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MICROBIOLOGICAL STUDY OF IDE (*LEUCISCUS IDUS* L.) FROM PONDS OF DIFFERENT TROPHY

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ABSTRACT. Microbiological status of water and ide (*Leuciscus idus* L.) reared in polyculture with common carp was evaluated. The fish were reared in the Ostróda-Warlity Fish Farm, in two ponds supplied with the water from Szeląg Wielki Lake, and in one biological pond supplied with treated domestic wastewater in Łężany Fish Farm. Quantitative analyses comprised counts of bacteria grown on standard agar in 20°C and 37°C, total counts of coliforms, fecal coliforms, fecal streptococci, ammonifying bacteria and fungi in water of the three ponds and in muscles, skin mucus, and digestive tract contents of ide. Qualitative analyses consisted of identification to the genus bacteria isolated on standard agar in 20°C, and on Endo medium in 37°C, from the digestive tract contents of ide.

Keywords: MICRO-ORGANISMS, BACTERIAL INDICES, IDE (*LEUCISCUS IDUS* L.), POND WATER.

INTRODUCTION

Microflora found in fish is related to various factors such as developmental stage of the fish, digestive tract structure, water temperature, site, food availability, and physiological state of the organism (Sugita et al. 1985).

Micro-organisms living in mucus on the skin surface, gills, and in the digestive tract of fish in different water bodies varies in terms of composition and abundance (Lésel 1979, Sugita et al. 1991, Spanggaard et al. 1993). Density of bacteria in mucus ranges from 10^2 to 10^7 per cm^2 of skin, the differences being related to different contamination of water with heterotrophic bacteria (Zaleski 1985). Abundant microflora is also present in fish intestines, reaching 10^3-10^8 per 1 g of gut contents. Microbiological study of tench from Dgał Wielki Lake (Zmysłowska et al. 2000b) revealed that numbers of heterotrophic bacteria in the digestive tract ranged from 10^4 to 10^5 cells per 1 g. Other studies (Horsley 1973, Trust and Sparrow 1974, Lésel 1976) showed that density of digestive tract microflora in salmonid fish reached a maximum of 10^8 per 1 g of the intestine contents, and in rainbow trout - 10^8 per 1 g of stomach contents,10^6 in the pyloric caeca, and 10^8 in the middle and posterior intestine.

Composition and density of the micro-organisms inhabiting various parts of fish digestive tract or body surface is related to their presence in feeds and in the

environment (Lewandowska 1998). Studies of Lésel and Péringer (1981) showed that microflora of digestive tract contents was more abundant than bacterial community of the gut wall itself. Bacteria may proliferate in the gut, the process being strongly affected by the food. Fish feeding with sterile food reduced bacterial counts in salmonid digestive tracts (Trust 1975). Sera and Ishida (1972) observed that feed absorption caused an increase of bacterial counts in the intestines of *Pagrus major* and *Erynnis japonica*. Also in *Cyprinus carpio*, *Ctenopharyngodon idella*, and *Tinca tinca* the highest densities of bacteria were found during active fish feeding.

Austin and Allen-Austin (1985) reported that such genera as *Achromobacter*, *Acinetobacter*, *Bacillus*, *Corynebacterium*, *Cytophaga*, *Flavobacterium*, *Micrococcus*, *Moraxella* and *Pseudomonas* were most common in aquatic ecosystems. These authors found mainly: *Aeromonas*, *Acinetobacter*, *Pseudomonas*, and *Enterobacter* in freshwater fish digestive tracts, while *Vibrio*, *Aeromonas*, and *Pseudomonas* were most frequent in marine fish. Yoshimizu et al. (1980) found different bacterial communities in juvenile and adult salmons. Similar differences were found in tilapia (Sugita et al. 1982), and sole, *Solea solea* (Cambell, Buswell 1983).

The objectives of the present study were to evaluate:

1) counts of bacteria indicatory of organic pollution and sanitary state, and abundance of fungi in water of three ponds of different trophy, and in body surface mucus, flesh, and digestive tract contents of ide reared in these ponds.
2) composition of bacterial genera isolated from ide digestive tracts on standard agar in 20°C, and on Endo medium in 37°C.

MATERIAL AND METHODS

1. Water. Samples were collected from 3 ponds:
   - No 12 and No 26 in Warlity Fish Farm near Ostróda,
   - B ("biological") in Łężany Fish Farm.

The ponds 12 and 26 were supplied with water from Szeląg Wielki Lake, and pond B – with treated wastewater from a three-phase sewage treatment plant in Łężany. Ide was reared in polyculture with common carp in all these ponds.

2. The ide (*Leuciscus idus* L.). The fish were collected from three ponds: 12, 26 and B.

Water and fish were sampled three times from each pond, at weekly intervals, in July 1995.

Totally 27 water samples and 45 fish samples were analysed.

3. Methods

Microbiological analyses of bacterial sanitary indices and of various physiological groups and species of bacteria and fungi were carried out using appropriate media and incubation conditions according to Table 1.

TABLE 1

Micro-organisms	Medium and incubation	References
Heterotrophic bacteria (TVC 20°C)	Standard agar (Bacto-agar DIFCO) 20°C / 72 h	Burbianka and Pliszka 1983
Heterotrophic bacteria (TVC 37°C)	Standard agar (Bacto-agar DIFCO) 37°C / 48 h	Burbianka and Pliszka 1983
Coliforms (TC)	Eijkman (MERCK) 37°C / 48 h *Endo 37°C / 48 h	Burbianka and Pliszka 1983
Fecal coliforms (FC)	Eijkman (MERCK) 44.5°C / 24 h *Endo 44.5°C / 24 h	Burbianka and Pliszka 1983
Fecal Streptococci (FS)	Enterococci confirmatory broth (DIFCO) 37°C / 72 h *Enterococci plus agar (DIFCO) *Endo 37°C / 48 h	Pawlaczyk-Szpilowa 1980
Sporaceous anaerobes (*C. perfringens*)	Wilson-Blair 37°C / 18 h samples pasteurised for 10 min. / 80°C before inoculation	Przesmycki 1953
Ammonifying bacteria	Broth-agar with 3% peptone 25°C / 72 h	Rodina 1968
Pseudomonas aeruginosa	Kinga A 42°C / 48 h	Burbianka and Pliszka 1983
Pseudomonas fluorescens	Kinga B 25°C / 72 h	Burbianka and Pliszka 1983
Aeromonas sp.	mA 37°C / 48 h	Rippey and Cabelli 1979
Fungi	Sabouraund (BIOMED) 28°C / 7 days	Pawlaczyk-Szpilowa 1980

Points 3, 4, 5 – MPN per 100 cm^3 of water
**- for fish*

The colonies grown on Kinga A were subjected to confirmatory test for *P. aeruginosa* (Dutka and Kwan 1977). The colonies on Kinga B were confirmed for *P. fluorescens* (Shewan et al. 1960 a).

4. Microbiological analyses

1. Quantitative analyses

Water – Samples were diluted with physiological NaCl solution (0.85%). Inoculations were performed in 3 replicates. The results were obtained using plate method, and counted colonies were recalculated as colony forming units (CFU) per 1 cm^3 of water. The most probable numbers (MPN) of bacteria were evaluated using three tube sets per each dilution. MPN was read from Mc Crady's tables per 100 cm^3 of water (Paluch 1973).

Fish – Samples were taken of mucus from 1 cm^3 of skin, 1 g of muscle tissue, and 1 g of digestive tract contents of the ide. They were transferred to sterile mortars with sea sand, diluted 10 times with physiological NaCl solution (0.85%), and thoroughly ground. Further dilutions were made and inoculated into appropriate media in 3 replicates. The colonies were counted after incubation, and recalculated per colony-forming units (CFU) per 1 g of flesh, 1 g of gut contents, and in case of mucus – per 1 cm^2 of skin.

2. Qualitative analyses

Composition of bacterial genera was determined in samples from digestive tracts. Samples were inoculated onto standard agar and incubated in 20°C for 72 h, and onto Endo medium (incubated in 37°C for 48 h). All different colonies were transferred separately to the same media to obtain pure strains. Each monoculture was subjected to morphological analyses after the incubation: gram-staining, evaluation of cell shape, motility and sporulation tests. The strains were identified to genus according to Shewan et al. (1960 a, b), and using "Enteroplast®" tests made by Plastomed.

Total number of 573 strains were studied, including 345 strains isolated on standard agar in 20°C, and 228 strains isolated on Endo medium in 37°C. Percentages of various bacterial genera in the digestive tract contents of ide from different ponds were evaluated.

RESULTS

Average zooplankton abundance in the ponds is shown in Table 2. The highest zooplankton biomass was observed in pond B ("biological") supplied with treated

domestic wastewater from the sewage treatment plant in Łężany. Ponds 12 and 26 supplied with the water from Szeląg Wielki Lake showed lower zooplankton abundance.

TABLE 2

Average zooplankton abundance in ponds in the period March-October 1995 (Guziur 1995)

Pond location	Zooplankton abundance mg dm^{-3}		Estimation of zooplankton abundance (relative index)
	Biomass (mean)	Range (min - max.)	
„biological" - B Łężany	34.69	1.25 - 74.79	360
nr 12 Warlity	22.95	0.75 - 158.7	230
nr 26 Warlity	9.96	1.83 - 34.53	100

TABLE 3

Average number of micro-organisms per 1 cm^3 and MPN per 100 cm^3.

Micro-organisms	Pond no. 12	Pond no. 26	Pond B
Heterotrophic bacteria TVC 20 °C	2 600	900	1 000 000
Heterotrophic bacteria TVC 37 °C	180	80	20 000
Coliform bacteria, TC*	450	450	250 000
Fecal coliform bacteria, FC*	250	230	9 500
Fecal streptococci, FS*	9 500	250	2 500
Ammonifying bacteria	600	100	1 000 000
Pseudomonas aeruginosa	0	0	10
Pseudomonas fluorescens	60	10	50
Aeromonas sp.	0	0	1
Fungi	280	370	450 000

* *MPN (most probable number) in 100 cm^3*

Counts of various groups and species of bacteria living in pond water are shown in Table 3. The highest densities of bacteria, except FS, were present in pond B, lower numbers were observed in pond 12, and the lowest – in pond 26. Also fungi were most abundant in pond B, considerably less numerous in 26, and the least numerous in the pond 12.

P. aeruginosa were absent from ponds 12 and 26. Sporaceous anaerobes were never found (*C. perfringens*) so no data are shown.

Quantitative data for fish are presented in Table 4. The highest numbers of bacteria were found in ide digestive tract contents, lower – in the mucus, and the lowest – in fish

flesh. TVC 20°C, TVC 37°C, ammonifying bacteria and fungi were more abundant than other micro-organisms in all samples. No coliforms (TC) were found in flesh of the fish from pond 12 and 26, and in the mucus of fish from pond 12. Fecal coliforms (FC) were absent from all samples of ide from ponds 12 and 26. No fecal streptococci were observed in the flesh of fish from these two ponds, or Aeromonas in flesh of any fish. No *C. perfringens*, *P. aeruginosa* or *P. fluorescens* were found in fish, so no results are shown.

TABLE 4

Average microbial counts in mucus (per 1 cm^2 of skin), muscle tissue (per 1 g), and digestive tract contents (per 1 g) of the ide (*Leuciscus idus* L.). M – muscle tissue, S – mucus, T – digestive tract contents

Micro-organisms	Pond no. 12			Pond no. 26			Pond B		
	M	S	T	M	S	T	M	S	T
Heterotrophic bacteria TVC 20 °C	170	7 000	20 500	90	7 000	288 000	1 670	3 050 000	7 900 000
Heterotrophic bacteria TVC 37 °C	120	5 900	15 500	90	8 000	341 000	1 750	2 430 000	900 000
Coliforms, TC	0	0	95	0	9	95	9	2 500	45 000
Fecal coliforms, FC	0	0	0	0	0	0	4	1 400	1 400
Fecal streptococci, FS	0	75	110	0	150	95	25	450	250
Ammonifying bacteria	170	8 700	14 400	0	11 000	370 000	1 260	2 800 000	9 800 000
Aeromonas	0	480	330	0	10	350	0	300	200
Fungi	10	3 000	400	2	660	360	44	47 000	30 000

M - meat, S - mucus , T - food tract

Composition of bacterial genera isolated from the digestive tract contents of ide on standard agar in 20°C is shown in Table 5. Fish from ponds 12 and 26 contained 6 taxa of bacteria, and fish from pond B – 4 taxa. Gram-negative *Aeromonas* (17%-30%), *Vibrio* (16%-22%), and *Pseudomonas* (13%-31%) were most common and present in all samples. Gram-positive *Bacillus* (7%-25%), and *Sarcina* (9%-12%) were also present. Unidentified strains comprised 4%-18% of bacteria.

Bacteria isolated from ide digestive tract contents on Endo medium in 37°C are shown in Table 6. Among *Enterobacteriaceae* family, five genera were identified in the samples from ponds 12 and 26, and four genera from pond B. The genera *Proteus* (13%-25%) and *Enterobacter* (12%-17%) were most common and present in all the samples. Among other bacteria, *Aeromonas* (8%-13%), and unclassified bacteria (10%-16%) were found.

TABLE 5

Composition of bacteria isolated from the ide digestive tract contents on standard agar in 20°C

Bacteria	Pond no. 12		Pond no. 26		Pond B	
	n	%	n	%	n	%
Aeromonas	36	30	20	17	28	25
Vibrio	26	22	18	16	21	19
Pseudomonas	17	14	36	31	14	13
Plesiomonas	13	11	-	-	-	-
Flavobacterium	-	-	6	5	-	-
Sarcina	15	12	10	9	-	-
Bacillus	8	7	9	8	28	25
Undetermined	5	4	16	14	19	18
Total	120	100	115	100	110	100

n – number of isolated strains

TABLE 6

Composition of bacteria isolated from the ide digestive tract contents on Endo medium in 37°C

Bacteria	Pond no. 12		Pond no. 26		Pond B	
	n	%	n	%	n	%
Enterobacteriaceae:						
Citrobacter	18	24	14	19	-	-
Serratia	12	15	16	21	-	-
Proteus	10	13	12	16	18	25
Enterobacter	12	15	9	12	13	17
Escherichia	-	-	5	7	16	21
Yersinia	8	10	-	-	10	13
Other:						
Aeromonas	10	13	8	11	6	8
Undetermined	8	10	11	14	12	16
Total	78	100	75	100	75	100

n – number of isolated strains

DISCUSSION

Water and fish samples were collected from the environments of different trophy. Ponds 12 and 26 in Warlity Fish Farm were supplied from Szeląg Wielki Lake, and pond B in Łężany Fish Farm – with treated domestic sewage. Differences in zooplankton abundance and the results of microbiological analyses indicate that pond 12 was more polluted compared to pond 26. Pond B was the most eutrophic, with zooplankton abundance several times higher, and bacterial counts 50-1000 times higher compared to ponds 12 and 26.

Many studies (Godlewska-Lipowa 1974, Zmysłowska 1987, Niewolak and Tucholski 1995) revealed that higher densities of heterotrophic micro-organisms were present in more eutrophic waters and higher densities of bacteria in fish (Zaleski 1985, Zmysłowska et al. 2000b).

The results of the present study showed that the highest counts of micro-organisms occurred in the digestive tract contents, lower densities were found in mucus, and the lowest – in the muscles. In the latter, no bacteria were often found, especially in fish reared in ponds supplied with lake water. Similar relationships were reported by Niewolak and Tucholski (1995) in the study of common carp reared in purified sewage ponds.

Pond 12 contained higher numbers of bacteria compared to pond 26, but the opposite was observed in fish: ide from pond 26 showed higher bacterial counts than the fish from pond 12. This was probably due to lower density of fish in pond 26 than in pond 12 (Zmysłowska et al. 2000a).

Microflora of fish digestive tract is very important from the point of view of aquaculture, thus the present study involved not only evaluation of bacterial counts, but focused also on the identification of heterotrophic bacteria grown on standard agar in 20°C (TVC 20°C) and Endo medium in 37°C. It was found that in the most polluted pond B, in which density of bacteria was the highest, there were less bacterial genera than elsewhere: 4 TVC 20°C and 5 on Endo medium in 37°C, whereas in ponds 12 and 26 there were 6 and 6 respectively. All ide digestive tract samples from all three ponds contained *Aeromonas*, *Vibrio*, *Pseudomonas* and *Bacillus* among the strains isolated on standard agar in 20°C, and *Proteus*, *Enterobacter* (*Enterobacteriaceae* family) and *Aeromonas* among the bacteria isolated on Endo medium in 37°C.

These results are consistent with the results of other studies (Yoshimizu 1980, Austin and Allen-Austin 1985, Zmysłowska et al. 2000b) which showed presence of *Aeromonas*, *Pseudomonas*, *Acinetobacter* and *Vibrio* in the fish digestive tracts. Analyses of the composition of bacterial community of tench digestive tract also revealed the presence of 6 genera of *Enterobacteriaceae* family, with *Enterobacter* comprising 20% and *Proteus* 9.1% (Zmysłowska et al. 2000b).

CONCLUSIONS

1. The ponds differed in terms of bacterial and fungal counts in water. These counts were higher in pond B supplied with treated wastewater than ponds 12 and 26 supplied with water from Szeląg Wielki Lake.

2. In the ide, the highest counts of micro-organisms were found in the digestive tracts, lower in the skin mucus, and the lowest in the muscles.
3. Higher numbers of micro-organisms in fish were related to higher abundance of bacteria in the pond water.
4. Among the bacteria isolated from ide digestive tract and grown on standard agar in 20°C, gram-negative bacteria predominated: *Aeromonas* (17%-30%), *Vibrio* (16%-22%), and *Pseudomonas* (13%-31%).
5. Among the bacteria grown on Endo medium in 37°C, 6 genera were isolated from the gut contents of fish from ponds 12 and 26, and 5 genera from fish of pond B. *Proteus* (13%-25%) and *Enterobacter* (12%-17%) of *Enterobacteriaceae* family were present in all samples, accompanied by *Aeromonas* (6%-13%), and unidentified strains (8%-16%).

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STRESZCZENIE

MIKROBIOLOGICZNE BADANIA JAZIA (*LEUCISCUS IDUS* L) ZE STAWÓW O RÓŻNYM STOPNIU TROFICZNOŚCI

Przeprowadzono mikrobiologiczne badania wody stawowej oraz tkanki mięsnej, śluzu ze skóry i treści przewodów pokarmowych jazi pochodzących ze stawów o różnym stopniu troficzności. Wychów jazi prowadzono w dwóch stawach (nr 12 i nr 26) zasilanych wodą z jeziora Szeląg Wielki w Gospodarstwie Rybackim Ostróda - Warlity i jednym stawie „biologicznym" (B) zasilanym wodą po oczyszczeniu ścieków bytowo-gospodarczych w Gospodarstwie Rybackim Łężany.

W badaniach ilościowych stwierdzono zróżnicowanie liczebności bakterii i grzybów w wodzie poszczególnych stawów, jak również w próbach ryb pochodzących z tych stawów. We wszystkich badanych próbach wody i ryb liczniej występowały bakterie TVC 20 °C, TVC 37 °C, amonifikacyjne i grzyby niż

pozostałe oznaczane drobnoustroje. Wyższe liczebności drobnoustrojów występowały w wodzie stawu B a niższe w wodzie stawów nr 12 i nr 26. W badaniach ryb stwierdzano zawsze najwyższe liczebności drobnoustrojów w treści przewodów pokarmowych, niższe w śluzie skóry i najniższe w tkance mięsnej.

W badaniach jakościowych bakterii, wyizolowanych z treści przewodów pokarmowych jazi, na podłożu agarowym zwykłym w temperaturze 20°C stwierdzono przewagę pałeczek gramujemnych, wśród których najwyższy procentowy udział miały rodzaje - *Aeromonas* (17% - 30%), *Vibrio* (16% - 22 %) i *Pseudomonas* (13% - 31%). W składzie bakterii wyizolowanych z treści przewodów pokarmowych jazi, pochodzących z trzech stawów, na podłożu Endo w temperaturze 37 °C, przeważały bakterie z rodziny *Enterobacteriaceae* a wśród nich z rodzajów *Proteus* (13% - 25%) i *Enterobacter* (12% - 17%).

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