2.6%.

No Escherichia were found in the fish gut contents, and no Edwardsiella in lake water.

DISCUSSION

The results of microbiological analyses of lake water and tench digestive tract contents concerning bacteria grown on standard agar in 20°C (TVC 20°C) and at 37°C (TVC 37°C), and coliform bacteria (TC) revealed considerable differences between spring (May) and autumn (October, November) in both types of environment, and between them. Micro-organisms of these groups were more numerous in spring than in autumn, as well in water samples as in tench digestive tract contents. No differences were found between the samples taken from both types of environment in October and November.

Density of bacteria, and other biological indices are related to physio-chemical environmental factors, and fluctuate within a range. Peak density of bacteria may occur at various time. In eutrophic Mikołajskie Lake the lowest numbers of bacteria were observed in spring, somehow higher in autumn, and the highest – in summer (Godlewska-Lipowa 1975). Similar results were obtained for Szczycieńskie lakes (Godlewska-Lipowa et al. 1974), and for Kortowskie Lake (Zmysłowska 1987). Czeczuga and Czerpak (1967) observed peak density of bacteria in Legińskie Lake in spring.

Bacterial microflora of fish belong to psychrophilic group (Prost 1980). This was confirmed by the results of the present study, showing that heterotrophic bacteria TVC 20°C were always more numerous in the water and fish samples compared to TVC 37°C.

Comparison of both types of environment shows that TVC 20°C, TVC 20°C, and coliforms (TC) were always more abundant in tench digestive tracts than in lake water. This is consistent with the results of other studies (Niewolak and Tucholski 1995 a, b, Zmysłowska et al. 2000). Similar results were also obtained by Esteve and Garay (1991) who observed 10^3-10^4 heterotrophic bacteria per cm^3 in the eel tank water, while in the eels themselves bacteria were more numerous – 10^5-10^6 per 1 g.

Different results were obtained in tank rearing of tench (Zmysłowska et al. 1999), when the numbers of heterotrophic bacteria in the water and fish gut contents were similar, ranging from 10^3 to 10^4 cell, and for TVC 20°C from 10^2 to 10^3 per 1 cm^3 or 1 g for TVC 37°C.

Additionally to quantitative relations between water and fish bacterial communities, also qualitative relationships were found. The data of these and other studies (Trust and Sparrow 1974, Lesel 1979 a, Lewandowska 1998, Zmysłowska et al. 2000) indicate that Gram-negative bacilli predominated in both types of environment.

Lesel (1979 b) observed that not all bacterial taxa present in water were found in fish digestive tracts. This was confirmed by the results of this study, which showed that such genera as Pseudomonas, Bacillus or Escherichia, which were present in water, were absent from tench digestive tract contents. Lesel (1979 b) observed 18 groups of bacteria in *Salmo gairdneri*, among which Pseudomonas and Enterobacteriaceae predominated. In the group of gram-positive microorganisms, corynebacteria and Streptococcus were most common.

According to Esteve and Garay (1991), among 17 bacterial strains isolated from the water Pseudomonas were most frequent, comprising 53%, followed by Acinetobacter

(9.5%), Moraxella (8.2%), and Micrococcus (6.1%). In bacterial community of eel also Pseudomonas predominated (37%), accompanied by Aeromonas (32.4%), *Micrococcus* (8.3%), and Acinetobacter (4.8%).

The results of this study showed considerable diversity of bacteria isolated from the water and tench gut contents. Among the bacteria grown on standard agar in 20°C, Aeromonas predominated, comprising 40.6% in fish digestive tracts and 30% in the water. They were accompanied by Acinetobacter, Vibrio, Sarcina, Plesiomonas, while Pseudomonas and Bacillus were present only in water samples. Among Enterobacteriaceae genus Yersinia was most common, as well in fish (38.1%) as in water (34%). The remaining isolated strains were identified as Enterobacter, Citrobacter, Proteus, Escherichia (only in water), and Serratia, Edwardsiella (only in fish).

CONCLUSIONS

1. Bacteria grown on standard agar in 20°C and in 37°C, and coliform bacteria were more numerous in spring (May) than in autumn (October, November), in water of Dgał Wielki Lake, and in tench digestive tract contents.
2. Numbers of bacteria grown on standard agar in 20°C and in 37°C, and of coliform bacteria in the fish gut contents were 10 to 100 times higher compared to lake water.
3. Among the isolated bacterial strains grown on standard agar in 20°C Aeromonas predominated comprising 30% in water and 40.6% in tench digestive tracts.
4. Among bacterial strains grown on Endo medium in 37°C, Yersinia were most frequent, comprising 34% in water and 38.1% in tench digestive tract contents.

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STRESZCZENIE

MIKROBIOLOGICZNE BADANIA LINA (*Tinca tinca* L) I WODY Z JEZIORA DGAŁ WIELKI

Przeprowadzono ilościowe i jakościowe badania treści przewodów pokarmowych lina i wody jeziora Dgał Wielki. Badania ilościowe obejmowały oznaczenia bakterii wskaźnikowych stopnia zanieczyszczenia (TVC 20 °C i TVC 37 °C) i stanu sanitarnego (TC, FC, FS), *Cl. perfringens* i *P. aeruginosa*. Drobnoustroje wymienionych grup występowały liczniej na wiosnę (maj) niż jesienią (październik, listopad) i to zarówno w próbach wody jak i treści pokarmowej lina. Na podstawie wyników badań stwierdzono, że liczby oznaczanych drobnoustrojów w treści przewodów pokarmowych lina były od kilku do stu razy wyższe niż w wodzie jeziorowej.

Badania jakościowe obejmowały identyfikację bakterii wyizolowanych na podłożu agarowym zwykłym w temperaturze 20 °C (TVC 20 °C) i na podłożu Endo w temp. 37 °C (z rodziny Enterobacteriaceae) z wody jeziornej i treści pokarmowej ryb.

Wśród bakterii TVC 20 °C wyizolowano 60 szczepów z wody reprezentujących siedem rodzajów (Aeromonas, Pseudomonas, Acinetobacter, Vibrio, Plesiomonas, Bacillus, Sarcina) i 180 szczepów z treści pokarmowej lina, reprezentujących pięć rodzajów (oprócz Pseudomonas i Bacillus takie same rodzaje jak w wodzie). Dominowały gramujemne pałeczki z rodzaju Aeromonas, które w wodzie stanowiły 30% a w treści przewodów pokarmowych lina 40,6 %. Nie sklasyfikowano 10 % szczepów pochodzących z wody i 12,8 % pochodzących z treści pokarmowej ryb. Wśród bakterii wyrosłych na podłożu Endo w 37 °C (z rodziny Enterobacteriaceae) wyizolowano 50 szczepów z wody i 110 z treści pokarmowej lina. Wśród zidentyfikowanych szczepów z rodziny Enterobacteriaceae dominowały pałeczki z rodzaju Yersinia, stanowiąc w wodzie 34 % a w treści pokarmowej ryb 38,1 %.

Pozostałe szczepy należały do rodzajów: Enterobacter, Citrobacter, Proteus, Escherichia (tylko w wodzie), Serratia, Edwardsiella (tylko w treści przewodów pokarmowych ryb).

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