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Occurrence of Bacteria in Water and in Vendace *(Coregonus Albula)* during Rearing in Tanks

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Abstract

Quantitative and qualitative bacteriological studies were carried out on vendace larvae and fry, in tank water in the course of fish rearing, and in fish feeds. Quantitative studies comprised bacteria indicatory of water pollution and sanitary state. Qualitative analyses paid attention to bacteria belonging to the different genera and to the family *Enterobacteriaceae.* The highest number of bacteria was observed for the groups of psychrophilic (TVC 20°C - total viable count of psychrophilic bacteria on broth-agar after 72 h incubation in 20°C), mesophilic (TVC 37°C - total viable count of mesophilic bacteria on broth-agar after 24 h incubation in 37°C), proteolytic and ammonifying microorganisms. Qualitative studies were used to reveal that bacteria from the genera *Aeromonas* and *Flavobacterium,* and from the family *Enterobacteriaceae* were present in fish, while tank water contained bacteria belonging to the genera *Pseudomonas, Bacillus* and *Flavobacterium,* and fish feed - those from the genera *Aeromonas, Pseudomonas* and *Bacillus.*

Keywords: bacteria, water pollution, vendace, vendace rearing, indices of water pollution, fish feed

Introduction

Vendace *{Coregonus albula)* is one of the most important and valuable fishes in Polish lake fisheries. This fish develops best in relatively deep (over 8 m) water bodies, with high water transparency and strong thermal stratification in summer. Vendace is a pelagic species, feeding on zooplankton. Itsjife-cycle is short and natural mortality is high. Survival on spawning grounds is usually low, from 0.5% to 1.0%, and natural recruitment is even lower. Hence, production of vendace is based on egg incubation in the hatcheries and fish stocking into natural lakes. Natural vendace reproduction in lakes has become almost non-existent in recent years. This is due to strong water eutrophication and pollution, which prevent spawning of some species. Consequently, fish rearing and their stocking into lakes has become one of the basic conditions of fisheries management as well as a method of preventing further lake degradation.

Only recently has attention been given to microbiological studies in fish hatcheries. So far studies have focused on bacteria isolation aimed at establishing causes of fish diseases and medication methods. No attention has been given to the composition of the microflora, its variations in time, and effect on fish. This is why the problem of bacterial flora in fish larvae, water and rearing equipment ought to be studied.

This study comprises quantitative and qualitative analysis of bacteria in vendace larvae and fry, fish feeds, and in water of the rearing tanks. Attention was also paid to bacteria elimination by a biological filter.

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Materials and Methods

Study Area

Experiments were carried out in the hatchery of the Faculty of Environmental Protection and Fisheries of the University of Warmia and Mazury in Olsztyn.The hatchery was operating in a closed recirculation system supplied with tap water. Water flow (induced by a water pump) was 15 1/min per one tank. Fish larvae and fry were reared in 160 1 tanks. Water from the rearing tanks was directed to a biological filter consisting of a row of three platforms (Fig. 1). (A detailed description of this water treatment devise can be found in Kolman [5]). Vendace larvae and fry were reared in tanks from 15 January to 15 March. Water temperature was 15°C, pH close to neutral. Fish were fed pellets - a starter feed produced by "Dana Feed" and distributed by automatic feeders. During the studies no fish diseases or mass losses were observed, so no treatment baths were made.

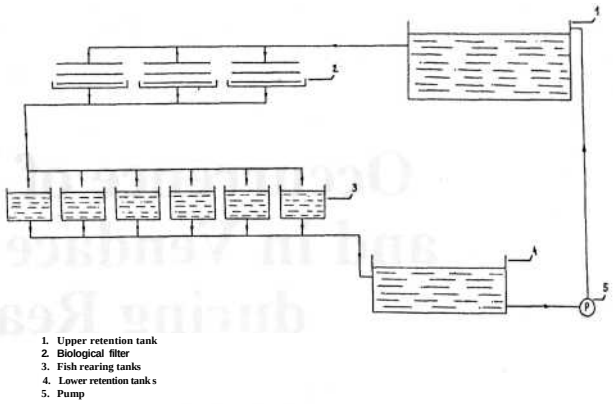

Fig. 1. Scheme of the hatchery.

Materials

Materials consisted of:

1. Vendace larvae and fry reared in tanks.
2. Water: a) inflowing to the rearing tank after treat-

Table 1. List of micro-organisms, appropriate media and conditions of incubation.

Micro-organisms	Medium and incubation	Source
1. Psychrophilic bacteria (TVC 20°C)[1]	Bacto-agar 20°C/72 h	Medium produced by "DIFCO"
2. Mesophilic bacteria (TVC 37°C)[2]	Bacto-agar 37°C/24 h	Medium produced by "DIFCO"
3. Proteolytic bacteria	Medium with gelatine Frazier 20°C/48 h	Rodina [10]
4. Ammonifiers	Nutritive agar medium 37°C/24 h	PN 76R-64791 [2]
5. Coliforms (TC) MPN	Eijkman 37°C/48 h *Endo 37°C/24 h	Medium produced by "MERCK" Burbianka, Pliszka [1]
6. Fecal coliforms (FC) MPN	Eijkman 44.5°C/24 *Endo 44.5°C/24 h	Medium produced by "MERCK" Burbianka, Pliszka [1]
7. Fecal streptococci (FS) MPN	Enterococci confirmatory broth 37°C/72 h *Enterococci plus agar 37°C/72 h	Medium produced by "DIFCO" Medium produced by "DIFCO"
8. *Clostridium perfringens*	Wilson-Blaira 37°C/18 h	Burbianka, Pliszka [1]
9. *Pseudomonas aeruginosa*	Kinga B 42°C/48 h	Burbianka, Pliszka [1]
10. *Pseudomonas fluorescens*	Kinga B 25°C/72 h	Burbianka, Pliszka [1]
11. *Aeromonas sp.*	Kinga B 37°C/48 h	Rippey, Cabelli [9]
13. Fungi (only in feed)	Sabouraud 28°C/7 days	Medium produced by "BIOMED"

* for samples of fish and feed

[1] Total viable count of psyehrophilic bacteria on broth-agar after 72 h incubation in 20°C

[2] Total viable count of mesophilic bacteria on broth-agar after 24 h incubation in 37°C

ment on the biological filter; b) from three platforms of the filter containing activated sludge; c) from fish rearing tank.

3. Fish pellets: starter produced by "Dana Feed."

Sampling and Analyses

1. Water samples (inflowing and from the tank) were collected directly into sterile 200 cm^3 glass bottles, three times: in January, February and March. Samples were also collected in February from three platforms of the biological filter. Water samples were diluted with physio logic salt solution (0.85% NaCl).

2. Vendace samples were collected twice from the same tank: in January (1-day old larvae) and in March (8-week old fry) into sterile glass vessels. 1 g samples of the fish were transferred (larvae and fry separately) to sterile mortars, diluted 10-fold with 0.85% NaCl solution, and thoroughly grinned.

3. Fish feed was sampled, at the beginning of the study, in January. 10 g were collected, diluted 10-fold with the same NaCl solution, homogenised for 10 min, and subsequently, diluted 10-fold.

Quantative analyses of water, fish, larvae and fry, and fish feed comprised determination of different physiologic groups and species of bacteria grown on appropriate media, as given in Table 1. Bacteria number was determined with plate method of Koch [1], and - after plate incubation - the results were given as a colony forming units (CFU) in 1 ml or 1 g. The most probable number (MPN) of bacteria in 100 ml of water was determined in the case of: total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS) [6]. All inoculations were made in three parallel repetitions.

Qualitative analyses were carried out on water, fish and fish feed. Colonies which developed on the standard agar plates (in 20°C/72h and 37°C/24h) inoculated by the samples of tank water, fish larvae and fry, and fish feed were transferred to nutritive broth and then to agar slants for morphological studies (motility, Gram stain, spore stain by Wurtz method) and as an inoculum for the oxidase and catalase tests and test for the utilization of glucose. It was performed on Hugh-Leifson medium in aerobic and anaerobic conditions. Further cultural and physiological studies of the pure cultures of bacteria were carried out according to standard microbiological methods. Classification to the genus was done according to Schewan [11] and Bergey's Manual [2].

Results

Table 2 presents the results of quantitative studies on some groups and species of bacteria found in water supplied to the fish tank, in the tank itself, in fish larvae and fry, and fish feed used during vendace rearing. The highest number was found as regards psychrophilic (TVC 20°C), mesophilic (TVC 37°C), proteolytic and ammonifying bacteria, and in the case of fish and feed samples - also from *Aeromonas* genus.

The highest number of particular groups of bacteria was recorded in fish samples, both larvae (January) and fry (March), compared to the results obtained for water and fish feed.

Water supplied to the tank after treatment on a biological filter, always contained lower number of bacteria (January, February, March) than water in the fish tank itself. The differences were about 1.5-fold for mesophilic bacteria (TVC 37°C), 3-fold for psychrophilic (TVC 20°C), from 3 to 7-fold for ammonifiers, from 3 to 6-fold for TC, 10-fold for *Aeromonas sp.*, 10-20-fold for proteolytic bacteria. Fish samples contained higher number of psychrophilic (TVC 20°C), mesophilic (TVC 37°C), proteolytic and ammonifying bacteria than tank water, the differences being of the order of 10^3. Water and fish samples were free of fecal coliforms (FC), and *CI. perfringens*, and there were no fecal streptococci (FS) in water supplied to the tank. *P.aeruginosa* and *P. fluorescens* were not present in the inflowing water, nor in fish samples.

Fig. 2 presents elimination of bacteria number during water treatment on the three platforms of the biological filter. As regards psychrophilic (TVC 20°C), mesophilic (TVC 37°C), proteolytic and ammonifying bacteria, as well as belonging to *Aeromonas* genus, permanent elimination was observed on particular platforms of the biological filter.

Fig. 2. Reduction of bacteria numbers during tank water treatment on particular platforms of the biological filter.

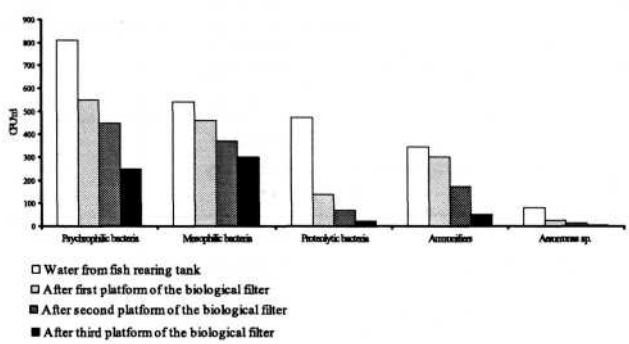

Table 3 presents percentage elimination of bacteria number in particular physiological groups on the separate platforms of the biological filter. The highest elimination was observed for proteolytic bacteria (95%), the lowest (44%) - for mesophilic ones.

Qualitative composition of bacterial microflora in the rearing tank (in January, February and March), in vendace larvae (January) and fry (March), and in fish feed (January) is presented in Table 4. Bacteria present in tank water were dominated by the genus *Aeromonas:* 96.5% in January, 90% in February, 80% in March. Other bacteria were: *Pseudomonas sp.* IV group - 1%, 4.5% and 5.5%, respectively, *Bacillus sp.* - 2.5, 5.5 and 12%, respectively, bacteria from the genus *Flavobacterium* appeared only in March (2.5%).

Samples of 1-day-old vendace larvae contained only *Aeromonas sp.* bacteria (100%). Examination carried out 8 weeks later revealed domination by *Aeromonas sp.* (92.5%), but the other bacteria were also found: *Flavobacterium sp.* (5%) as well as the bacteria from the family *Enterobacteriaceae* (2.5%).

Table 2. Mean numbers of bacteria in water (CFU/ml and MPN), vendace larvae, fry and fish feed (CFU/g).

Micro–organisms	Month	Water inflow to the tank	Tank water	Vendace	Feed
Psychrophilic bacteria (TVC 20°C)	January	135	440	850,000	1350
	February	250	810	–	–
	March	185	690	435,000	–
Mesophilic bacteria (TVC 37°C)	January	360	490	774,000	2250
	February	300	540	–	–
	March	290	470	402,000	–
Coliforms (TC)*	January	2.5	4.5	75	25
	February	0.4	2.5	–	–
	March	0.7	1.6	75	–
Fecal coliforms (FC)*	January	0	0	0	0
	February	0	0	–	–
	March	0	0	0	–
Fecal streptococci (FS)*	January	0	0	45	45
	February	0	0.4	–	–
	March	0	0.9	25	–
Proteolytic bacteria	January	75	750	247,000	60
	February	22	475	–	–
	March	30	415	> 300,000	–
Ammonifiers	January	120	390	274,000	550
	February	50	345	–	–
	March	85	365	> 300,000	–
Clostridium perfringens	January	0	0	0	0
	February	0	0	–	–
	March	0	0	0	–
Pseudomonas aeruginosa	January	0	2	0	35
	February	0	1	–	–
	March	0	1	0	–
Pseudomonas fluorescens	January	0	5	0	3
	February	0	1	–	–
	March	0	1	0	–
Aeromonas sp.	January	5	45	> 300,000	360
	February	7	80	–	–
	March	12	120	> 300,000	–
Fungi	January	–	–	–	10

* - MPN in 100 ml of water

Table 3. Bacteria elimination (%) in the biological filter during tank water treatment.

Groups of bacteria		Initial number	Bacteria elimination (%)			
			B	Z_1	Z_2	Z_3
CFU/ml	TVC 20°C	810	100	32	44	69
	TVC 37°C	540	100	15	32	44
	Proteolytic bacteria	475	100	71	85	95
	Ammonifiers	345	100	13	49	85
	Aeromonas sp.	80	100	64	81	91
MPN	TC*	2.5	100	64	84	84
	FC*	0	100	0	0	0
	FS*	0.4	100	0	0	0

* - MPN in 100 ml of water
B - water in fish rearing tank
Z - (1, 2,3) platforms of the biological filter

Table 4. Qualitative composition (%) of bacterial microflora in particular samples.

Bacteria	Tank water			Fish		Fish feed
				Larvae	Fry	
	January	February	March	January	March	January
Aeromonas sp.	96.5	90	80	100	92.5	60
Flavobacterium sp.	–	–	2.5	–	5	–
Pseudomonas sp. IV gr.	1	4.5	5.5	–	–	35
Bacillus sp.	2.5	5.5	12	–	–	5
Enterobacteriaceae	–	–	–	–	2.5	–

Bacteria from the genus *Aeromonas* also dominated in feed samples, representing 60% of the population. *Pseudomonas sp.* IV group were also found (35%) as well as bacteria from the genus *Bacillus* (5%).

Discusssion

The highest number of bacteria found in water and vendace were those belonging to groups of psychrophilic (TVC 20°C), mesophilic (TVC 37°C), proteolytic and ammonifying bacteria. Number of psychrophilic bacteria (TVC 20°C), in vendace larvae amounted to 8.5 x 10^5 CFU/g and decreased to 4.5 x 10^5 CFU/g after 8 weeks of fish rearing. The number of mesophilic bacteria (TVC 37°C), was more or less the same, respectively 7.7 x 10^5 CFU/g and 4 x 10^5 CFU/g of the sample. Vendace larvae develop their food tracts at the age of about 10 days. Due to this, microflora observed at the beginning of the experiment was represented exclusively by bacteria present in slime on the fish body surface. Microflora related to the food tract develops only when the fish commence active feeding.

A much lower bacteria number was observed in tank water used for fish rearing. The respective number was from 440 to 810 CFU for psychrophilic bacteria (TVC 20°C) and 470 to 540 CFU for mesophilic (TVC 37°C) ones in 1 ml. This agrees with the results obtained by Esteve and Gray [4], who reared eel in tanks and found that the number of heterotrophic bacteria in tank water varied from from 10^3 to 10^4 CFU/ml, whereas 10^5 to 10^6 CFU/g was of fish samples.

Proteolytic bacteria number found in vendace fry in our study ranged from 2.5 x 10^5 to 3 x 10^5 CFU in g of the sample. On the other hand, proteolytic bacteria in water decreased from 750 at the beginning of the experiment to 415 CFU/ml at the end. A high number of proteolytic bacteria at the beginning of the experiment was probably caused by high content of proteins in water, originating from egg membranes and hatching enzymes. Enzymatic hydrolysis of egg membranes yields pectide proteins and amino acids not related to the membrane itsef [12]. It can be assumed that proteins present in tank water at the beginning of the experiment favoured development of proteolytic bacteria. As the proteins became used by the bacteria, the number of proteolytic micro-organisms decreased. On the other hand, these bacteria were most strongly eliminated during tank water treatment in the biological filter.

Qualitative examination of bacterial flora of the fish revealed that only *Aeromonas sp.* were present at the moment of vendace hatching (100%). By the end of the rearing period also *Flavobacterium sp.* (5%) and bacteria from the family *Enterobacteriaceae* (2.5%) appeared.

Aeromonas sp. bacteria dominated also in tank water; their share amounted to 96.5% at the beginning of the experiment, and 80% at the end. Also share of *Bacillus sp.* increased in course of the experiment, from 2.5% to 12%, and so did bacteria belonging to *Pseudomonas sp.* IV group (from 1% to 5.5%). Bacteria from the genus *Flavobacterium* appeaered in the last stage of the experiment, representing 2.5% of the entire population. Cambell and Buswell [3], who reared *Solea solea,* observed similar quantitative and qualitative composition of bacteria in water and fish larvae. Bacteria isolated from water were dominated by *Pseudomonas sp.* group IV and *Alcaligenes sp.,* which represented 53% of the microflora present in water and 64% of the one present in fish. Bacteria belonging to the genera: *Vibrio, Pseudomonas, Flavobacterium* and *Cytophaga* were less numerous.

Qualitative examination of the microflora present in tench food tracts [15] revealed that *Aeromonas sp.* bacteria dominated at all times (three sampling dates), representing 40-42% of a bacterial population, while bacteria in lake water were dominated by *Pseudomonas sp.,* in October and *Aeromonas sp.* in November (30% each). In addition to these genera, both lake water and the fish contained *Enterobacter sp., Acinetobacter sp., Achromobacter sp., Vibrio sp., Sarcina sp.,* and *Bacillus sp.* Studies on the microflora in lake and pond water showed these the two environments contained similar bacteria: *Aeromonas sp., Pseudomonas sp., Plesiomonas sp., Micrococcus sp, Achromobacter sp., Flavobacterium sp.,* and bacteria from the family *Enterobacteriaceae* [14].

Trust and Sparrow [13] stated that microflora of the food tract in freshwater fish was dominated by *Enterobacteriaceae* and *Aeromonas sp.,* while *Pseudomonas sp., Bacillus sp., Flavobacterium sp.,* and *Achromobacter sp.* occurred in lower numbers. Lesel [6] found that there were qualitative relationships between water microflora and microflora present in fish food tracts, but not all taxonomic groups present in water were also present in fish.

The rearing tank in our experiment was supplied with

recirculated water (Fig. 1). Water outflowing from the tank passed a three-platform biological filter, and returned to the tank. The obtained results suggest that the filter was characterized by relatively high efficiency of elimination of the majority of bacteria, some of which were even reduced 100%. Only elimination of mesophilic bacteria was relatively low; treated water still contained 56% of live bacteria count in relation to their initial levels.

Feed used in the experiment corresponded to Polish Standard PN-76/R-64791 [8], which defines acceptable number of bacteria and fungi in g of feed. According to this standard, they are: 5000 for proteolytic bacteria, 25000 for ammonifiers, and 500 CFU for fungi per g of feed. As regards feed used in our experiment, number of proteolytic bacteria amounted to 60, of ammonifiers to 550 and fungi - to 10 CFU in g. Hence, feed was of very good microbiological quality.

Fish feeding commenced on the 10th day of rearing. Automatic feeders were used, and the feeding rates were adapted to fish stock weight. Thanks to this, the feed was readily consumed and no remains were left at the bottom. Good feed quality as well as balanced feeding rates resulted in the fact that feeding had no direct effect on the presece and development of bacteria in water.

Conclusions

1. The highest bacteria cell number found in water, fish and fish feed was represented by psychrophilic (TVC 20°C), mesophilic (TVC 37°C), proteolytic and am monifying bacteria.

2. Higher bacteria number was always noted in vendace larvae and fry than in tank water.

3. The biological filter used in the experiment resulted high elimination of bacteria in water used in the recir culation system. Efficiency of the elimination of bacteria was 44% to 100%.

4. Microflora of vendace larvae consisted only of the genus *Aeromonas.* Bacteria of vendace fry (8-week old) were also dominated by *Aeromonas sp.* (92.5%), but *Flavobacterium sp*.(5%) and bacteria from the family *Enterobacteriaceae* (2.5%) were found, too.

5. Samples of tank water, similarly to fish samples, were dominated by *Aeromonas* sp.(from 96.5% to 80%), but there was a gradual increase of other bacteria numb er: *Pseudomonas sp.* group IV (from 1% to 5.5%), *Bacillus sp.* (from 2.5% to 12%) and *Flavobacterium sp.* (2.5%).

References

1. BURBIANKA M, PLISZKA A. Mikrobiologia zywnosci. PZWL, Warszawa, **1983.**
2. Bergey's Mannual of Determinative Bacteriology. The Will iams, Wilkins Company / Baltimore, **1994.**
3. CAMPBELL A. C, BUSWELL J. A. The intestinal micro flora of farmed Dover sole *(Solea soled)* at different stages of fish development. J. Appl. Bacterid., **55,** 215, **1983.**
4. ESTEVE C, GARAY E. Heterotrophic bacterial flora asso ciated with European eel *Anguilla anguilla* reared in fresh water. Nippon Suisan Gakkaishi, 57, 1369, **1991.**
5. KOLMAN R. Efektywnosc biologicznego filtru pukowego zastosowanego do uzdatniania wody w systemie recyrkulacyjnym przy wychowie pstr^ga. Arch. Ryb. Pol., **1,** 1, **1992.**
6. LESEL R. Donnees preliminaires sur la microflore du tube digestif de la truite arcen-ciel *Salmo gairdneri* Richardson. Revue de Biologie et Ecologie mediterraneenne, 6, 167, **1979.**
7. PALUCH J. Mikrobiologia wod. PWN, Warszawa, **1973.**
8. POLSKA NORMA, PN-76/R-64791, Pasze sypkie. Wymagania i badania mikrobiologiczne z zakresu obecnosci drobnoustrojow czynnych i grzybow toksynotworczych. Polski Komitet Normalizacji i Miar.
9. RIPPEY S. R., CABELLI V. J. Membrane filter procedure for enumeration of *Aeromonas hydrophila* in fresh waters. Appl. Environ. Microbiol. **38,** 108, **1979.**
10. RODINA A. Mikrobiologiczne metody badania wod. PWR i L, Warszawa, **1968.**
11. SHEWAN J. M, HOBBS G., HODGKISS W. A determi native scheme for the identyfication of certain genera of Gram-negative bacteria, with special reference to the Pseudomonaceae. J. Appl. Bacterid., **23,** 379, **1960.**
12. SCHOOTS A. F. M., SACKERS R. J., OVERCAMP P. S. G., DENUCE J. N. Hatching in the teleost Oryzias latipes: Limited proteolysis causes egg envelope swelling. J. Exp. Zool., **226, (1),** 93, **1983.**
13. TRUST T. J., SPARROW R. A. H. The bacterial flora in the alimentary tract of freshwater salmonid fishes. Can. J. Microbiol, **20,** 1219, **1974.**
14. ZMYSLOWSKA I, LEWANDOWSKA D, GUZIUR J. Microbiological study of ide (Leuciscus idus L.) from ponds of different trophy. Pol. Arch. Ryb. **8,** (2), 259, **2000.**
15. ZMYSLOWSKA I, LEWANDOWSKA D, PIMPICKA E. Microbiological evaluation of water and digestive tract con tents of tench *{Tinea tinea* L.) during tank rearing. Pol. Arch. Ryb. 8, (1), 95, **2000.**