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ABILITY OF BACTERIOLOGICAL SELF-PURIFICATION OF COMMON CARP, TENCH AND CRUCIAN CARP FINGERLINGS REARED IN POND RECEIVING THE DISCHARGE FROM SEWAGE TREATMENT PLANT AND THEN KEPT IN RUNNING RIVER WATER OF DIFFERENT QUALITY

S. Niewolak, S. Tucholski

Warmia and Masuria University in Olsztyn

ABSTRACT. Studies were carried out on bacteriological self-purification of muscles, skin, and digestive tract contents of common carp (1+), tench (2+), and crucian carp (1+) reared in a pond supplied with a discharge from sewage treatment plant in Jedwabno (Masurian Lakeland) and then kept for 21 days in a concrete pond supplied with the effluents from trout culture, and for another 4 days – in a plastic tank with the Łyna River water. The study was carried out in autumn 1996 on 5 individuals of each species, before and after fish cleaning in running water. The following bacteriological indices were evaluated: total number of bacteria on broth agar at 20 and 37°C, number of coliforms, fecal coliforms, fecal streptococci, *Clostridium perfringens*, *Pseudomonas aeruginosa*, Aeromonas sp., and Salmonella sp. No *Clostridium perfringens* and *Pseudomonas aeruginosa* were found in the tissues and digestive tract contents of the fish. Fish kept in running water eliminated majority of the bacteria from their tissues and digestive tract. In case of the muscles, however, contents of bacteria remained too high. Total numbers of bacteria on broth agar in 20 and 37°C were only slightly reduced, and numbers of fecal streptococci even increased. On the other hand numbers of coliforms, fecal coliforms, and Salmonella sp. were considerably reduced. Numbers of bacteria in the muscles decreased more in common carp and tench than in crucian carp.

Keywords: FRY, AQUACULTURE, WASTEWATER, BACTERIAL INDICES, CLEANING, RIVER WATER

INTRODUCTION

The ability of fish to get rid of the micro-organisms by releasing them to water (Morse et al. 1978, Lesel and LeGac 1983, Buras et al. 1987, Phelps and Stiebel 1991) may be used in practice to purify fish reared in sewage-supplied ponds. These fish usually contain high numbers of bacteria (including pathogenic micro-organisms: *Aeromonas*, *Pseudomonas*, *Salmonella* etc.) in the digestive tract, mucus, gills, internal organs and flesh (Buras et al. 1987). Fish flesh for human consumption must be thoroughly devoid of these bacteria. Static fish cleaning in tanks filled with clean water is usually not sufficient due to bacteriological contamination of water, resulting

in a possibility of a secondary fish infection. Bocek et al. (1992) reported *Salmonella typhimurium* in silver carp 14 days after experimental fish infection, followed by their transfer to clean water. *Salmonella panama* was isolated from channel catfish intestine after 30 days of fish keeping in clean water. According to Buras et al. (1987), the muscles of wastewater-reared common carp kept for 14 days in clean running water contained excessive numbers of bacteria and were not suitable for consumption. Similar studies on tilapia containing low numbers of bacteria in the gut and almost no micro-organisms in the muscles revealed considerable reduction of gut contamination, and sterile muscles after 14 days in clean running water. The authors reported that this procedure was not effective when bacteria were present in fish muscles, and was effective only when the concentration of bacteria in fish organs was low and running water was used.

The study on purification of table common carp reared in wastewater-supplied pond (Niewolak and Tucholski 1995) revealed that fish flesh was bacteriologically safe when the fish were transferred for 14 days into running river water at the end of the rearing season. No literature data were found on bacteriological contamination and purification of other species of fish reared in ponds receiving the discharge from sewage treatment plant

Bacteriological contamination of the muscles, skin and digestive tract content of common carp (1+), tench (2+), and crucian carp (1+) from wastewater culture was evaluated in the present study and compared with the results obtained for the same fish kept for 21 days in a tank supplied with trout culture discharge water, and for another 4 days in a tank with running Łyna River water. The study was performed in 1996 on fish reared at the sewage treatment plant in Jedwabno.

MATERIAL AND METHODS

FISH SPECIES

Common carp and crucian carp fingerlings aged 1+ and tench 2+ were studied. The fish were reared in a pond supplied with biologically treated sewage from the local sewage treatment plant in Jedwabno, Masurian Lakeland. Common carp were reared in the pond for 6 months (from spring stocking with 39.3 g fry to the harvest in autumn 1996). Tench spent about 18 months in the pond (from stocking with 1.2 g fry in spring 1995 to the harvest in autumn 1996). Crucian carp fingerlings were the offspring of spawners brought to the pond in spring 1995 and

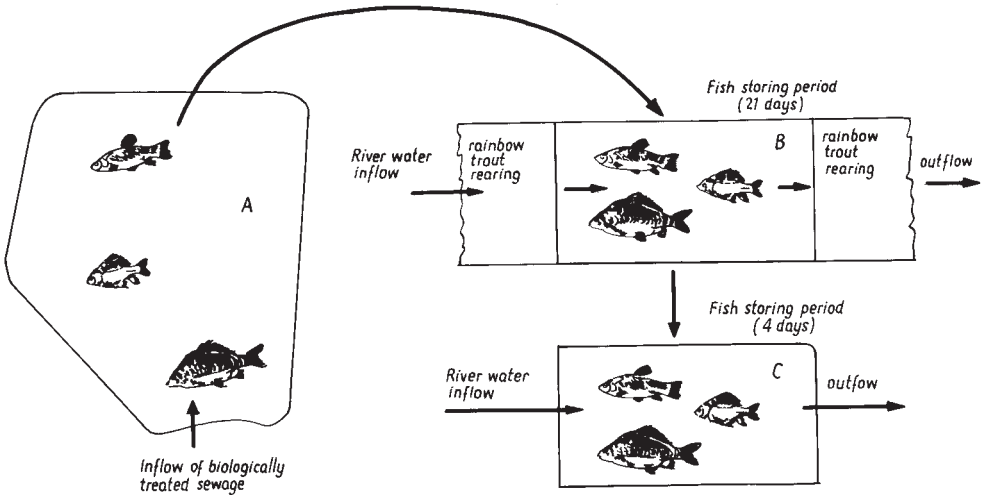

Fig. 1. Experimental design (explanation in the text).

spawned in summer 1995. The entire development of crucian carp fingerlings took place in the pond (Fig. 1A).

FISH PURIFICATION

Thirty individuals of each species were transferred to the Trout Farm in Ruś operating on Łyna River water (Fig. 1B). The fish were kept for 21 days (Oct. 18 – Nov. 8, 1996) between trout raceways, in a concrete flow-through pond supplied with trout culture effluents having purity class II. Then, for the next 4 days (Nov. 8 – 12), the fish were transferred to a plastic tank with Łyna River water of purity class I (Fig. 1C). After 25 days of fish cleaning procedure (21 days in trout water and 4 days in pure river water), the fish were subjected to the same sanitary and bacteriological analyses as the fish harvested directly from the sewage-supplied pond (Niewolak and Tucholski 2000). Common carp, tench and crucian carp of these groups had similar body weight (g ind.$^{-1}$): common carp 305.5-422.3, tench 65.0-93.0, and crucian carp 34.1-45.8.

SAMPLING AND DISSECTION

Five fish of each species (common carp, tench, and crucian carp) were harvested from the pond in autumn 1996, and 5 fish were taken from the cleaning tank. The fish

were immediately transferred to the laboratory in containers with pond or river water respectively. They were dissected according to Buras et al. (1987). Skin was sampled above the lateral line, under the dorsal fin, muscles and digestive tract were isolated and placed in sterile glass vessels. The tissues were weighed under sterile conditions, ground in a mortar with sea sand, and suspended in NaCl physiological solution (10 ml of the solution for each 1 g of the tissue or digestive tract content). The suspensions were homogenised using Universal Laboratory Aid Type MPW-309 homogenizer, at 1000 rpm, for 10 minutes. The homogenates were diluted 1:10-1:10000 depending on the tissue, and inoculated into culture media. Time lag from fish collection to the analyses did not exceed 6 hours. Water from sewage-supplied pond in Jedwabno, concrete pond in Ruś, and from Łyna River was sampled simultaneously with fish sampling.

MICROBIOLOGICAL ANALYSES

The following analyses were performed of the fish tissues and digestive tract contents:

1. total number (CFU g^{-1} wet weight) of bacteria on broth agar, after 72 hours of incubation at 20°C (TVC 20°C);
2. total number (CFU g^{-1} wet weight) of bacteria on broth agar, after 24 hours of incubation at 37°C (TVC 37°C);
3. number (CFU g^{-1} wet weight) of coliform bacteria (TC) on Endo medium, after 48 hours of incubation at 37°C;
4. number (CFU g^{-1} wet weight) of fecal coliform bacteria (FC) on Endo medium, after 24 hours of incubation at 44.5°C;
5. number (CFU g^{-1} wet weight) of fecal Streptococci (FS) on m-Enterococcus Agar, after 72 hours of incubation at 37°C;
6. number (CFU g^{-1} wet weight) of anaerobic sulfite-reducing bacteria (*Clostridium perfringens*) on Wilson-Blair medium, after 18 hours of incubation at 37°C (in the samples pasteurised at 80°C for 10 min.);
7. number (CFU g^{-1} wet weight) of *Pseudomonas aeruginosa* (Pa) on mPa Agar, after 48 hours of incubation at 37°C;
8. number (CFU g^{-1} wet weight) of Aeromonas sp. (Ae) on Rimler-Shotts medium, after 24 hours of incubation at 37°C;
9. presence or lack of Salmonella sp. on selective Kauffman's medium with sodium

tetrathionate, after 24 hours of incubation at 37°C, and then on separating medium with xylose, lysine and sodium desoxycholate (XLD), under the same incubation conditions (Bordner et al. 1978).

TVC 20°C, TVC 37°C, TC, FC and FS numbers in wastewater pond, concrete tank in Ruś, and Łyna River water were evaluated in 3 replicates, according to the Standard Methods (1992). Concentrations of bacteria (1-8), and presence of Salmonella sp. (9) in the muscles, skin and digestive tract contents of common carp, tench and crucian carp were also evaluated in 3 replicates of the same sample. All confirmation tests for coliforms, fecal coliforms, fecal streptococci, *Clostridium perfringens*, *Pseudomonas aeruginosa*, Aeromonas sp., and Salmonella sp. were carried out according to techniques described by Niewolak and Tucholski (2000). Single water samples were analysed, and total number of 90 samples of the muscles, skin and digestive tracts from 30 fish (10 of each species).

RESULTS

WATER

TVC 20°C, and TVC 37°C in Jedwabno wastewater pond measured on the day of fish sampling were 10500 and 7000 CFU ml^{-1} respectively. Numbers of coliforms, fecal coliforms, and fecal streptococci were 15000, 14000, and 140000 MPN 100 ml^{-1} respectively. Water of concrete pond (trout culture effluents) contained 5730 and 210 TVC 20°C and TVC 37°C CFU ml^{-1}. Concentrations of coliform bacteria, fecal coliforms, and fecal streptococci reached 1100, 43, and 140000 MPN 100 ml^{-1} respectively. Łyna River water samples contained 1350 and 37 CFU ml^{-1} of TVC 20°C, and TVC 37°C, and 25, 3 and 43 MPN 100 ml^{-1} of coliforms, fecal coliforms, and fecal streptococci (Tab. 1).

FISH

No sulfite-reducing dormant anaerobes (*Clostridium perfringens*) or *Pseudomonas aeruginosa* were found in the tissues and digestive tract of common carp, tench and crucian carp fingerlings from the sewage-supplied pond. Only some individuals contained pathogenic Salmonella sp. Bacteria identified on broth agar in 20 and 37°C, coliforms, fecal coliforms, fecal streptococci, and Aeromonas sp. were usually less numerous in common carp tissues and gut compared to tench and crucian carp (Tab. 2-4). As regards common carp muscles, bacteria determined on broth agar in 20°C

TABLE 1

Concentrations of indicatory bacteria in water of the ponds and Łyna River used for rearing and purification of common carp, tench and crucian carp

Water	[1]TVC 20ºC	[2]TVC 37ºC	[3]TC	[4]FC	[5]FS
	CFU ml^{-1}		MPN 100 ml^{-1}		
Wastewater-supplied pond	10500	7000	15000	14000	140000
Concrete pond supplied with trout culture effluents	5750	210	1100	43	140000
Plastic tank supplied with Łyna River running water	1350	35	25	3	43

[1]*TVC 20ºC – Total viable count at 20ºC*
[2]*TVC 37ºC – Total viable count at 37ºC*
[3]*TC – Total coliforms*
[4]*FC – Faecal coliforms*
[5]*FS – Faecal streptococci*

TABLE 2

Recovery of bacteria from carp organ before and after depuration for 25 days in flowing river water in autumn 1996. Mean (for five fish) and range for the number of bacteria in CFU per 1 g wet wt. (in brackets per cent reduction)

Carp	TVC 20ºC	TVC 37ºC	TC	FC	FS	Aeromonas sp.	Salmonella sp.
Before depuration							
Fish organs							
Muscle	$2.6x10^3$ $0.5x10^3$ - $5.7x10^3$	$0.5x10^3$ $0.2x10^3$ - $0.9x10^3$	$0.045x10^3$ 0 - $0.1x10^3$	$0.04x10^3$ 0 - $0.1x10^3$	$0.08x10^3$ $0.01x10^3$ - $0.2x10^3$	$0.03x10^3$ 0 - $0.045x10^3$	+(1)
Skin	$16.2x10^3$ $3.9x10^3$ - $47x10^3$	$18.9x10^3$ $2.1x10^3$ – $38.0x10^3$	$9.1x10^3$ $0.35x10^3$-$40.8x10^3$	$2.8x10^3$ $0.3x10^3$ - $8.5x10^3$	$0.26x10^3$ $0.07x10^3$ - $0.6x10^3$	$0.9x10^3$ $0.05x10^3$ - $3.5x10^3$	+(1)
Intestine tract	$17.2x10^6$ $7.5x10^6$ - $28.5x10^6$	$6.3x10^6$ $2.0x10^6$ - $10.0x10^6$	$3.3x10^6$ $0.35x10^6$ - $7.5x10^6$	$1.9x10^6$ $0.1x10^6$ - $4.8x10^6$	$5.9x10^3$ $0.33x10^3$-$14.5x10^3$	$170x10^3$ $31x10^3$ - $243x10^3$	NF
After depuration							
Fish organs							
Muscle	$0.73x10^3$ $0.3x10^3$ - $1.2x10^3$ (54.3)	$0.45x10^3$ $0.35x10^3$ - $0.5x10^3$ (90.2)	$0.05x10^3$ 0 - $0.25x10^3$ (0)	0 0 (100)	$0.3x10^3$ $0.025x10^3$ - $0.47x10^3$ (73.1)	7 0 - $0.035x10^3$ (77.0)	NF (100)
Skin	$3.2x10^3$ $0.7x10^3$ - $5.6x10^3$ (80.3)	$2.16x10^3$ $0.2x10^3$ - $5.0x10^3$ (88.6)	0 0 (100)	0 0 (100)	$1.1x10^3$ $0.2x10^3$ - $3.1x10^3$ (0)	$0.1x10^3$ 0 - $0.4x10^3$ (89.0)	NF (100)
Intestine tract	$12.3x10^6$ $0.3x10^6$ - $40.0x10^6$ (22.7)	$4.4x10^6$ $34x10^3$-$17.6x10^6$ (30.1)	$0.15x10^6$ $7x10^3$ - $0.27x10^6$ (95.5)	$14.9x10^3$ 0 - $41.3x10^3$ (99.2)	$2.0x10^3$ $0.6x10^3$ - $58.0x10^3$ (65.7)	$134x10^3$ $8.6x10^3$ -$380x10^3$ (20.8)	NF –

NT – not tested
NF – not found
+(1) – found in one fish
Clostridium perfringens and Pseudomonas aeruginosa not found

predominated; those evaluated in 37°C were less numerous. Coliforms, fecal coliforms, fecal streptococci, and Aeromonas sp. were the least abundant. All these bacteria were present at much higher densities in the skin and digestive tracts of the fish. Salmonella sp. was found in skin of 1 individual only (Tab. 2).

Tench muscles contained similar densities of fecal streptococci as common carp flesh, and the other groups of bacteria were at least 10 times more abundant. Salmonella sp. were present in 4 out of 5 fish. Also skin of tench contained 10-20 fold more bacteria compared to common carp skin, but it did not contain pathogenic Salmonella sp. TVC 20°C, TVC 37°C, and fecal streptococci counts in tench digestive tract were similar to the values observed for common carp. Numbers of coliforms and fecal coliforms were 10 times higher, and of Aeromonas sp. – 10^3 fold higher than in common carp guts (Tab. 3).

TABLE 3

Recovery of bacteria from tench organs before and after depuration for 25 days in flowing river water in autumn 1996. Mean (for five fish) and range for the number of bacteria in CFU per 1 g wet wt. (in brackets per cent reduction)

Tench	TVC 20°C	TVC 37°C	TC	FC	FS	Aeromonas sp.	Salmonella sp.
Before depuration							
Fish organs							
Muscle	$0.18x10^6$ $52.0x10^3$-$0.38x10^6$	$0.15x10^6$ $49.0x10^3$-$0.34x10^6$	$7.8x10^3$ $0.16x10^3$-$25.0x10^3$	$0.9x10^3$ $0.42x10^3$ - $1.7x10^3$	$0.09x10^3$ $0.02x10^3$-$0.14x10^3$	$0.5x10^3$ 0 - $7.0x10^3$	+(4)
Skin	$0.66x10^6$ $34.3x10^3$ -$0.93x10^6$	$0.36x10^6$ $0.11x10^6$-$0.63x10^6$	$40.7x10^3$ $31.8x10^3$-$53.0x10^3$	$20.0x10^3$ $2.3x10^3$ - $83.0x10^3$	$0.46x10^3$ $0.1x10^3$ - $0.7x10^3$	$13.0x10^3$ 0 - $30.0x10^3$	NF
Intestine tract	$8.16x10^6$ $1.4x10^3$ - $25.3x10^6$	$6.7x10^6$ $3.5x10^6$ - $15.2x10^6$	$600x10^3$ $53x10^3$ - $1100x10^3$	$40.2x10^3$ $7.1x10^3$ - $1.22x10^6$	$2.4x10^3$ $1.5x10^3$-$3.3x10^3$	$0.17x10^6$ 0 - $0.4x10^6$	+(1)
After depuration							
Fish organs							
Muscle	$12.6x10^3$ $3.1x10^3$ - $22.4x10^3$ (90.0)	$6.5x10^3$ $2.0x10^3$ - $14.0x10^3$ (95.9)	$0.34x10^3$ $0.05x10^3$-$0.49x10^3$ (95.7)	$0.08x10^3$ 0 - $0.2x10^3$ (91.5)	$1.0x10^3$ $0.2x10^3$ - $2.7x10^3$ (0)	$1.8x10^3$ $0.02x10^3$ - $4.9x10^3$ (0)	NF
Skin	$0.25x10^6$ $8.5x10^3$ - $0.86x10^6$ (96.0)	$0.11x10^6$ $2.9x10^3$ - $0.43x10^6$ (70.0)	$25.6x10^3$ $3.7x10^3$-$66.0x10^3$ (37.1)	$5.6x10^3$ $0.1x10^3$-$19.6x10^3$ (72.2)	$39.3x10^3$ $0.25x10^3$-$0.16x10^6$ (0)	$36.7x10^3$ $2.2x10^3$ - $100x10^3$ (0)	NF
Intestine tract	$0.82x10^6$ $0.20x10^6$-$2.75x10^6$ (89.1)	$0.27x10^6$ $0.11x10^6$-$0.78x10^6$ (96.0)	$57.9x10^3$ $30.0x10^3$ - $101.5x10^3$ (99.5)	$10.5x10^3$ 0 - $28.0x10^3$ (73.8)	$22.6x10^3$ $1.2x10^3$ - $0.10x10^6$ (0)	$100x10^3$ $46.0x10^3$ -$280x10^3$ (0)	NF

NT – not tested
NF – not found
+1/4 – found in one/four fish
Clostridium perfringens and Pseudomonas aeruginosa not found

Crucian carp muscles contained 10^3-10^6 bacteria determined on broth agar in 20 and 37°C. Number of coliforms ranged within 10^3-10^5, concentration of fecal coliforms and Aeromonas sp. did not exceed 10^3, and of fecal streptococci – 10-10^2. Muscles of one fish only contained pathogenic Salmonella sp. TVC 20°C, and TVC 37°C in the skin of crucian carp ranged within 10^2-10^7, and 10^2-10^6 respectively, and numbers of TC from 10^4 to 10^7. FC concentrations were similar in all fish, those of FS ranged from 10^2 to 10^4, and of Aeromonas sp. from 10^3 to 10^5. Skin of 2 out of 5 fish contained pathogenic Salmonella sp. Counts of bacteria determined on broth agar in 20 and 37°C in the intestine content of crucian carp ranged within 10^2-10^7 and 10^2-10^6 respectively. Numbers of coliforms and Aeromonas sp. were similar. Concentrations of fecal coliforms and fecal streptococci ranged from 10^4 to10^5 and from 10^2 to 10^5. Salmonella sp. were found in 2 fish (Tab. 4).

TABLE 4

Recovery of bacteria from crucian carp organs before and after depuration for 25 days in flowing river water in autumn 1996. Mean (for five fish) and range for the number of bacteria in CFU per 1 g wet wt. (in brackets per cent reduction)

Crucian carp	TVC 20°C	TVC 37°C	TC	FC	FS	Aeromonas sp.	Salmonella sp.
Before depuration							
Fish organs							
Muscle	$0.76x10^6$ $8.4x10^3$-$1.26x10^6$	$0.40x10^6$ $3.9x10^3$-$0.93x10^6$	$71.6x10^3$ $3.0x10^3$-$0.17x10^6$	$0.7x10^3$ 0 - $2.1x10^3$	$0.02x10^3$ 0 - $0.04x10^3$	$0.28x10^3$ 0 - $1.08x10^3$	+(1)
Skin	$4.7x10^6$ $0.13x10^3$ -$19.0x10^6$	$0.71x10^6$ $0.40x10^3$ -$1.12x10^6$	$0.23x10^6$ $40.8x10^3$ -$37.6x10^6$	$57.2x10^3$ $52.0x10^3$ -$76.0x10^3$	$6.7x10^3$ $0.8x10^3$ - $22.0x10^3$	$110x10^3$ $1.2x10^3$ - $190x10^3$	+(2)
Intestine tract	$7.4x10^6$ $0.13x10^3$-$19.0x10^6$	$3.2x10^6$ $0.76x10^3$ - $6.6x10^6$	$0.45x10^6$ $0.11x10^6$ -$0.95x10^6$	$0.35x10^3$ $20.0x10^3$ -$0.73x10^6$	$21.0x10^3$ $0.2x10^3$-$0.10x10^6$	$620x10^3$ $100x10^3$ -$1700x10^3$	+(2)
After depuration							
Fish organs							
Muscle	$0.61x10^6$ $96.0x10^3$ - $1.5x10^6$ (19.4)	$0.54x10^6$ $78.0x10^3$ - $1.3x10^6$ (0)	$3.6x10^3$ $0.8x10^3$ - $12.6x10^3$ (95.0)	$0.39x10^3$ 0 - $1.0x10^3$ (43.6)	$0.35x10^6$ $37.2x10^3$ -$0.83x10^6$ (0)	$0.41x10^3$ 0 - $1.45x10^3$ (0)	NF
Skin	$4.3x10^6$ $38.0x10^3$ -$17.8x10^6$ (8.2)	$5.7x10^6$ $15.5x10^3$ -$17.2x10^6$ (0)	$11.7x10^3$ $2.5x10^3$-$22.5x10^3$ (95.0)	$4.4x10^3$ 0 -$17.0x10^3$ (92.3)	$6.8x10^6$ $9.5x10^3$ - $12.2x10^6$ (0)	$54.0x10^3$ $0.09x10^3$ -$150x10^3$ (0)	NF
Intestine tract	$0.35x10^6$ $66.0x10^3$ -$0.76x10^6$ (95.3)	$84.8x10^3$ $11.6x10^3$-$0.16x10^6$ (97.4)	$51.3x10^3$ 0 – $0.10x10^6$ (88.5)	$0.73x10^3$ 0 – $2.66.0x10^3$ (99.8)	$0.67x10^6$ $53.0x10^3$ - $1.6x10^6$ (0)	$13.5x10^3$ $8.3x10^3$ - $20.6x10^3$ (97.8)	NF

NF – not found
+1/2 – found in one/two fish
Clostridium perfringens and Pseudomonas aeruginosa not found

FISH CLEANING

Common carp, tench and crucian carp fingerlings from sewage-supplied pond kept for 21 days in trout culture effluents, and for 4 days in pure river water were able to eliminate most of the bacteria determined on broth agar in 20 and 37°C. They also got rid of the majority of coliforms and fecal coliforms from the muscles, skin and digestive tract contents. Common carp eliminated fecal streptococci and Aeromonas sp. No pathogenic Salmonella sp. were found in cleaned fish. Level of purification was, however, different for various species and tissues. For example, TVC 20°C in common carp muscles decreased by 54.3%, and TVC 37°C by 90.2%. Fecal coliforms were entirely eradicated. Concentrations of fecal streptococci and Aeromonas sp. decreased by 73.1 and 77.0% respectively. On the other hand, fecal coliform numbers increased. Reduction of most bacteria (except fecal streptococci) ranged from 80.3 to 100% in the skin, and from 20.8% to 30.1% of Aeromonas sp. and bacteria evaluated on broth agar at 20 and 37°C were eliminated from the digestive tracts. Reduction of TC, FC, and FS ranged from 65.7 to 99.1%. Tench muscles eliminated 90.0-95.9% of bacteria evaluated on broth agar in 20 and 37°C, and of coliforms. Numbers of fecal streptococci and Aeromonas sp. increased. TVC 20°C , TVC 37°C, and number of coliforms in the digestive tract contents were reduced by 89.1-99.5%. Fecal coliform density decreased by 73.8%, and concentration of fecal streptococci and Aeromonas sp. increased. In crucian carp muscles reduction of TVC 20°C was 19.4%, of coliforms – 95.0%, and of fecal coliforms – 43.6%. Numbers of bacteria determined on broth agar in 37°C, of fecal streptococci, and Aeromonas sp. increased. Crucian carp skin eliminated 8.2% of TVC 20°C and 92.3-94.9% of coliforms and fecal coliforms. TVC 37°C, concentration of fecal Streptococci and Aeromonas sp. increased. From the digestive tract contents 88.5-99.8% of TVC 20°C , TVC 37°C, coliforms, fecal coliforms, and Aeromonas sp. were eliminated, but concentration of fecal Streptococci increased.

DISCUSSION

Comparison of TVC 20°C, TVC 37°C and fecal coliform counts in Jedwabno sewage treatment plant wastewater with water quality criteria (Cabejszek et al. 1960) suggests considerable contamination of the first. According to the criteria of Albinger (1992), pond water contained moderate amount of organic matter easily decomposable by heterotrophic bacteria, and moderate amount of human and animal faeces. Fecal

coliform concentrations (detailed data available from the authors) exceeded the values for all classes of water purity (Decree of the Minister of Environment Protection, Natural Resources and Forestry of Nov. 5, 1991). Numbers of these bacteria in the concrete pond of Ruś Trout Farm were typical for slightly and/or considerably polluted surface waters, containing moderate amount of easily decomposable organic matter and human and animal faeces. Such water may be classified as being in class II or III of purity. Prevalence of fecal streptococci over fecal coliforms in the tank might have resulted from pollution by gulls which fed on trout, and/or with faeces of trout fed contaminated feeds (Geldreich and Clarke 1966). Bacteriological contamination of trout culture effluents was also reported by Austin and Austin (1985). Del Rio Rodriguez et al. (1997) observed that *Escherichia coli* may proliferate in rainbow trout digestive tract and is released to water. In Łyna River water numbers of indicatory bacteria were typical of slightly polluted waters containing low amounts of easily decomposable organic matter, human and animal faeces. This water fell in I/II class of purity.

The data on the concentrations of indicatory bacteria in the muscles, skin and digestive tract contents of common carp, tench, and crucian carp reared in wastewater-supplied pond reveal considerable bacteriological contamination of the fish. This resulted from particularly high concentration of the bacteria evaluated on agar in 20 and 37°C, coliforms, fecal coliforms, and fecal streptococci in water. Counts of these bacteria were high also in the concrete cleaning pond in which the fish were kept for 21 days before their final purification in class II river water. According to Geldreich and Clarke (1966), bacterial microflora of the muscles, skin, gills and gut content reflects concentration of bacteria in water. If *Escherichia coli* and Enterococci (fecal streptococci) are abundant in water, not only fish digestive tracts but also their muscles may contain numerous bacteria (Buras et al. 1987, Fattal et al. 1993). At 1×10^6 – 1×10^7 CFU ml^{-1} of bacteria in water, their numbers isolated from the organs and muscles of the fish were high (Buras et al. 1987). When bacteriological contamination of water was low, they were observed in low numbers in fish kidneys, spleen, and liver, but never in the muscles. Bacteria in muscles of common carp, tench and crucian carp fingerlings reared in sewage-supplied pond in Jedwabno were found when values of TVC 20^0C and TVC 37^0C in water were 10.5×10^3 and 7.0×10^3 CFU ml^{-1}respectively. Numbers of coliforms, fecal coliforms, and fecal Streptococci were 15.0×10^3, 14.0×10^3, and 140×10^3 MPN 100 ml^{-1} respectively. These values were similar to those reported for sewage-supplied ponds (1.0×10^4 CFU ml^{-1} Standard Plate Count). According to

Buras et al. (1987), if concentration of bacteria in water exceeded this level, they were found in the muscles of silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*), and tilapia aurea (*Sarotherodon molitrix*) reared in the ponds for 90 to 120 days. In the present study, fish muscles contained not only numerous coliform bacteria, fecal coliforms, fecal streptococci, and Aeromonas sp., but in some individuals also pathogenic Salmonella sp. were detected. Presence of thermotolerant Aeromonas sp. in the muscles of fish reared in wastewater-supplied ponds was reported also by other authors (Buras et al. 1987, Fattal et al. 1992). Their concentration reached 2.4×10^3 CFU g^{-1} of fresh weight, and number of enterococci amounted to 40 CFU g^{-1}. No fecal coliforms or coliphages F+ were, however, found. Slabbert et al. (1989) reared *Oreochromis mossambicus* fry in humus tank effluents supplemented with commercial trout pellets, in an intensive flow through and recirculation system at Dasport, Pretoria. The effluents contained 1×10^5 fecal coliforms, and 1×10^6 of coliforms and coliphages in 100 ml, but no pathogenic micro-organisms. Van den Heever and Frey (1994) analysed Salmonella content in muscles of fish reared in maturation ponds of the Bloemspruit Sewage Works. Despite high concentrations of fecal coliforms and coliphages, no Salmonella were found in water or fish flesh. Efficiency of self-purification of wastewater-reared fish transferred to pure water depends mainly on the initial bacteriological contamination of fish. Low level of purification of common carp, tench and crucian carp reared in sewage-supplied pond and then transferred for 21 days to the tank with poor quality trout farming wastewater, and for another 4 days to pure running river water, may be explained by considerable contamination of the fish themselves, and of trout culture effluents. According to the data of other authors (Buras et al. 1987, Bocek et al. 1992), additional cleaning for 4 days in good-quality river water was too short to result in a satisfactory outcome. Better purification of common carp muscles, skin and digestive tract content compared to other fish species might have resulted from lower initial contamination. Poor self-purification of tench and crucian carp may be specific for these species.

CONCLUSIONS

1. Muscles, skin, and digestive tract content of common carp fingerlings (1+), tench (2+), and crucian carp (1+) reared in the pond supplied with biologically treated domestic sewage from the sewage treatment plant in Jedwabno were considerably contaminated with bacteria, including pathogenic ones.

2. Concentration of indicatory bacteria in the muscles, skin, and digestive tract contents of the fish transferred for 21 days to the flow-through concrete tank supplied with trout culture effluents and for another 4 days to pure running river water, was more or less reduced, and pathogenic Salmonella were entirely eradicated.
3. Level of reduction of indicatory bacteria in muscles, skin, and digestive tract contents of common carp, tench, and crucian carp depended on their initial concentration in the tissues. Common carp fingerlings containing the lowest numbers of bacteria eliminated them more efficiently compared to tench and crucian carp.

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STRESZCZENIE

SKUTECZNOŚĆ BAKTERIOLOGICZNEGO OCZYSZCZANIA SIĘ KROCZKÓW KARPIA, LINA I KARASIA HODOWANYCH W STAWIE ZASILANYM ODPŁYWAMI ŚCIEKOWYMI I PRZETRZYMYWANYCH W BIEŻĄCEJ WODZIE RZECZNEJ RÓŻNYCH KLAS CZYSTOŚCI

Badano stopień oczyszczania bakteriologicznego mięśni, skóry i treści przewodu pokarmowego kroczka karpia (1+), lina (2+) i karasia (1+), hodowanych w stawie zasilanym biologicznie oczyszczonymi ściekami przy oczyszczalni ścieków w Jedwabnie (Pojezierze Mazurskie) i przetrzymywanych 21 dni w stawie betonowym, zasilanym wodą bieżącą odpływającą z produkcji pstrąga i dodatkowo 4 dni w basenie z tworzywa sztucznego, zasilanego czystą bieżącą wodą z rzeki Łyny. Badania przeprowadzono jesienią 1996 r. na 5 osobnikach każdego gatunku przed i po oczyszczeniu. Bakteriologicznymi parametrami oceny skuteczności oczyszczania się tych ryb były ogólna liczba bakterii oznaczana na agarze bulionowym w temperaturze 20 i 37°C, liczba bakterii z grupy pałeczki okrężnicy, liczba kałowych bakterii z grupy pałeczki okrężnicy (*Escherichia coli*), liczby paciorkowców kałowych, beztlenowych bakterii redukujących siarczyny (*Clostridium perfringens*) oraz bakterii potencjalnie chorobotwórczych *Pseudomonas aeruginosa*, Aeromonas sp. i Salmonella sp. W tkankach i treści przewodu pokarmowego badanych ryb przed i po oczyszczeniu nie stwierdzono *Pseudomonas aeruginosa* i *Clostridium perfringens*. Stopień redukcji pozostałych bakterii wskaźnikowych z wyjątkiem Salmonella sp., zwłaszcza w mięśniach badanych ryb, był wyraźnie niezadowalający. Najmniejszej redukcji podlegała liczba bakterii oznaczana na agarze bulionowym w temperaturze 20 i 37°C; największej liczba bakterii z grupy pałeczki okrężnicy, zwłaszcza *Escherichia coli*. Najmniej bakterii ulegało redukcji w mięśniach karasia, najwięcej w mięśniach karpia. W tkance skórnej karpia oraz w mięśniach, tkance skórnej i treści przewodu pokarmowego lina i karasia wzrosła wyraźnie liczba paciorkowców kałowych, w mięśniach i tkance skórnej karasia – również Aeromonas sp.

ADRESY AUTORÓW:

Prof. dr hab. Stanisław Niewolak
Uniwersytet Warmińsko-Mazurski w Olsztynie
Katedra Mikrobiologii Środowiskowej
10-957 Olsztyn-Kortowo

Dr Stefan Tucholski
Uniwersytet Warmińsko-Mazurski w Olsztynie
Katedra Melioracji i Kształtowania Środowiska
10-957 Olsztyn-Kortowo