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BACTERIOLOGICAL EVALUATION OF WATER, FEED AND STURGEON *(ACIPENSER BAERI* BRANDT) FRY QUALITY DURING INTENSIVE REARING IN COOLING WATER

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ABSTRACT. Skin mucus and the digestive tract contents of Siberian sturgeon (*Acipenser baeri* Brandt), feed and water were analyzed during intensive tank rearing. The analyses included the total number of heterotrophic bacteria on common agar at 22°C (TVC 22°C) and 37°C (TVC 37°C), the number of coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS). The water was evaluated for nitrogen cycle bacteria - proteolytic, ammonifying and nitrifying bacteria of phases I and II. The lowest numbers of TVC 22°C (83600 CFU cm^{-3}) and TVC 37°C (7040 CFU cm^{-3}) occurred in inflow water. The highest numbers of TVC 22°C (169200 CFU cm^{-3}) were present in the tanks, while TVC 37°C (7280 CFU cm^{-3}) were the most numerous in outflow water. Statistical analysis confirmed the influence of sturgeon rearing on the densities of these bacteria in the water. No such relationship was detected for sanitary indicator bacteria (TC, FC and FS), the numbers of which did not significantly differ between inflow or outflow water.

Key words: SIBERIAN STURGEON (*ACIPENSER BAERI*), BACTERIA, COOLING WATER

INTRODUCTION

Various forms of intensive fish rearing have been developed in recent years. The adaptive abilities, homeostasis and resistance to diseases which fish develop during their evolution are stronger under natural conditions than in aquaculture. This explains the higher frequency of diseases and the more severe symptoms in cultured fish (Kolman 1999). The difference between rearing fish under natural and controlled conditions does not stem from pathogenicity, but is related to the varied reactions of the fish to pathogens in the two types of environment.

Aquaculture methods are continuously changing in response to general tendencies in animal rearing, and modern technologies are applied to achieve mass production and shorten the production cycle. This is related to creating rearing conditions which are considerably different from the natural ones. The prevention and treatment of fish diseases and microbiological water pollution is a difficult and complex issue under such conditions. This necessitates the microbiological evaluation of quantitative and qualitative changes in microbial communities, especially those of pathogenic

bacteria present in the fish skin mucus, digestive tract and water. Data on this aspect of Siberian sturgeon *(Acipenser baeri* Brandt*)* rearing are few, therefore microbiological studies of this species under intensive rearing conditions are necessary.

In intensive culture, fish excrete large amounts of metabolites and feces which contain intestinal bacteria into the water, and these which can alter the quantitative and qualitative composition of aquatic microbial communities (Zmysłowska et al. 2000a). Pollution of the aquatic environment and microbiological contamination with sanitary indicator bacteria and human or warm-blooded animal pathogenic bacteria also affect the microbiological status of fish. This was confirmed by the results obtained by Niewolak and Tucholski (1995a, b) for common carp (*Cyprinus carpio* L.) reared in ponds supplied with water from a sewage treatment facility that treated communal sewage and vegetable processing wastewater. The microbiological contamination of these fish decreased after they were transferred to clean water.

Feed can also be a source of bacterial contamination in fish (Zmysłowska et al. 2000b). The microbiological status of feed depends on the quality of each component, processing conditions and storage – particularly temperature and humidity (Zmysłowska and Lewandowska 2000). However, the application of appropriate feeding methods and feed production technologies allows for water contamination to be reduced (Mamcarz et al. 1992, Świątecki 1994, 1997, Lewandowska et al. 2001). The presence of sanitary indicator bacteria and pathogens in fish mucus and digestive tract contents depends on their presence and survival in the water and feed. Data obtained by various authors (Del Rio-Rodriguez et al. 1997, Zmysłowska et al. 2001) indicate that the presence and survival of fish intestinal bacteria strongly depend on environmental conditions, mainly temperature.

The aim of the present study was to evaluate the microbiological contamination and sanitary status of cooling water, fish skin mucus, digestive tract contents and the feed of Siberian sturgeon during intensive tank rearing. The evaluation also includes nitrogen cycle bacteria - proteolytic, ammonifying - and nitrifying bacteria (of phases I and II).

MATERIAL AND METHODS

The study was carried out in May at the Fish Culture Facility in Wąsosze (Wielkopolska) during intensive rearing of Siberian sturgeon fingerlings at the age 0+ (average body weight 356 g). The fish were reared in flow-through tanks measuring $8 \times 3 \times 1.5$ m which were supplied with water from Lake Wąsoskie. The tanks were

stocked with 500 indiv. m^{-2} and water flow was 150 l min^{-1}. The water was introduced into the recirculation system once, and evaporation was compensated for. The fish were fed Trouvit Classic pellets by Nutreco, Holland, with a nominal composition of protein 46%, fat 14%, carbohydrates 21.5%, ash 9.0% and fiber 1.5%. The fish were fed manually, and the daily feeding rates were adjusted according to sturgeon feeding principles (Kolman 1998).

The water temperature fluctuated within the range of 21-25°C, oxygen saturation did not decrease below 50% and ammonia and nitrite concentrations did not exceed permissible levels for sturgeon (Kolman 1999).

Water was sampled directly into sterile bottles at a depth of 0.3 m from three sites, I - water inflow, II - fish tank and III - outflow. The samples were transported in cold boxes (4°C) to the laboratory where the analyses were done within six hours.

Three fish from each tank were sampled and transported to the laboratory in plastic bags filled with tank water and pure oxygen in order to perform microbiological analyses of skin mucus and digestive tract contents. The mucus was collected from a 1 cm^2 of skin surface using sterile cotton plugs and transferred to a 0.85% NaCl solution. Intestinal contents were collected aseptically from isolated digestive tracts. The contents were placed in sterile vessels, weighed and diluted 10 times with a 0.85% NaCl solution. The feed was sampled into sterile vessels, weighed and homogenized to a 10-fold dilution with 0.85% NaCl.

METHODS OF MICROBIOLOGICAL ANALYSES

The analyses of all samples included:

- total number of bacteria on common agar at 22°C (TVC 22°C) after 72 h incubation, and at 37°C (TVC 37°C) after 24 h incubation;
- most probable number (MPN 100 cm^{-3}) of coliforms (TC) on Eijkman medium, after 48 h incubation at 37°C;
- most probable number (MPN 100 cm^{-3}) of fecal coliforms (FC) on Eijkman medium after 24 h incubation at 44.5°C;
- most probable number (MPN 100 cm^{-3}) of fecal streptococci (FS) on Slanetz & Bartley medium after 72 h incubation at 37°C.

All analyses were done according Standard Methods (1975). The results for TVC 22°C and TVC 37°C were calculated as colony forming units (CFU) per 1 cm^3 of water, mucus from 1 cm^2 of skin surface, 1 g of digestive tract contents and 1 g of feed. The

most probable number (MPN) was calculated according to McCredy's tables (Meynell and Meynell 1970).

Additionally, the bacteria participating in the nitrogen cycle were analyzed in the water samples using common methods of water analysis (Rodina 1968). The analyses included the number of proteolytic bacteria (as CFU cm^{-3}), the number of ammonifying bacteria (as CFU cm^{-3}) and the most probable number of nitrifying bacteria (of phases I and II) (as MPN 100 cm^{-3}).

The results obtained were subjected to statistical analysis using the nonparametric Wilcoxon test ($P < 0.05$) (Łomnicki 1999).

RESULTS

The lowest number of TVC 22°C (83600 CFU cm^{-3}) was observed at the inflow in the electric power plant cooling water discharge channel, while the highest number of these bacteria occurred in the fish tank water (169200 CFU cm^{-3}) (Table 1). In the mucus, digestive tract contents and feed, the numbers of TVC 22°C were 6020 CFU cm^{-2}, 4000000 CFU g^{-1} and 283 CFU g^{-1}, respectively. Statistical analysis ($n = 6$, $P = 0.027709$) revealed significant differences in bacteria numbers between water inflow and outflow.

TABLE 1

The average numbers of bacteria on common agar (TVC 22°C and TVC 37°C), coliforms (TC), fecal coliforma (FC) and fecal streptococci (FS) per 1 cm^3 of water, in the mucus from 1 cm^2 of skin, per 1 g of digestive tract contents and feed

Groups of bacteria	Unit	Sample					
		Water-site			Mucus	Digestive tract contents	Feed
		I inflow	II tank	III outflow			
Bacteria TVC 22°C	CFU	83600	169200	126000	6020	4000000	283
Bacteria TVC 37°C	CFU	7040	7110	7280	1060	180000	140
TC	MPN	1500	4500	1500			
FC	MPN	450	250	40			
FS	MPN	0	90	0			
Proteolytic bacteria	CFU	1390	1750	650			
Ammonifying bacteria	CFU	2450	930	480			
Phase I nitrifying bacteria	MPN	250	1500	950			

The highest number of TVC 37°C bacteria was observed at the water outflow – 7280 CFU cm^{-3}, and the lowest in the water supplying fish tank - 7040 CFU cm^{-3} (Table 1). The average number of bacteria in digestive tract contents was

180000 CFU g^{-1}, in mucus - 1060 CFU cm^{-2} and in feed - 140 CFU g^{-1} (Table 1). Similarly to TVC 22°C, statistical analysis revealed a significant difference in the numbers of bacteria between water inflow and outflow.

The ratio of skin mucus TVC 22°C bacteria to the number of these bacteria in the tank water was 0.04, while for TVC 37°C bacteria it was 0.15. These ratios for the numbers of bacteria in digestive tract contents and tank water were 23.64 (TVC 22°C) and 25.32 (TVC 37°C) (Table 2).

TABLE 2

The ratios of numbers of bacteria cultured on common agar (TVC 22°C and TVC 37°C) between samples

Samples	TVC 22°C	TVC 37°C
Mucus/water-site II	0.04	0.15
Digestive tract contents/water-site II	23.64	25.32

The TVC 22°C: TVC 37°C for tank water was 23.80, for mucus 5.68, for digestive tract contents 22.22 and for feed 2.02 (Table 3).

TABLE 3

The ratios of bacteria cultured on common agar at 22°C and at 37°C (TVC 22°C to TVC 37°C) in various samples

Sample	Ratio of bacteria numbers TVC 22°C:TVC 37°C
Water - site I	11.8
Water - site II	23.0
Water - site I	17.1
Mucus	5.8
Digestive tract contents	22.2

The highest average number of coliforms (TC) occurred in the fish tanks (4500 MPN 100 cm^{-3}), while in the inflow and outflow water it was 1500 MPN 100 cm^{-3}. Fecal coliforms (FC) were the most numerous at the water inflow (450 MPN 100 cm^{-3}), while their lowest number was found at the outflow (40 MPN 100 cm^{-3}) (Table 1). No FC: FS ratio was calculated due to the negligible numbers of fecal streptococci (below 100 cells per 100 cm^3). Statistical analysis did not reveal that sturgeon rearing had any significant influence on water quality with regard to sanitary indicator bacteria TC, FC and FS.

The average numbers of proteolytic and phase I nitrifying bacteria were the highest in fish tanks at 1750 CFU cm^{-3} and 1500 MPN 100 cm^{-3}, respectively, while ammonifying bacteria were the most abundant in inflow water at 2450 CFU cm^{-3} (Table 1). No phase II nitrifying bacteria were found in the samples.

DISCUSSION

The microbiological status of the water in which fish culture takes place depends on organic matter content, temperature, pH, oxygen saturation, feeding intensity, feed type and fish species (Sugita et al. 1985a). The results of the present study showed that intensive fish rearing in electric power plant cooling water did not considerably affect its sanitary and bacteriological status. The results concerning the numbers of TVC 22°C and TVC 37°C bacteria in the water revealed slight differences among the sampling sites. The highest number of TVC 22°C in the fish tank (site II) was related to the high availability of nutrients from unconsumed feed and from fish feces. TVC 37°C bacteria were the most abundant at the water outflow (site III).

The numbers of indicator bacteria – coliforms and fecal streptococci - were the highest in the fish tank (site II). This was probably related to the presence of fish feces, which, as Sugita et al. (1985b) reported, are particularly abundant in these bacteria. Fecal coliforms were the most abundant in inflow water (site I), which might have been related to water temperature which strongly affects bacterial development and survival in cooling and other heated waters (Świątecki 1994, Zmysłowska et al. 2001).

The digestive tract microbial communities of feeding fish are usually abundant (Zaleski 1985). This was confirmed by the results of the present study which showed about 10^7 bacteria in 1 g of Siberian sturgeon digestive tract contents. The relationship between the numbers of TVC 22°C and TVC 37°C bacteria in the skin mucus and the numbers of them in the water and digestive tract contents is particularly interesting. The ratio of TVC 22°C bacteria in mucus from 1 cm^2 of skin to the number in 1 cm^3 of tank water was 0.04, while for TVC 37°C bacteria it was 0.15 (Table 2). This index shows a significant decrease in the number of these bacteria in mucus as compared to the water. The ratio of mucus TVC 22°C to TVC 37°C was also lower compared to the levels in the water (sites I, II, III) and digestive tract contents 5.68 (Table 3). These results indicate that sturgeon mucus is considerably bactericidal and efficiently reduces the abundance of microorganisms. Moreover, it appears that the mucus of Siberian sturgeon is selectively active and causes a stronger reduction in TVC 22°C numbers in comparison with TVC 37°C. No data concerning this issue was found in the literature, therefore it is necessary to undertake more detailed studies to explain the mechanisms of the bactericidal effects of mucus in sturgeon.

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STRESZCZENIE

BAKTERIOLOGICZNA OCENA JAKOŚCI WODY, PASZY I NARYBKU JESIOTRA SYBERYJSKIEGO (*ACIPENSER BAERI* BRANDT) PODCZAS INTENSYWNEGO CHOWU W WODACH POCHŁODNICZYCH

Badano bakteriologiczną jakość wody w czasie intensywnego chowu jesiotra syberyjskiego (*Acipenser baeri* Brandt), mikroflorę bakteryjną występującą w śluzie, treści przewodu pokarmowego i paszy. Prace

badawcze prowadzone były w Ośrodku Hodowli Ryb w Wąsoszach koło Konina. W wodzie basenowej, w której prowadzony był chów, średnia liczba bakterii na agarze zwykłym w temp. 22°C (TVC 22°C) i 37°C (TVC 37°C) wynosiła odpowiednio 169200 CFU cm^{-3} i 7110 CFU cm^{-3} (tab. 1), natomiast średnie liczebności bakterii TC, FC i FS: odpowiednio 4500, 250 i 90 MPN 100 cm^{-3} (tab. 1). Ze względu na minimalne liczebności paciorkowców kałowych (poniżej 100 komórek w 100 cm^3) nie obliczano stosunku ilościowego FC:FS w wodzie (stanowisko I, II, III). Średnie liczebności bakterii TVC 22°C i TVC 37°C były najwyższe w przewodzie pokarmowym, gdzie wynosiły odpowiednio: 4000000 CFU g^{-1} i 180000 CFU g^{-1} (tab. 1). W śluzie z powierzchni 1 cm^2 skóry liczba bakterii TVC 22°C wynosiła 6020 CFU cm^{-2}, a TVC 37°C 1060 CFU cm^{-2} (tab. 1). Najmniejsze liczebności tych bakterii stwierdzono w 1 g paszy: 283 (TVC 22°C) i 140 (TVC 37°C). Stosunek liczby bakterii przewodu pokarmowego TVC 22°C do takich samych w wodzie wynosił 23,64, a w przypadku bakterii TVC 37°C 25,32 (tab. 2). Natomiast stosunek ilościowy bakterii TVC 22°C ze śluzu z powierzchni skóry do bakterii z wody basenowej kształtował się na poziomie 0,04, a w przypadku bakterii TVC 37°C wynosił 0,15 (tab. 2). Być może śluz jesiotra wpływa bakteriobójczo, powodując redukcję liczebności drobnoustrojów w sposób wybiórczy. W wodzie badane były również bakterie biorące udział w przemianach związków azotu (proteolityczne, amonifikacyjne oraz I i II fazy nitryfikacji). Najwięcej bakterii amonifikacyjnych stwierdzono w wodzie dopływającej do basenu (2450 CFU cm^{-3}), proteolitycznych w wodzie basenowej (1750 CFU cm^{-3}), a nitryfikacyjnych w wodzie odpływającej z basenu (950 MPN 100 cm^{-3}) (tab. 1). Analiza statystyczna wykazała istotne różnice w liczebności bakterii TVC 22°C i TVC 37°C w odpływie i dopływie wody.

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