MICROBIOLOGICAL EVALUATION OF WATER AND DIGESTIVE TRACT CONTENTS OF TENCH (*Tinca tinca* L.) DURING TANK REARING

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ABSTRACT. Microbiological studies of tank water at three temperatures, and of tench (*Tinca tinca* L.) digestive tract contents were carried out. Analyses of water included quantitative evaluation of bacteria indicatory of sanitary state: the genera Pseudomonas, Aeromonas and Acinetobacter, and fungi. Studies of tench digestive tract contents involved quantitative evaluation of bacteria indicatory of water pollution, coliforms, and qualitative analyses of coliforms, fecal coliforms, fecal streptococci, bacteria from Pseudomonas, Aeromonas and Acinetobacter genera, and fungi. Bacteria indicatory of pollution and sanitary state i.e. Pseudomonas, Aeromonas, Acinetobacter, and fungi found in water were usually also present in the fish digestive tract contents. Heterotrophic bacteria grown on standard agar medium in 20°C and 37°C, and coliforms reached from 7.3×10^3 to 3.1×10^4 , from 1.25×10^3 to 8×10^3 , and from 0.78×10^3 to 3.3×10^3 cells per 1 g of fish digestive tract content respectively.

Keywords: WATER, FISH (Tinca tinca), BACTERIA, FUNGI.

INTRODUCTION

Microbiological studies indicate that micro-organism communities found in fish are variable and their composition and densities depend on many factors.

Density and composition of micro-organisms present in fish are affected not only by environmental factors (Trust and Sparrow 1974, Esteve and Garay 1991, Niewolak and Tucholski 1995), but also by intrinsic factors such as developmental stage of fish, species and condition (Sugita et al. 1988 a, b), structure of the digestive system, type of food ingested by the fish, and physiological factors (Sugita et al. 1985 b). Sugita et al. (1991) showed differences in the densities and composition of microorganisms in digestive tracts of various fish species living in the same environment. Enterobacter, Aeromonas and Acinetobacter predominate in freshwater fish (Trust and Sparrow 1974), or Vibrio-Aeromonas, Enterobacteriaceae, Pseudomonas, and Bacteroides of A and B types (Sugita et al. 1983, 1985 a, 1990). It is not quite clear whether these microorganisms are or are not autochthonous to fish digestive tract. Lesel (1979) claims that no autochthonous bacteria occur in fish, and only resident micro-organisms may be considered, the functions of which should be defined. Sugita et al. (1988 a, b) found certain species of bacteria (*Aeromonas hydrophila, A. punctata*) in *Carassius carassius* digestive tracts at various developmental stages and described them as autochthonous microbial community.

The aim of the present study was to evaluate composition and densities of bacteria and fungi indicatory of water pollution and sanitary state, and of the same micro-organisms in the digestive tracts of tench (*Tinca tinca* L.) during tank rearing at three temperatures (13°C-17°C, 18°C and 20°C).

MATERIAL AND METHODS

A study on the effect of temperature upon oogenesis in tank-reared tench was carried out in 1992-1995. Microbiological analyses were carried out in the same experiment, from March to August 1993 and in May 1994. Tench digestive tract contents and tank water were analysed.

1. Conditions of fish rearing. Tench (*Tinca tinca* L.) fry hatched in June were obtained from rearing ponds in Łężany and stocked at the beginning of November 1992 into 3 rearing tanks of different water temperature. The aim of the ichthyological experiment was to evaluate the effect of water temperature on oogenesis in tench.

Rearing tanks were placed in the laboratory and supplied with water from Kortowskie Lake. Three water temperatures stimulating vitellogenesis in tench were applied (Fig. 1):

A – 300 l aquarium of temperature varying from 13° C to 17.5° C (without regulation), stocked with 1630 tench.

 B_L – 3 interconnected tanks, 250 l each, with water temperature maintained at 20°C, stocked with 1630 tench.

 B_P - 3 interconnected tanks, 250 l each, water temperature maintained at 18°C, stocked with 1630 tench.

The fish were fed carp pellets to satiation. Non-consumed food was removed daily.

2. Sampling. Samples of water were collected from 3 tanks A, B_L, and B_P to sterile bottles of 100 cm³, at monthly intervals from March to August 1993, and in May 1994.

Samples of fish were taken simultaneously with water samples. Nine fish were collected each time (3 fish from each tank) in 1993, and 6 fish (3 individuals from B_L and B_P) in May 1994. Digestive tract contents were squeezed out to sterile glass jars.



Fig. 1. Design of the rearing tank arrangement

The samples were weighed, diluted 10 times with physiological NaCl solution, and homogenized. Quantitative analyses were performed twice: in June 1993 and in May 1994.

3. Microbiological analyses. The analyses involved evaluation of the indices of pollution and sanitary state viz. certain species of bacteria and fungi present in water and tench digestive tract contents, grown on appropriate media (Tab. 1).

Quantitative analyses of tank water were carried out for all groups shown in Tab. 1. Only bacteria grown on standard agar medium in 20°C, 37°C, and coliforms were evaluated in tench digestive tract contents. Physiological NaCl solution (0.85%) was used for quantitative analyses. The cultures were grown in 3 replicates. The results were obtained using plate method, colonies were counted (CFU) and calculated per 1 cm³ of water or 1 g of fish digestive tract content. The most probable number (MPN) of bacteria was evaluated only in water, using three tube sets for each dilution. MPN was read from McCrady's tables, and recalculated per 100 cm³ of water (Paluch 1973). Qualitative microbiological analyses of fish digestive tract contents were performed using scratch method, on appropriate selective media.

Water quality parameters, such as DO, ammonia, nitrite, and nitrate concentration were measured during the experiment (Tab. 2).

TABLE 1

Micro-organisms	Medium and incubation	References
Heterotrophic bacteria (TVC 20°C)	Standard agar (Bacto-agar DIFCO) 20°C / 72 h	Burbianka, Pliszka 1983
Heterotrophic bacteria (TVC 37°C)	Standard agar (Bacto-agar DIFCO) 37°C / 48 h	Burbianka, Pliszka 1983
Coliforms (TC)	Eijkman (MERCK) 37°C / 48 h *Endo 37°C / 24 h	Burbianka, Pliszka 1983
Fecal coliforms (FC)	Eijkman (MERCK) 44.5°C / 24 h *Endo 44.5°C / 24 h	Burbianka, Pliszka 1983
Fecal Streptococci (FS)	Enterococci confirmatory broth (DIFCO) 37°C / 72 h *Enterococci plus agar (DIFCO) *Endo 37°C / 72 h	Pawlaczyk-Szpilowa 1980
Acinetobacter sp.	30°C / 24 h	Beacham et al. 1992
Aeromonas hydrophila	MA 37°C / 48 h	Rippey, Cabelli 1979
Pseudomonas fluorescens	Kinga B 25°C / 72 h	Burbianka, Pliszka 1983
Pseudomonas aeruginosa	Kinga A 42°C / 48 h	Burbianka, Pliszka 1983
Fungi	Sabouraund (BIOMED) 28°C / 7 days	Pawlaczyk-Szpilowa 1980

* for fish

points 3, 4, 5 – in water - MPN per 100 cm^3

TABLE 2

	1 ,1	0 1	
Content	Aquarium with lake water (13 [°] C – 17 [°] C) A	Tank with water temp. $20^{\circ}C$ B _L	Tank with water temp. 18 ^o C B _P
oxygen O ₂ mg dm ⁻³	7.2 - 8.2	7.2 - 9.6	7.1 – 9.3
nitrites NO ₂ mg dm ⁻³	0.005 - 0.026	0.006 - 0.047	0.014 - 0.032
nitrates NO3 mg dm ⁻³	0.32 - 0.67	2.04 - 10.59	2.14 - 9.43
ammonia NH ₄ mg dm ⁻³	0.086 - 0.320	0.007 - 0.024	0.003 - 0.035

Values of water quality parameters during the experiment

RESULTS

Quantitative results for the bacteria indicatory of sanitary state and water pollution in 3 rearing tanks, and qualitative data for fish digestive tract contents are shown in Tab. 3.

TABLE 3

The results of microbiological analyses of tank water and fish									
		TVC 20 °C	TVC 37 °C	TC		FC		FS	
Date	Tank	in 1 cm ³ of water	in 1 cm ³ of water	NPL 100 · cm ⁻³ of water	fish	NPL 100 · cm ⁻³ of water	fish	NPL 100 · cm ⁻³ of water	fish
05.03.	А	14 000	1 560	240	+	0	-	240	+
1993	Br	8 300	180	240	+	0	-	93	+
	BP	19 000	3 100	1 100	+	0	-	23	-
05.04.	А	18 000	2 800	460	+	4	-	93	+
1993	B_L	8 000	2 260	39	-	0	-	240	+
	$B_{\rm P}$	15 200	10 000	1 100	+	15	-	240	+
06.05.	А	8 200	1 500	460	+	0	-	240	+
1993	B_L	6 000	7 000	21	-	0	-	43	-
	BP	26 000	18 300	93	+	0	-	120	+
05.06.	А	4 180	280	240	+	0	-	4	-
1993	B_L	4 880	530	93	+	0	-	23	-
	BP	900	220	39	+	0	-	0	-
05.07.	А	58 800	23 600	39	-	0	-	43	-
1993	B_L	3 700	2 000	7	-	9	-	93	+
	B _P	2 800	2 200	23	-	3	-	43	-
04.08.	А	14 000	11 000	93	+	4	-	14	-
1993	B_L	2 400	400	4	-	0	-	9	-
	BP	3 200	350	93	+	0	-	23	-
11.05.	А	4 100	540	240	-	0	-	0	-
1994	B_L	4 800	200	9	+	0	-	0	-
	B_P	1 230	150	23		0		0	

A – aquarium (13°C-17.5 °C), B_L – left tank (20 °C), B_P – right tank (18 °C)

 $TVC 20^{\circ}C$ – bacteria counted on standard agar after 72 hours of incubation in $20^{\circ}C$, $TVC 37^{\circ}C$ – bacteria counted on standard agar after 24 hours of incubation in $37^{\circ}C$, TC – coliforms, FC – fecal coliforms, FS – fecal streptococci

Total number of bacteria grown on standard agar medium in 20°C ranged from 4100 to 58800 per 1 cm³ in tank A (with variable water temperature 13°C-17.5°C), from 2400 to 8300 in tank B_L (20°C), and from 900 to 26000 in B_P (18°C) (Fig. 2).



Fig. 2. Dynamics of bacteria grown on standard agar in 20°C (TVC 20°C) per 1 cm³ of water.



Fig. 3. Dynamics of bacteria grown on standard agar in 37°C (TVC 37°C) per 1 cm³ of water.

Bacteria grown on standard agar in 37°C were less numerous compared to those grown in 20°C, and their densities were: 280-23600 (A), 180-7000 (B_L), and 150-18300 (B_P) per 1 cm³ of water respectively (Fig. 3).

Coliform bacteria were present in all tanks: A, B_L , and B_P , and their numbers amounted respectively to: 39-460, 4-240, and 23-1100 cells per 100 cm³ of water. Analyses of tench digestive tract contents did not always reveal the presence of coliforms. They were absent from all fish sampled in July, and from B_L tench in April, May and August 1994, and in May 1994.

No fecal coliforms were found in any water samples taken in March, May and June 1993, and in May 1993. In other months their number ranged from 3 to 15 cells. Fish digestive tract contents were free from fecal coliforms.

MPN of fecal streptococci ranged from 0 (June 1993 in B_P) to 240 (March and May in A, April in B_L and B_P) per 100 cm³ of water in 1993, while these bacteria were not found in 1994. Fish digestive tract contents were free from fecal streptococci in June and August 1993, and in May 1994. In other samples they were found when MPN in water was over 93 per 100 cm³.

The results of analyses of certain microbial groups: quantitative for tank water and qualitative for fish digestive tract contents are shown in Tab. 4.

Average numbers of Acinetobacter ranged from 0 to 410 cells per 1 cm³ of water. In A tank they were always present, ranging from 10 to 410 cells per 1 cm³. Their densities were similar in B_L and B_P : 0-50 and 0-80 cells per 1 cm³ respectively. These bacteria were absent from both tanks in March, April, June and July 1993, and from tank B_L also in May 1994. No Acinetobacter were found in the digestive tract contents of B_L and B_P tench in March and April.

Aeromonas hydrophila were present in all samples of the digestive tract content. They were, however, absent from water of B_P tank in May 1993, and from B_L in May 1994. In other water samples their densities ranged from 1 to 165 cells per a cm³.

No *Pseudomonas fluorescens* were found in any water or fish sample. *P. aeruginosa* were also absent, or their densities were very low, in water (0-20 cells per 1 cm^3). They were absent from the fish of A and B_L tanks in June, and from all fish in August 1993.

Fungi were found in all water and tench digestive tract content samples. Their numbers in water ranged from 100 to 2900 cells per 1 cm 3 .

Numbers of bacteria grown on standard agar medium in 20°C, 37°C, and of coliforms in tench digestive tract contents are shown in Tab. 5. Bacteria grown in 20°C

TABLE 4 ups in water (per 1 cm^3)

Average numbers of micro-organisms from various systematic groups in water (per 1 cm³), and in tench (*Tinca tinca* L.) digestive tract contents

Date Tank	Acinetobacter sp.		Aeromonas hydrophila		Pseudomonas fluorescens		Pseudomonas aeruginosa		Fungi		
	in 1 cm ³ of water	fish	in 1 cm ³ of water	fish	in 1 cm ³ of water	fish	in 1 cm ³ of water	fish	in 1 cm ³ of water	fish	
05.03.	А	20	+	40	+	0	-	20	+	300	+
1993	B_L	0	-	10	+	0	-	0	+	300	+
	B_P	0	-	5	+	0	-	0	+	100	+
05.04.	А	25	+	35	+	0	-	10	+	310	+
1993	B_L	0	-	20	+	0	-	10	+	1 1 3 0	+
	B _P	0	-	15	+	0	-	10	+	300	+
06.05.	А	410	+	40	+	0	-	0	+	2 900	+
1993	B_L	10	+	1	+	0	-	20	+	450	+
	BP	70	+	0	+	0	-	20	+	2 000	+
05.06.	А	10	+	22	+	0	-	0	-	200	+
1993	B_L	0	+	12	+	0	-	0	-	1 420	+
	BP	0	+	10	+	0	-	0	+	400	+
05.07.	А	10	+	60	+	0	-	10	+	2 700	+
1993	B_L	0	+	17	+	0	-	10	+	450	+
	BP	0	+	14	+	0	-	0	+	2 700	+
04.08.	А	170	+	165	+	0	-	0	-	300	+
1993	B_L	50	+	100	+	0	-	10	-	380	+
	BP	70	+	20	+	0	-	0	-	900	+
11.05.	А	110	+	60	+	0	-	10	+	510	+
1994	B_L	0	+	0	+	0	-	10	+	360	+
	BP	80		20		0		20		570	

A – aquarium (13°C-17.5 °C), B_L – left tank (20 °C), B_P – right tank (18 °C)

TABLE 5

Range and average densities of bacteria ($x \, 10^3$ cells) per 1 g of tench digestive tract contents

Date	Tank	TVC 20 °C	TVC 37 °C	TC
05.06.1993	А	20.0 18.0 – 28.0	2.53 2.17 – 3.0	1.7 1.36 – 2.18
	BL	16.2 13.3 – 18.0	7.36 7.1 – 8.0	2.77 2.2 – 3.3
	B _P	16.4 12.8 – 18.0	3.59 3.0 – 4.2	1.15 1.0 – 1.25
11.05.1994	B _L	20.0 12.5 – 31.0	2.0 1.25 – 3.6	1.51 1.0 – 253
	B _P	150 7.3 – 27.0	2.2 1.55 – 3.8	1.25 0.78 – 1.7

A – aquarium, B_L – left tank, B_P – right tank

TVC 20°C – bacteria counted on standard agar after 72 hours of incubation in 20°C

TVC 37°C – bacteria counted on standard agar after 24 hours of incubation in 37°C

TC-coliforms

were most numerous in 1 g of fish gut contents (15-20 thousand), followed by the bacteria grown in 37°C (2-7.36 thousand), and coliforms (1.15-2.77 thousand).

DISCUSSION

Microbial communities of fish digestive tract are diversified and fairly abundant. Many authors (Lesel 1979, Lesel and Peringer 1981, Sugita et al. 1988 a, b, Esteve and Garay 1991, Niewolak and Tucholski 1995, Lewandowska 1998) observed that composition of fish microflora depended on the composition of micro-organisms in water and on various environmental factors. This was also confirmed by the results of the present study.

According to the data obtained by Lesel (1979), numbers of bacteria per 1 g of fish digestive tract content ranges from 10^3 to 10^8 cells, and according to Sugita et al. (1991) they are from 1.1 x 10^6 to 3.1 x 10^9 . Lewandowska (1998) observed about 10^7 cells of heterotrophic bacteria per 1 g of the digestive tract contents of Coregonus fry. Microbiological study of tench from Dgał Wielki Lake revealed that densities of bacteria grown on standard agar medium in 20° C and 37° C were 10^{3} - 10^{5} and 10^{3} per 1 g of gut contents respectively (Zmysłowska et al. in print) The results obtained by Trust and Sparrow (1974), Lesel (1979), Lewandowska (1998), and Zmysłowska et al. (1999) showed that Gram-negative bacilli predominated in fish digestive tract content. The results of this study indicate that density of bacterial cells per 1 g of tench gut contents ranged from 7.3 x 10^3 to 3.1 x 10^4 (for bacteria grown on agar in 20°C), and from 1.25×10^3 to 8×10^3 (for bacteria grown on agar in 37°C). Low densities of these bacteria were related to their low numbers in water. No significant differences in bacterial densities were observed in the tanks with water recirculation, which might have resulted from stable thermal conditions ($10^{\circ}C$ in B_P, and $20^{\circ}C$ in B_L). In the flow-through tank A of variable temperature (13°C-17.5°C), numbers of bacteria were higher compared to the tanks of constant water temperature. Development and survival in the fish digestive tracts of the bacteria indicatory of sanitary state of water depend on water temperature (Del Rio-Rodriguez et al. 1997). Lower temperatures increase bacterial survival in water.

In the present study various groups of bacteria showed different frequencies in water and in tench digestive tract contents. Presence of bacteria indicatory of sanitary state must be related to water quality. The results obtained by Trust and Sparrow (1974) indicate that fish are usually only the carriers of bacteria. Our own data revealed that Acinetobacter, *Aeromonas hydrophil*, and *Pseudomonas aeruginosa* were often present in water and in fish. Similar results were obtained by Lewandowska (1998) for digestive tract contents of Coregonus fry and Legińskie Lake water. Trust and Sparrow (1974) stated that these bacteria belonged to proper microflora of fish.

CONCLUSIONS

- 1. Densities of bacteria indicatory of pollution (evaluated on standard agar medium after 72 hours of incubation in 20°C, and after 24 hours in 37°C), and of bacteria indicatory of pollution (coliforms, fecal coliforms, fecal streptococci), Acinetobacter, *Pseudomonas fluorescens, Pseudomonas aeruginosa, Aeromonas hydrophila* and fungi, were similar in tanks B_L and B_P with water recirculation, and higher in flow-through tank A of variable water temperature.
- 2. Micro-organisms found in water were usually present also in fish digestive tract contents.
- 3. Average numbers of bacteria grown on standard agar in 20°C and 37°C, and coliforms ranged from 15×10^3 to 20×10^3 , from 2×10^3 to 7.36×10^3 , and from 1.15×10^3 to 2.77×10^3 per 1 g of tench gut contents respectively.

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STRESZCZENIE

MIKROFLORA WODY I TREŚCI PRZEWODÓW POKARMOWYCH LINA (*Tinca tinca* L.) PODCZAS WYCHOWU BASENOWEGO

Przeprowadzono mikrobiologiczne badania treści przewodów pokarmowych lina i wody basenowej o trzech wariantach temperaturowych (13 °C - 17 °C, 18 °C i 20 °C), w których hodowano te ryby. Badania obejmowały oznaczenia bakterii stanu zanieczyszczenia (liczba bakterii oznaczonych na podłożu agarowym zwykłym po 72 godz. inkubacji w temperaturze 20 °C i liczba bakterii oznaczonych na podłożu agarowym zwykłym po 24 godz. inkubacji w temperaturze 37 °C) i sanitarnego (bakterie z grupy pałeczki okrężnicy, bakterie z grupy pałeczki okrężnicy typu kałowego i paciorkowce kałowe), Acinetobacter, *Pseudomonas aeruginosa, Aeromonas hydrophila* i grzybów. Wykazano, że drobnoustroje, które występowały w wodzie stwierdzano je zazwyczaj i w treści przewodów pokarmowych. Na ogół bakterie te występowały liczniej w wodzie z basenu A (13 °C –17,5 °C) niż w basenie B_L (20 °C) i B_P (18 °C). Nie stwierdzono obecności bakterii *Pseudomonas fluorescens* zarówno w wodzie jak i w treści przewodów pokarmowych lina. W ilościowych oznaczeniach bakterii heterotroficznych wyrosłych na podłożu agarowym zwykłym w temperaturze 20 °C, 37 °C i bakterii z grupy pałeczki okrężnicy w treści przewodów pokarmowych lina stwierdzono odpowiednio: od 7,3 x 10³ do 3,1 x 10⁴, od 1,25 x 10³ do 8 x 10³ i od 0,78 x 10³ do 3,3 x 10³ komórek w 1 g.

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