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SANITARY AND BACTERIOLOGICAL STUDIES OF WATER AND EUROPEAN CATFISH (*SILURUS GLANIS* L.) DURING WINTERING

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ABSTRACT. Sanitary and bacteriological studies were conducted during European catfish (*Silurus glanis* L.) wintering from January 3, 2001 to March 7, 2001. Samples of water, skin and digestive tract contents were collected every two weeks. Qualitative analyses included culturing heterotrophic bacteria on a broth-agar medium at temperatures of 4°C for 7 days of incubation (TVC 4°C), 22°C for 72 hours of incubation (TVC 22°C) and 37°C for 24 hours of incubation (TVC 37°C). The total number of coliforms (TC), the total number of fecal coliforms (FC) and the total number of fecal streptococci (FS) were determined. The results obtained confirmed that the sanitary and bacteriological state of the water in the wintering pond was good. The number of microbes per cm^2 of skin varied from 10^3 to 10^4, and in 1 g of digestive tract contents from 10^3 to 10^5.

Key words: EUROPEAN CATFISH (*SILURUS GLANIS*), BACTERIA, WINTERING PONDS, WATER, SKIN, INTESTINAL CONTENTS

INTRODUCTION

The biology of the majority of cultivated cyprinid species requires that these fish be wintered. The most difficult form of fish to winter are fry. This is due to the low weight of the fish and the meager amount of stored reserves. The successful wintering of fry, i.e. without large losses, depends on the good condition of the fish and proper supervision in the wintering ponds. This includes ensuring that there is a constant supply of water with the appropriate physico-chemical and biological properties. Of these, the sanitary and bacteriological quality of the water plays a significant role. When the water temperature falls during the wintering period the metabolism of the fish slows; this is manifested by the fish not feeding and the limited excretion of metabolic wastes. The fish meet their energy needs from the reserves they have accumulated during the vegetation period. The wintering period is that when temperatures fall below 8 - 10°C and, in the temperate zone, it normally lasts from between 130 - 160 days (Guziur and Woźniak 1993). During this period, the bacterial flora in the digestive system of the fish undergoes both qualitative and quantitative changes. Limited or suspended feeding causes only a decline in the abundance of bacteria in the diges-

tive system and not its total disappearance (Lésel 1979a, b). A low level of organic substances in the water, low temperature, good oxygenation and the appropriate pH assures that the bacteriological status of the water is good and that the abundance of heterotrophic bacteria remains low. Large amounts of bacterial flora in wintering ponds can contribute to the deterioration of the condition of the fish. This renders them more vulnerable to disease and mass death.

The presence of bacteria in the aquatic environment which can be used to evaluate its sanitary and bacteriological state also reflect the state of the fish living in it (Zmysłowska et al. 2000b). The survival of these bacteria is dependent on the conditions prevailing in the aquatic environment and fish are often simply their hosts (Trust and Sparrow 1974, Del Rio-Rodriguez et al. 1997). The difference between the abundance of bacteria in the external fish mucus and on fish skin itself is the result of the fish inhabiting environments with varying degrees of heterotrophic bacterial flora (Zaleski 1985, Zmysłowska et al. 2000a).

The majority of published works on fish in wintering ponds deal with carp *Cyprinus carpio* L. (Jirăsek 1987, Trzebiatowski et al. 1987), and very few of these works are microbiological studies. This prompted the authors to undertake the current study, the goal of which was to use data on bacterial occurrence to describe the contamination level and sanitary state of water and European catfish (*Silurus glanis* L.) fry during wintering.

MATERIAL AND METHODS

STUDY SITE

The study was conducted at the Komorowie Fisheries Facility near Łukta in northeastern Poland. The water used at the facility came from a lake. The studied fish were wintered in a cement pond with a volume of 2.5 m^3. The water was exchanged every 18 minutes. The studied wintering stock was comprised of six thousand European catfish fry with a specimen weight of 4.9 g.

MICROBIOLOGICAL TESTS

Both the water and the catfish fry were subjected to microbiological tests during the wintering period. These were conducted from January 3 to March 7, 2001. Samples were taken bi-weekly. Ten fry specimens were collected for each sampling session. Skin samples with a surface area of 1 cm^2 and 1 g of digestive tract contents were

collected under sterile conditions. The samples were then ground in a sterile mortar with sea sand using a 0.85% NaCl solution as a dilutor. The microbiological tests conducted on samples of the wintering water and the skin and digestive tract contents of European catfish fry indicated the following:

- total count of heterotrophic bacteria on broth-agar after 7 days of incubation at 4°C (TVC 4°C);
- total count of heterotrophic bacteria on broth-agar after 72 h of incubation at a temperature of 22°C (TVC 22°C);
- total count of heterotrophic bacteria on broth-agar after 24 h of incubation at 37°C (TVC 37°C);
- total count of coliform bacteria on Endo medium (Burbianka and Pliszka 1983) after 48 hours of incubation at 37°C (TC);
- number of fecal coliform bacteria on Endo medium (Burbianka and Pliszka 1983) after 24 h of incubation at 44.5°C (FC);
- number of fecal streptococci on Slanetz and Bartley medium (Merck) according to Standard Methods (1980) after 72 h of incubation at 37°C (FS).

The above counts were done using the pour plate method. The results were recalculated and reported in colony-forming units (CFU) per 1 cm^3 of water, 1 cm^2 of skin or 1 g of the digestive tract contents of catfish fry.

The water and fry samples collected at the wintering pond were transported to the laboratory in a refrigeration unit at temperatures ranging from 4-6°C. The time from sample collection to performing the tests did not exceed six hours.

CHEMICAL TESTS

In addition to microbiological tests, the basic physico-chemical parameters of the water drained from the wintering pond were measured; these included temperature, oxygen saturation, pH, ammonia contents and total phosphorous.

STATISTICAL TESTS

Following recommendations in the literature, the t-Student's test for dependent variables at a significance level of $\alpha = 0.05$ was used to describe the significance of differences between the various bacterial groups indicating contamination level and sanitary state (Stanisz 1998). STATISTICA PL was used for statistical analyses.

RESULTS

The total count of heterotrophic bacteria on broth-agar ranged from several to several tens of thousands of CFU per 1 cm^3 of water depending on temperature and incubation time (Table 1). The lowest average count was noted in bacteria after 7 days of incubation at 4°C (260 CFU cm^{-3}), a higher count was recorded at 37°C (3,070 CFU cm^{-3}) and the highest bacteria count was after 72 h of incubation at 22°C (5,010 CFU cm^{-3}). During the first six weeks of the experiment, the fall in the total bacteria counts on broth-agar after 7 days of incubation at 4°C and the total bacteria counts determined after 72 h of incubation at 22°C were accompanied by a rise in the total bacteria count after 24 h of incubation at 37°C. No similar tendencies in counts of the studied heterotrophic bacteria groups were observed during the remainder of the study (Fig. 1).

TABLE 1

Counts of bacterial indicators of contamination levels and sanitary state of the water during European catfish fry wintering (averages and ranges)

Bacteria group in water samples	Bacteria counts
TVC 4°C	260 (18 - 1,000)
TVC 22°C	5,010 (630 - 22,000)
TVC 37°C	3,070 (5 - 10,000)
TC	10,400 (1,000 - 32,000)
FC	14 (1 – 27)
FS	6 (0 – 16)

TVC 4°C - total viable counts (CFU cm^{-3}) on broth-agar at 4°C after 7 days of incubation

TVC 22°C - total viable counts (CFU cm^{-3}) on broth-agar at 20°C after 72 h of incubation

TVC 37°C - total viable counts (CFU cm^{-3}) on broth-agar at 37°C after 24 h of incubation

TC - number of total coliforms (CFU 100 cm^{-3}) on Endo medium at 37°C after 48 h of incubation

FC - number of fecal coliforms (CFU 100 cm^{-3}) on Endo medium at 44.5°C after 24 h of incubation

FS - number of fecal streptococci (CFU 100 cm^{-3}) on Slanetz and Bartley medium at 37°C after 72 h of incubation

Of the bacteria which indicate sanitary status (Table 1), the lowest average count was of fecal streptococci at 6 CFU per 100 cm^3 of water. Faecal coliforms had a slightly higher count at an average of 14 CFU per 100 cm^3, while the highest total bacteria count was observed for coliforms at an average of 10,400 CFU in 100 cm^3 of water. No regular tendency in the counts of indicator bacteria was observed in the water samples (Fig. 2).

The results of the level of bacteriological contamination of European catfish during wintering is presented in Table 2. The average count on a 1 cm^2 area of skin was

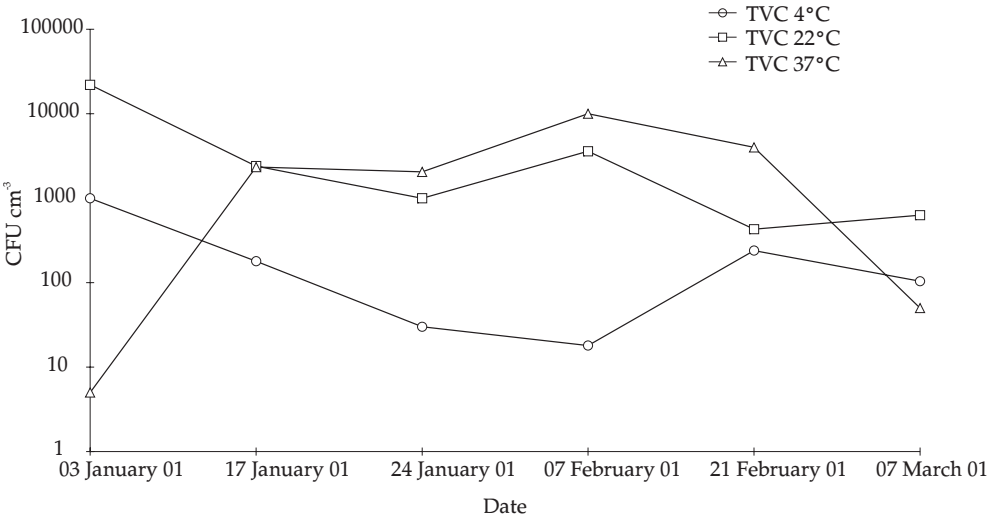
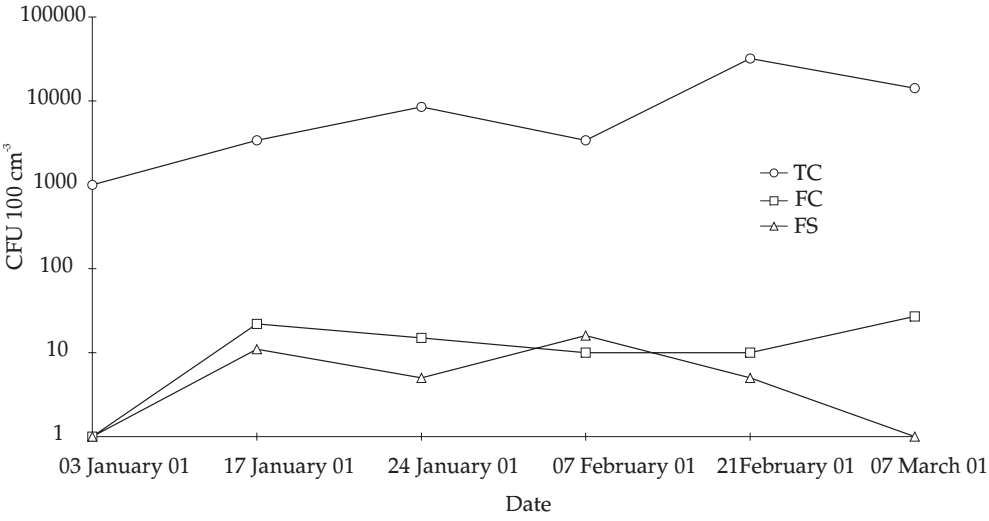


Fig. 1. Average count of heterotrophic bacteria in 1cm^3 water cultivated on broth-agar following incubation at 4, 22 and 37°C.



Rys. 2. Average counts of sanitary state indicator bacteria (TC, FC, FS) in 100 cm^3 water observed during European catfish fry wintering.

the lowest at TVC 4°C, higher at TVC 37°C and the highest at TVC 22°C at 2,000, 5,000 and 16,000 CFU cm^{-2}, respectively. The bacteria which indicated the sanitary state of 1 cm^2 of skin occurred as follows: FC - 1 CFU cm^{-2}; TC - 300 CFU cm^{-2}; FS - 2,000 CFU cm^{-2}.

TABLE 2

Counts of bacterial indicators of contamination levels and sanitary state per cm^2 of skin and in 1 g of digestive tract contents during European catfish fry wintering (averages and ranges)

Microbial group	Bacteria counts on the skin surface (CFU cm^{-2})	Bacteria counts in the digestive tract (CFU g^{-1})
TVC 4°C	2,000	27,000
	(500 – 4,000)	(10,000 - 42,000)
TVC 22°C	16,000	70,000
	(4,000 - 18,000)	(27,000 - 131,000)
TVC 37°C	5,000	10 000
	(3,000 - 9,000)	(7,000 - 14,000)
TC	300	1,000
	(0 - 1,000)	(1,000 - 3,000)
FC	1	4
	(0 - 10)	(1 - 14)
FS	2,000	6,000
	(0 - 4,000)	(5,000 - 7,000)

TVC 4°C - total viable counts (CFU cm^{-2} or g^{-1}) on broth-agar at 4°C after 7 days of incubation

TVC 22°C - total viable counts (CFU cm^{-2} or g^{-1}) on broth-agar at 20°C after 72 h of incubation

TVC 37°C - total viable counts (CFU cm^{-2} or g^{-1}) on broth-agar at 37°C after 24 h of incubation

TC - number of total coliforms (CFU cm^{-2} or g^{-1}) on Endo medium at 37°C after 48 h of incubation

FC - number of fecal coliforms (CFU cm^{-2} or g^{-1}) on Endo medium at 44.5°C after 24 h of incubation

FS - number of fecal streptococci (CFU cm^{-2} or g^{-1}) on Slanetz and Bartley medium at 37°C after 72 h of incubation

The highest count of bacteria in 1 g of digestive tract contents was observed at TVC 22°C (average - 70,000 CFU g^{-1}). It was lower at TVC 4°C (average - 27,000 CFU g^{-1}) and the lowest at TVC 37°C (average - 10,000 CFU g^{-1}). At the same time, the average count of indicator bacteria in 1 g of digestive tract contents was as follows: FC - 2 CFU g^{-1}; TC - 1,000 CFU g^{-1}; FS - 6,000 CFU g^{-1}.

Table 3 presents the results of the chemical analyses of water drained from the wintering ponds. An average temperature of 3.7°C and an average oxygen saturation of 55% were recorded during the study. The average pH was 7.25. However, the average ammonia content recorded in the drained water was 0.18 mg NH_4 dm^{-3} and the average total phosphorus was 0.21 mg P dm^{-3}.

TABLE 3

Physico-chemical water parameters in the pond during European catfish fry wintering (averages and ranges)

Parameters	Unit	Number
Temperature	°C	3.7 (3.6 – 3.8)
Oxygen	%	55 (52 – 57)
pH		7.25 (7.15 – 7.35)
Ammonia	mg NH_4 dm^{-3}	0.18 (0.10 – 0.25)
Total phosphorus	mg $P\text{-}PO_4$ dm^{-3}	0.21 (0.16 – 0.23)

DISCUSSION

The average physico-chemical parameters of the pond water which were recorded during the experiment, including temperature (3.7°C), oxygen saturation (55%) and pH (7.25), correspond to those prescribed for wintering ponds and also ensured that the European catfish fry had appropriate conditions for wintering (Guziur 1997).

Providing fish with an optimal habitat during the winter, and especially ensuring that wintering pond water meets the necessary requirements, limits losses and guarantees good survival. The microbiological state of fish is a reflection of the microbiological contamination of the aquatic environment (Lewandowska et al. 2001). The results of the authors' own studies indicate that counts and variations of bacterial indicators of contamination levels and the sanitary state of the water, on the skin and in the digestive tracts of the studied fish was dependent on the amount of feed matter present in the aquatic environment. The occurrence of heterotrophic bacteria which grows at 22°C indicates the presence of readily available organic matter which may come from water supplying the wintering pond or from fish fecal matter. Low temperature and limited fish feeding certainly limited the development of bacterial microflora. The lower counts of heterotrophic bacteria on broth-agar at 4°C resulted from the longer period required for their generation at the low temperatures during wintering. However, the higher count of heterotrophic bacteria on broth-agar at 37°C was due to its higher survival at this temperature (Zmysłowska 1993, Zmysłowska et al. 2000b).

The values of the sanitary indicators TC, FC and FS show that bacteriological contamination was low. This is confirmed by the count of fecal coliform bacteria (FC) which did not exceed 27 CFU in 100 cm^3 water (average - 14 CFU in 100 cm^3). The

results obtained for this group of bacteria meet the requirements set by the World Health Organization (1989) for water used in the fish cultivation, which permit 10^3 fecal bacteria from the coliform group per 100 cm^3 of water. Sanitary and bacteriological tests of water are essential during the cultivation of fish as contaminated water can be a source of infection for the fish. The presence of sanitary state bacterial indicators in the digestive tract depends on water quality. If the bacteriological quality of the fish is insufficient, they can transmit disease-causing bacteria to humans (Trust and Sparrow 1974). As a food-product and a raw material in the fish processing industry, fish should be subjected to bacteriological quality control.

The permissible counts of heterotrophic bacteria in the mucus of 1 cm^2 of skin ranges from 10^2 to 10^7; variations are linked to the degree of water contamination and water temperature (Lésel and Peringer 1981, Zaleski 1985, Zmysłowska et al. 2000a). Basic test results indicated that the counts of heterotrophic bacteria on the surface of fish skin were not high and ranged from 10^3 to 10^4 per 1 cm^2. These counts were influenced by both the low counts of heterotrophic bacteria in the water and the low water temperature.

Many authorities report that, following the mucus covering the exterior of the fish, the area with the highest counts of bacterial flora is the digestive tract with a range of 10^3 - 10^9 per 1 g of digestive tract content (Lésel 1979a, Zaleski 1985, Sugita et al. 1991, Zmysłowska et al. 2000c). A variety of factors have an influence on the quantitative and qualitative composition of the bacteria, including environmental (contamination, temperature, oxygen saturation, pH), diet type, developmental stage of the fish, species and fish condition, winter apathy and the structure of the digestive tract. These factors definitely had an impact on the values of the bacterial flora counts that ranged from 10^3 to 10^5 per 1 g of the digestive tract contents of the European catfish fry which were obtained in the current study. Water temperature had a significant impact on the relatively low count of bacteria. European catfish feed most intensively at water temperatures ranging from 20 to 25°C and cease feeding at those below 5°C (Horoszewicz 1971, Brylińska et al. 1986, Filipiak et al. 1993). During the current study, the water temperature was 3.7°C. This is below the temperature at which the studied fry in the wintering pond would feed. The authors' own study results indicate that when fish cease feeding this leads to a decrease in the bacterial flora in the digestive tract, but not to its total disappearance, which coincides with the results reported by Lésel (1979a, b).

CONCLUSIONS

1. The heterotrophic bacteria count on broth-agar at temperatures of 4, 22, 37°C, sanitary indicator (TC, FC, FS) counts and the physico-chemical values of the water (temperature, oxygen saturation, pH, ammonia content, total phosphorous level) all indicate that the studied European catfish fry had the appropriate environmental conditions for wintering.
2. Counts of bacterial flora on the skin surface in the range of 10^3 - 10^4 per cm^2 are evidence of the good sanitary and bacteriological state of the water in the wintering pond.
3. The low count of intestinal heterotrophic bacteria in the range of 10^3 - 10^5 per 1 g was linked to the good bacteriological state of the water as well as the low temperature and fact that the fry were not feeding.

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STRESZCZENIE

BADANIA SANITARNO-BAKTERIOLOGICZNE WODY I NARYBKU SUMA (*SILURUS GLANIS* L.) W CZASIE ZIMOWANIA

Badania sanitarno-bakteriologiczne prowadzone były w czasie zimowania suma (*Silurus glanis* L.) od 3 stycznia 2001 do 7 marca 2001 roku. Próby wody i narybku pobierano co dwa tygodnie. Każdorazowo do badań przeznaczano 10 szt. narybku suma. Próby skóry o powierzchni 1 cm^2 i treści przewodów pokarmowych o wadze 1 g pobierano z zachowaniem warunków sterylności. Oznaczenia ilościowe obejmowały: bakterie heterotroficzne wyrosłe na agarze odżywczym w temperaturach: 4°C po 7 dniach inkubacji (TVC 4°C), 22 °C po 72 godzinach inkubacji (TVC 22°C) i 37°C po 24 godzinach inkubacji (TVC 37 °C). Oznaczano również wskaźniki sanitarne: ogólną liczbę bakterii z grupy pałeczki okrężnicy (TC), liczbę kałowych bakterii z grupy pałeczki okrężnicy (FC) i liczbę paciorkowców kałowych (FS). Na podstawie uzyskanych wyników stwierdzono dobre warunki środowiskowe zimowania narybku suma (tab. 3). Potwierdzeniem jest dobry stan sanitarno-bakteriologiczny wody w zimochowie (tab. 1, rys. 1, 2), niska liczba bakterii heterotroficznych na 1 cm^2 skóry, rzędu 10^3 - 10^4 oraz liczebność mikroflory bakteryjnej w 1 g treści przewodu pokarmowego, rzędu 10^3 - 10^5 (tab. 2). Na tą ostatnią istotny wpływ miała niska temperatura wody i zaprzestanie pobierania pokarmu przez ryby.

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