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HISTOLOGY OF CARP (*CYPRINUS CARPIO*, L.) GILLS AND POND WATER QUALITY IN SEMIINTENSIVE PRODUCTION

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Abstract

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Gills of carp from ponds of the “Vrsacki ritovi” fish farm were studied, and the results discussed with reference to the results of physical and chemical properties of pond water.

Subepithelial edema and gill hyperemia that are classified as mild and reparable were found the most frequently. High ammonia level that occurred periodically and increased pH values induced gill epithelial hyperplasia. Symptoms of environmental gill disease were observed in less than quarter of samples examined. Parasites invasion was recorded in all samples and could be related to the increased organic matter content. Although different gill changes were found, there were always parts of the gill apparatus still functionally normal and able to adapt to environmental changes that in this study, exceeded values recommended for carp production only temporarily.

Key words: gill histology, fish ponds, carp, water quality

Abbreviations: BOD₅ - Biochemical oxygen demand; GC - Circulatory changes found on gills; GP - Progressive changes found on gills; GR - Regressive changes found on gills; HE - Haematoxylin and eosin; IF - Importance factor; V-1 to V-7 - Fish ponds number

Introduction

Aquatic organisms in a fish pond are exposed to a range of environmental conditions that depend on a production cycle and agrotechnical and hydrotechnical measures applied in order to increase production (Papoutsoglou, 1992; Poleksic et al., 1999; Poleksic et al., 2002 and Pillay, 2004).

It is well known that fish gills are among the most

sensitive organ, which reacts first in changed environment since respiration, osmoregulation and excretion are performed through the gills (Mallatt, 1985; Poleksic and Mitrovic-Tutundzic, 1994 and Lease et al., 2003). Their delicate structure responds to environmental changes by structural alterations that are not irritant specific but effect of factors' intensity and duration of exposure, especially in cases of sublethal concentration of pollutants (Evans, 1987; Lindesjoo

and Thulin, 1994; Karan et al., 1998 and Poleksic et al., 2004). Moreover, a rather small numbers of different tissues in the gill apparatus origin a relatively limited pathological response (Poleksic and Mitrovic-Tutundzic, 1994). All mentioned characteristics of this organ system make gill histology a method of choice for monitoring effects of environmental factors on cultured fish in fish farms (Haaparanta et al., 1997; Poleksic et al., 1999 and Poleksic et al., 2002).

The aim of this study was to relate histological changes on the gills of carp from the fish farm to environmental conditions monitored simultaneously during two growing seasons.

Material and Methods

Field studies were carried out at "Vrsacki ritovi" carp fish farm (Figure 1). This farm comprises of win-

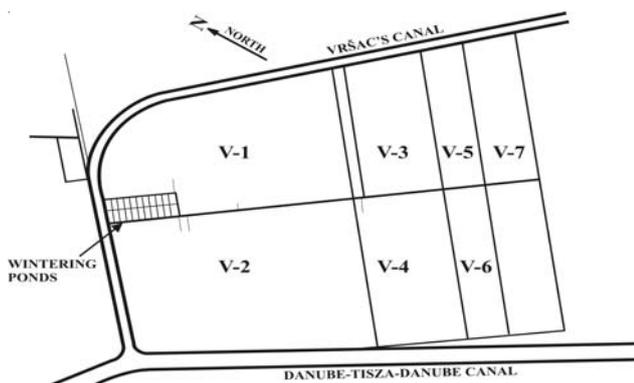


Fig. 1. Plan of "Vrsacki ritovi" fish farm

tering ponds and 7 rearing ponds. For market size carp growing, ponds V - 1, 2, 3, 4, 6, and 7 are used (Table 1). Table 1 also represents stock characteristics of the investigated ponds.

Hydrobiological studies

Hydrobiological investigations were carried out twice a month during the 2003 and 2004 rearing season. In total samples were taken 11 times in 2003 (14 and 27 May, 10 and 26 June, 15 and 28 July, 14 and 28 August, 18 September, 2 and 23 October), and 10 times in 2004 (11 and 25 May, 10 and 28 June, 19 July, 9 and 25 August, 14 September, 5 and 20 October). Sampling included measurement of water physical (temperature and turbidity) characteristics and sampling for chemical analysis. Measurements were carried out in each pond at the same period whenever it was possible. Water temperature was measured with a laboratory thermometer calibrated to 100°C (0.1°C accuracy) by immersing the thermometer 0.05 m below water surface. Water turbidity was measured with Secchi disk.

For chemical analysis water was taken from the middle part of each pond, at 0.3 m depth, in 1l plastic bottles. Measurements of dissolved oxygen, electroconductivity, and pH were performed using a MULTI 340i/SET, WTW, Germany. Electrode was immersed in water at 0.3 m below water surface. Samples for BOD₅ measurements were taken in Winkler's bottles. Consumption of KMnO₄ was determined in acid medium by titration. Spectropho-

Table 1
Pond and stock characteristics (2 years old and marketable size carp)

Pond	V - 1	V - 2	V - 3	V - 4	V - 6	V - 7
Depth (m)	1.30	1.30	1.25	1.25	1.15	1.40
Surface area (h)	180	225	74	93	42	40
Pond volume (000 m ³)	2 340	2 925	925	1 162	483	560
Average mass (g) in 2003	-	192	380	345	358	-
Number of individuals in 2003	-	106 837	53 494	63 437	40 471	-
Average mass (g) in 2004	989	471	1 090	1 053	1 093	1 000
Number of individuals in 2004	86 517	148 833	28 969	31 027	29 093	16 799

tometer UNICAM 5625 UV/VIS was used to determine concentrations of nitrates, total phosphorus and orthophosphate, and ionized ammonia (method by Nessler). Unionized ammonia was determined from the amount of ionized ammonia in water at the specific temperatures and from the standard tables (Piper, 1982). Total ammonia was calculated by adding together ionized and unionized ammonia.

Histological studies

Simultaneously with water sampling for physical and chemical analyses, gills were sampled after fish were caught and killed (5 fish per sampling site/pond, in total 55 fish from each pond investigated in 2003 and 50 fish in 2004). The second left gill arch was taken and fixed in 4% formaldehyde. After fixation, tissue was dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin according to standard histological techniques. Sections of 5–7 µm were cut and stained with haematoxylin and eosin before examination under a light microscope.

Gills were examined, and microphotographs taken using a Leica DMLS light microscope equipped with the DC 300 camera. For description of histological changes and assessment of the degree of pollution a modified method proposed by Bernet et al. (1999) was used. According to this method pathological changes are classified in five reaction patterns, namely: circulatory, regressive, progressive, inflammatory, and neoplastic. An “importance factor” ranging from 1 (minimal alteration) to 3 (marked importance) is assigned to each alteration pointing out the relevance of a lesion, its pathological importance. Depending of the degree and extent of lesions a “score value”, ranging from 0 (unchanged) to 6 (severe occurrence) is determined for each sampling date. Using importance factor and score value an index for each alteration is determined. The frequency of appearance for each alteration is calculated for all sampling dates and presented in a table. The modification consisted of: more detailed alterations list and inclusion of changes induced by parasites invasion, with importance factor of 1. For each gill sample 500 secondary lamellae were examined.

All the analyses were done in laboratories for Zoology, Microscopy, and Fishery of the Faculty of Agriculture, University of Belgrade.

Results and Discussion

Histopathological changes of the gills of carp as indicator of pond water quality (biological method), were related to physical and chemical parameters measured (physical and chemical method).

Results of physical and chemical characteristics of pond water are presented in Table 2 (for the 2003 growing season), and Table 3 (for the 2004 growing season). Both tables represent the range of values found, i.e. minima and maxima for each parameter measured during the season.

Nearly all parameters measured were within the range of optimal values for carp production (Tables 2 and 3) (Alabaster and Lloyd, 1982; Boyd, 1982; Piper et al., 1982 and Svobodova et al., 1993). It holds particularly for electroconductivity, nitrate concentration, KMnO_4 consumption (as a measure of organic matter content), total phosphorus, and orthophosphate in both investigate seasons. Water temperature varied depending of the meteorological conditions. Dissolved oxygen was in the range of acceptable values, except in ponds V-3 and V-4 (in 2003) and V-7 (in 2004), when it was below the value of 5 mg/l. It must be stressed that dissolved oxygen was measured once a day when field investigation were carried out and that in fish ponds daily variations of the oxygen concentration occur, depending on organisms activity, i.e. whether autotrophes release oxygen from photosynthesis (during sunlight), or oxygen is used for respiration (Boyd, 1982). Turbidity in all ponds in both years of investigation varied depending on algal and phytoplankton development, as well as of increase of suspended material, especially when carp is using bottom fauna, when stocking density is high, or when there are debris of decomposing living material and unused feed. Maximum pH values in all ponds exceed the recommended value of 8.5 for carp production (Tables 2 and 3). Increased pH in carp fish farms can affect fish both directly and indirectly by increasing the tox-

Table 2

Results of physical and chemical properties in 2003 growing season. Each parameter is represented by the range (from minimal to maximal value) and throughout the whole season (from May to October)

Parameter	Unit	Optimal values	V-2	V-3	V-4	V-6
Temperature	°C	22-26 (28)	22.8-30.0	23.5-27.8	22.5-28.6	23.3-30.0
Turbidity	m	0.2-0.3	0.16-0.30	0.17-0.30	0.18-0.40	0.17-0.30
pH		6.5-8.5	8.46-9.36	8.06-8.86	7.73-9.00	8.01-8.53
Electroconductivity	µS/cm	>300	291-376	410-510	404-564	443-570
Total ammonia	mg/l	<0.5	0.22-0.55	0.39-0.82	0.27-0.87	0.30-0.64
Nitrate NO ₃	mg/l	2 (1-4)	<0.3	0.30-0.80	0.3-1.15	0.3-0.5
Consumption KMnO ₄	mg/l	<80 (120)	49.6-77.3	32.1-54.7	23.5-39.0	35.8-57.1
Total hardness	°dH	>10	4.82-8.60	8.04-11.35	8.8-12.7	10.85-14.96
Total phosphorus, P	mg/l	-	0.22-0.42	0.14-0.28	0.08-0.18	0.10-0.25
Orthophosphate, P	mg/l	0.1-0.5	0.01-0.02	0.01-0.08	0.01-0.10	0.02-0.12
BOD ₅ , O ₂	mg/l	<8.0	11.7-25.5	2.6-14.8	1.7-6.8	3.2-13.5
Dissolved oxygen, O ₂	mg/l	>5	8.80-19.33	4.93-14.2	4.25-10.22	6.89-10.70

Table 3

Results of physical and chemical properties in 2004 growing season. Each parameter is represented by the range (from minimal to maximal value) and throughout the whole season (from May to October)

Parameter	Unit	Optimal values	V-1	V-2	V-3	V-4	V-6	V-7
Temperature	°C	22-26	15.8-25.1	13.2-24.9	16.1-26.4	16.6-25.9	16.1-26.5	16.6-26.9
Turbidity	m	0.2-0.3	0.16-0.22	0.16-0.40	0.16-0.57	0.18-0.38	0.16-0.30	0.16-0.23
pH		6.5-8.5	7.71-8.84	7.48-9.17	7.83-9.00	7.63-8.98	7.97-8.73	7.91-9.05
Electroconductivity	µS/cm	>300	473-583	380-483	494-591	402-579	505-549	459-714
Total ammonia	mg/l	<0.5	0.17-0.79	0.16-0.33	0.20-1.02	0.12-0.35	0.30-0.52	0.24-0.63
Nitrate NO ₃	mg/l	2 (1-4)	0.2-0.7	0.2-0.8	0.3-1.1	0.2-1.3	0.2-1.8	0.3-1.3
Consumption KMnO ₄	mg/l	<80 (120)	30.9-40.5	27.9-54.8	25.8-40.0	23.1-37.6	33.7-46.8	38.5-59.8
Total hardness	°dH	>10	9.71-11.65	7.65-10.64	10.92-12.90	8.00-11.91	11.42-12.50	9.40-19.41
Total phosphorus, P	mg/l	-	0.19-0.30	0.09-0.29	0.12-0.27	0.11-0.29	0.15-0.24	0.09-0.23
Orthophosphate, P	mg/l	0.1-0.5	0.04-0.11	0.01-0.04	0.01-0.09	0.02-0.06	0.02-0.08	0.02-0.07
BOD ₅ , O ₂	mg/l	<8.0	3.9-12.5	3.8-8.1	2.6-5.3	1.6-10.8	4.0-8.3	4.2-9.0
Dissolved oxygen, O ₂	mg/l	>5.00	5.64-9.62	5.21-14.50	5.40-17.7	5.15-9.50	6.23-8.10	4.48-10.46

icity of ammonia and other components (Piper et al., 1982 and Svobodova et al., 1993). Maximum values for ammonia were above the recommended ones for carp culture in the majority of the ponds (all ponds except V-2, V-4 in 2004) which could result in fish autointoxication since ammonia excretion is blocked when there is high ammonia in water (Das et al., 2004).

In this investigation BOD₅ was very often above the optimal value. It exceeded the recommended value of 8 mg/l in all ponds, except in ponds V-4 in 2003, and V-3 in 2004. Results of histological analysis and scoring of carp gills are shown in Table 4 for year 2003 and Table 5 for year 2004, respectively. Histological changes found in this study could be classified

in three main groups: circulatory changes (GC), progressive changes (GP) and regressive changes (GR)

Circulatory changes are frequently found in this study, but all the changes in this group did not have significant importance factor, so the score is not high for all circulatory changes. First subgroup of circulatory changes (GC1) includes gill hyperemia, blood stasis and hematomas, and a second subgroup GC2, includes lamellar telangiectasia. Most frequent circulatory change, not just in this group, but in the whole study, was gill hyperemia (GC1) indicating increased blood supply in order to cope with impaired gas exchange. Lamellar telangiectasis is also very often found, but it has to be stressed that telangiectasia can occur as a consequence of sampling techniques (Crespo et al., 1988 and Takashima and Hibiya, 1995).

Marginal canal and blood lacunae dilatation can result in hematoma (Figure 2) and stasis. Hemorrhages were not observed in the study indicating that circulatory changes on the gills of carp from fish farm "Vrsacki ritovi" are reparable. From the table it can be noted that in second year of field study circulatory changes were more present than in the first year.

Second group of histological changes includes progressive changes on gill epithelium (GP1 and GP2) and supporting tissue (GP3 and GP4). Hypertrophy of epithelium (GP1) and supporting tissue (GP3) were not frequently found in this study, resulting in low scoring level.

Contrary to hypertrophy, different levels of hyperplasia of the primary epithelium, from distended tips of the secondary lamellae and/or different degree of lamellar fusion, and finally, to a complete fusion of some

Table 4

Types of histological changes on carp gills and their frequency in year 2003; IF – importance factor; HA – histopathological alteration

Histopathological changes / Pond	V-2	V-3	V-4	V-6	IF	
Hyperemia	5.3	5.3	5.3	6.0	GC1	1
Hematomas	4.0	4.7	3.3	5.3	GC1	1
Stasis	4.0	2.7	3.3	3.8	GC1	1
Lamellar telangiectasia	4.7	2.7	2.7	2.3	GC2	1
Hypertrophy of the respiratory epithelium	0.0	0.7	1.3	0.0	GP1	1
Distended tips of secondary lamellae	2.0	6.7	4.0	6.0	GP2	2
Chaotic hyperplasia of primary epithelium	3.3	7.7	4.0	4.5	GP2	2
Fusion of tips of the primary lamellae	4.3	10.0	7.3	7.5	GP2	2
Fusion of several secondary lamellae	2.7	5.3	7.3	6.0	GP2	2
Complete fusion of secondary lamellae	0.7	1.3	2.7	3.8	GP2	2
Hypertrophy of the chloride cells	0.7	2.0	2.0	1.5	GP3	1
Hyperplasia of eosinophylic cells	2.0	6.0	5.3	4.5	GP4	2
Hyperplasia of the mucous cells	0.7	5.3	0.0	1.5	GP4	1
Parasites - Trichodina	6.0	5.7	4.3	3.8	GP5	1
Parasites - Trematodes	6.0	5.7	4.7	3.8	GP5	1
Lifting of the secondary epithelium	5.3	5.0	4.3	3.4	GR1	1
Thinning of respiratory epithelium	1.7	3.3	4.7	7.5	GR5	2
Necrosis	2.0	8.0	12.0	4.5	GR6	3
Chloride and mucous cells in secondary lamellae	2.7	4.0	4.0	2.6	GR7	1

Table 5

Types of histological changes on carp gills and their frequency in year 2004;
IF – importance factor; HA – histopathological alteration

Histopathological changes / Pond	V1	V2	V3	V4	V6	V7	IF	
Hyperemia	6.0	6.0	5.1	6.0	6.0	6.0	GC1	1
Hematomas	5.1	2.6	4.7	1.2	3.6	5.0	GC1	1
Stasis	3.4	3.8	4.3	0.6	3.6	1.0	GC1	1
Lamellar telangiectasia	4.3	2.3	4.3	0.0	3.6	3.0	GC2	1
Hypertrophy of the respiratory epithelium	3.4	1.5	0.9	1.2	2.4	0.0	GP1	1
Distended tips of secondary lamellae	6.9	4.5	5.1	12.0	8.4	4.0	GP2	2
Chaotic hyperplasia of primary epithelium	7.7	3.8	3.4	3.6	3.6	7.5	GP2	2
Fusion of tips of the primary lamellae	6.0	3.0	4.3	4.8	6.0	8.0	GP2	2
Fusion of several secondary lamellae	12.0	10.5	6.0	9.6	8.4	8.0	GP2	2
Complete fusion of secondary lamellae	1.7	1.5	3.4	2.4	4.8	6.0	GP2	2
Hypertrophy of the chloride cells	2.1	0.8	1.7	2.4	2.4	1.0	GP3	1
Hyperplasia of eosinophylic cells	9.4	6.0	5.1	8.4	7.2	8.0	GP4	2
Hyperplasia of the mucous cells	0.0	7.5	4.3	2.4	0.0	2.0	GP4	1
Parasites - Trichodina	4.3	5.6	6.0	4.2	6.0	5.5	GP5	1
Parasites - Trematodes	3.4	4.9	3.4	3.6	3.6	5.0	GP5	1
Lifting of the secondary epithelium	6.0	4.5	4.3	6.0	4.8	6.0	GR1	1
Thinning of respiratory epithelium	10.3	6.0	6.9	7.2	7.2	10.0	GR5	2
Necrosis	14.1	13.5	7.7	7.2	5.4	4.5	GR6	3
Chloride and mucous cells in secondary lamellae	1.3	3.8	0.9	3.6	1.2	4.0	GR7	1

secondary lamellae were frequently found (GP2; Figures 3 and 4). This type of change occurs as a defense mechanism in order to increase water-blood diffusion distance and thus decrease the respiratory area (Roberts, 1989; Poleksic and Mitrovic-Tutundzic, 1994; Takashima and Hibiya, 1995). Different degree of secondary lamellae fusion could be related to increased level of ammonia. Whenever ammonia was higher than 0.5 mg/l for a longer period epithelial cell proliferation was found: in ponds V-3 and V-4 in 2003 from May 14 to June 26 ammonia level was higher than 0.55 mg/l (not shown in the table). Later, in the mentioned ponds ammonia level was below the critical values, and epithelial hyperplasia was not recorded. Similar effects on the gills of fish were found in a number of studies on increased ammonia and nitrite concentration (Svobodova et al., 1993; Cardoso et al.,

1996; Frances et al., 1998; Svobodova et al., 2005; Das et al., 2004). Complete fusion of secondary lamellae that correspond to the environmental gill disease-EGD (Klontz, 1995; Poleksic et al., 1999) was found less frequently in gill samples examined except for the ponds V-6 and V-7 where this change had higher score values.

The histological analysis has revealed hyperplasia of eosinophylic cells (GP4; Figure 5). It is suggested that this cell type belongs to the group that proliferates from the primary epithelium in order to give the differentiated cells needed for adaptation to environmental changes, especially when there is chronic injury and/or inflammation (Roberts, 1989; Ferguson, 1989; Poleksic et al., 1999; Poleksic et al., 2002). Eosinophylic cells proliferation was more frequently found in 2004 and particularly on gills from pond V-

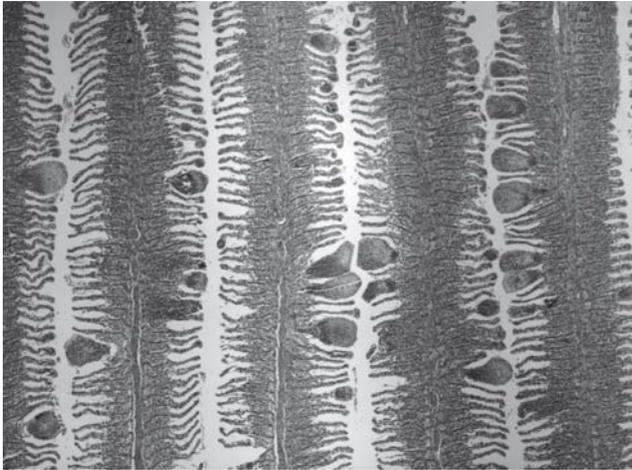


Fig. 2. Hematomas (carp from fish pond V-1; 11. 05. 2004.; HE x50)

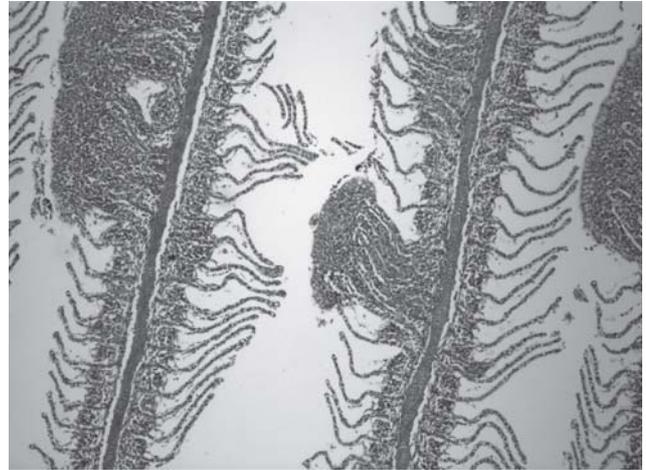


Fig. 3. Fusion of several secondary lamellae (carp from fish pond V-3; 28.07.2003.; HE x100)

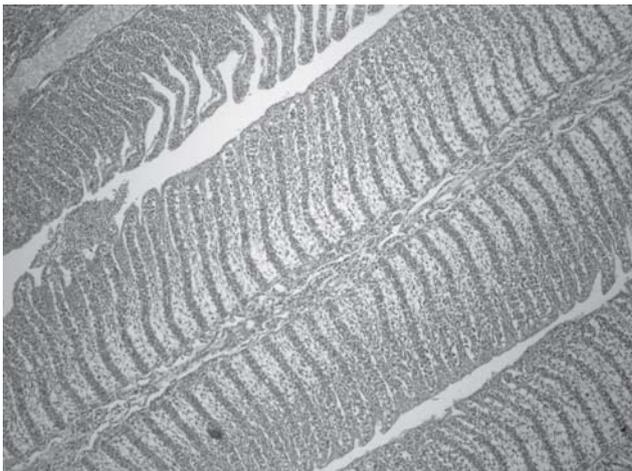


Fig. 4. Complete fusion of secondary lamellae (carp from fish pond V-6; 28.06.2004.; HE x100)

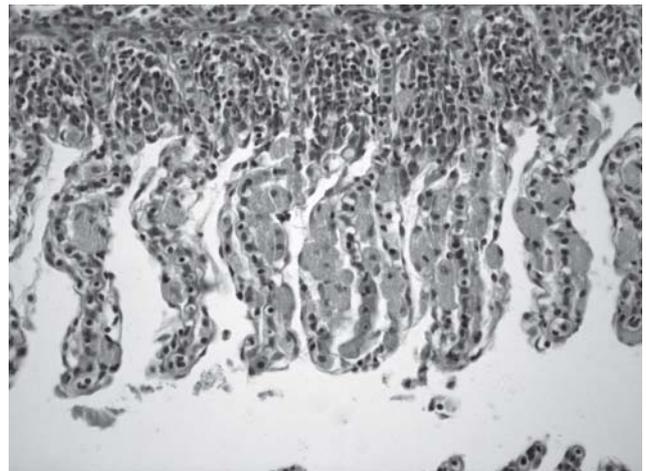


Fig. 5. Hyperplasia of eosinophylic cells (carp from fish pond V-3; 10. 06. 2003.; HE x200)

1. Changes that affect chloride and mucous cells (GP4; Figure 6) were not frequent and were found to a different extent in the ponds investigated. In the pond V-4 in 2003, V-1 and V-6 in 2004 this alteration was not encountered, but in pond V-2 in 2004 it was found with very high score of 7.5 (Table 5). Similar frequency of appearance was found for chloride cells hypertrophy, but more pronounced in 2003. While chloride cells are in charge of the acid-base regulation, mucous proliferate in presence of irritants (Roberts, 1989; Goss et al., 1998; Lease et al., 2003; Svobodova et

al., 2005). In the present study changes of chloride and/or mucous cells indicate impaired environmental conditions in the ponds but could not be attributed to change of a specific physical or chemical parameter.

From changes that belong to the group of architectural and structural alterations (GR1), the lifting of respiratory epithelium (Figure 7) is the only one founded. This change is found in all ponds investigated on almost all analyzed samples with the very high frequency of appearance. On some samples the entire filament had lifted secondary epithelium. Lifting

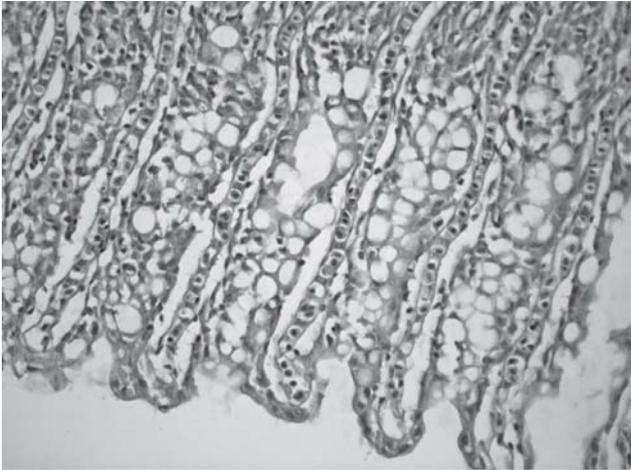


Fig. 6. Hyperplasia of the mucous cells (carp from fish pond V-3; 23. 10. 2003.; HE x200)

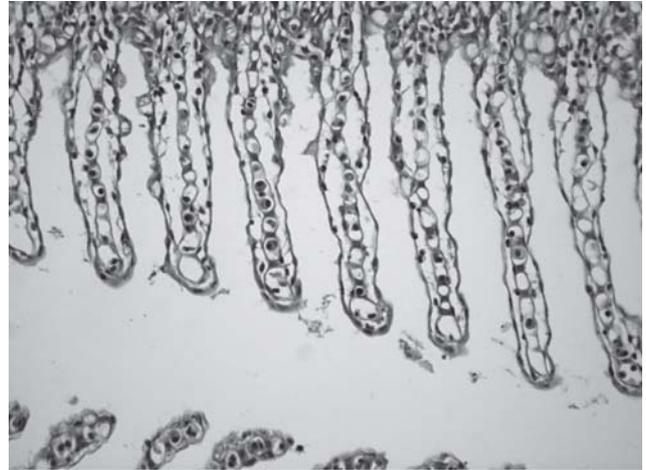


Fig. 7. Lifting of the respiratory epithelium (carp from fish pond V-4; 15. 07. 2003.; HE x400)

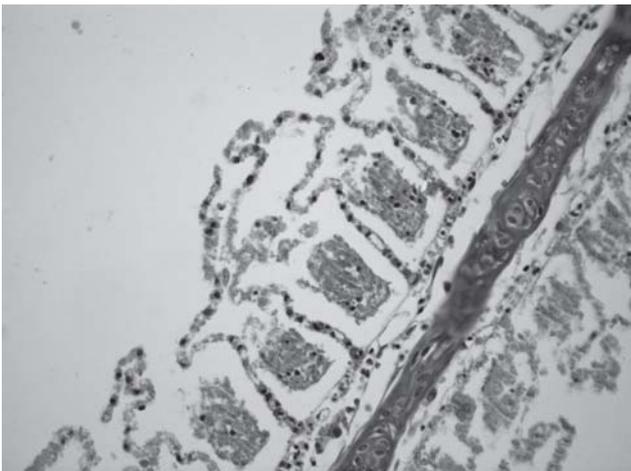


Fig. 8. Necrosis (carp from fish pond V-4; 27. 05. 2003.; HE x400)

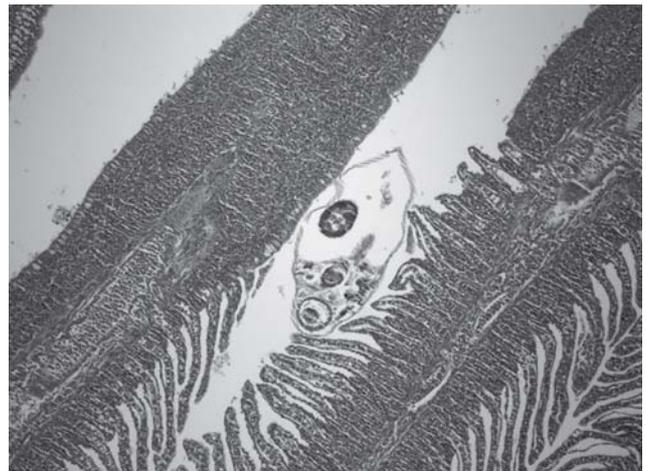


Fig. 9. Parasites-Trematodes (carp from fish pond V-7; 10. 06. 2004.; HE x100)

of the respiratory epithelium is considered as mild and reparable change (Poleksic and Mitrovic-Tutundzic, 1994).

Necroses are irreversible changes that occur following exposure to toxicants and/or chronic damage of the gills (Poleksic and Mitrovic-Tutundzic, 1994; Takashima and Hibiya, 1995). Gill necroses (Figure 8) detected in this investigation were focal. In 2003 necroses were prominent on gills of fish from ponds V-4, and in 2004 the largest score was found in gills from fish ponds V-1 and V-2.

In addition to Bernet et al., (1999) scoring we

considered alterations induced by parasites. The scoring system by Bernet et al. (1999), used in this field experiment is slightly modified. Being primarily based on histological analysis of fish population in wild, its negative side, when it is applied on fish at fish farms, is that it is not taking into consideration different ectoparasites. These parasites (usually Trichodina and Trematodes, as well as Myxosporidia) are common at carp fish farms, and they can contribute to certain degree of hyperplasia of primary epithelium. The degree of hyperplasia caused by parasites is never massive and that is the reason for assigning importance

factor of 1, even though Bernet et al. (1999) have assigned importance factor 2 to hyperplasia of epithelium.

Parasites were present in all samples examined. Most abundant were Trichodina and Trematodes (Figure 9); parasites cysts were found on all samples examined.

Myxosporidia were observed only in V-6 in 2004. Parasites abundance can be explained by environmental conditions in the pond such as stocking density and/or stress (Roberts, 1989; Airkoviæ, 2003 and Nowak et al., 2004). Increased organic matters are good substrate for parasites. By the results of the BOD₅ level it can be seen that the level of organic matters that is degraded was often high (Tables 2 and 3) and that consequently dissolved oxygen varied during the day (Boyd, 1982). Increased organic pollution that is often a result of excess of added feed and its decomposition affects fish gills making them more sensitive to pathogens and parasites (Klontz, 1995; Poleksic et al., 1999; Nikolic et al., 2003; Cirkovic, 2003). On the other hand, gill tissue reaction to parasites such as focal hyperplasia and mucous cells proliferation didn't affect gill function and most of the gills invaded by parasites were functionally normal.

Conclusions

In fish farms with semiintensive production aquatic environment is altered due to different measures used to maintain and enhance production. As a consequence changes in gill histology occur since they are in permanent contact with surrounding water and perform vital functions.

In ponds investigated at the fish farm "Vrš àèki ritovi" with periodically deteriorated water quality, alteration of the gill apparatus were observed. Their extent varied from slight alterations to, occasionally, serious ones dependent of the environmental conditions in the pond.

In the present study subepithelial edema and gill hyperemia that are amongst first defense mechanisms and are classified as mild and reparable were found

the most frequently - in all ponds investigated with score values above 3.4 for subepithelial edema and above 5.1 for hyperemia in all ponds investigated.

High ammonia level induced epithelial hyperplasia on the gills of carp. In less than quarter of gill samples examined symptoms of environmental gill disease (EGD) were observed, especially in 2004.

Parasites, especially mobile peritrichs (i.e., *Trichodina*) and Trematodes, were recorded in all ponds during each time period and occurred at higher frequencies in ponds with high biological oxygen demand and increased organic matter.

It is important to stress that although different gill changes were found, there were always parts of the gill apparatus still functionally normal and able to adapt to environmental changes that in this study exceeded values recommended for carp production only temporarily.

References

- Alabaster, J. S. and R. Loyd**, 1982. Water quality criteria for fresh water fish, *Butterworths*, London, 315 pp.
- Boyd, C. E.**, 1982. Water Quality Management of Pond Fish Culture. *Dev. Aquacult. Fish. Sci. 9*, Elsevier, Amsterdam, 318 pp.
- Bernet, D., H. Schmidt, W. Meier, P. Burkhardt-Holm and T. Wahli**, 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. *J. Fish Dis.*, **22**: 25-34.
- Cardoso, E. L., H. Chiarini-Garcia, R. M. A. Ferreira and C. R. Poli**, 1996. Morphological changes in the gills of *Lophiosilurus alexandri* exposed to un-ionized ammonia. *J. Fish Biol.*, **49**: 778-787.
- Crespo, S., F. Padros, R. Sala and M. J. Marlasca**, 1988. Gill structure of cultured *Salmo trutta fario* related to sampling techniques. *Dis. Aquat. Org.*, **4**: 219-221.
- Cirkovic, M.**, 2003. Carp fry protozoal diseases. In: T. Vulic (editor) *Trout and carp fishery seminar* (Proceedings of trout and carp fishery seminar, 23-24 September 2003) Belgrade, Serbia, pp. 51-61 (Sr).
- Das, P. C., S. Ayyappan, J. K. Jena and B. K. Das**, 2004. Acute toxicity of ammonia and its sub-lethal effects on selected haematological and enzymatic parameters of

- mrigal, *Cirrhinus mrigala* (Hamilton). *Aquacult. Res.*, **35**: 135-143.
- Evans, D. H.**, 1987. The fish gill: site of action and model for toxic effects of environmental pollutants. *Environ. Health. Perspect.*, **71**: 47-58.
- Ferguson, W. H.**, 1989. Systemic Pathology of fish. *Iowa State University Press*, Ames, USA, 263 pp.
- Frances, J., L. A. Geoff and F. B. Nowak**, 1998. The effects of nitrite on the short-term growth of silver perch, *Bidyanus bidyanus*. *Aquaculture*, **163**: 63-72.
- Goss, G. G., S. F. Perry, J. N. Fryer and P. Laurent**, 1998. Gill Morphology and Acid-Base Regulation in Freshwater Fishes. *Comp. Biochem. Physiol. A*, **119**: 107-115.
- Haaparanta, A., E. T. Valtonen and R. W. Hoffmann**, 1997. Gill anomalies of perch and roach from four lakes differing in water quality. *J. Fish Biol.* **50**: 575-591.
- Karan, V., S. Vitorovic, V. Tutundzic and V. Poleksic**, 1998. Functional enzymes activity and gill histology of carp after copper sulfate exposure and recovery. *Ecotoxicol. Environ. Saf.*, **40**: 49-55.
- Klontz, W. G.**, 1995. Care of Fish in Biological Research. *J. Anim. Sci.* **73**: 3485-3492.
- Lease, H. M., J. A. Hansen, H. L. Bergman and J. S. Meyerc**, 2003. Structural changes in gills of Lost River suckers exposed to elevated pH and ammonia concentrations. *Comp. Biochem. Physiol. C*, **134**: 491-500.
- Lindesjoo, E. and J. Thulin**, 1994. Histopathology of skin and gills of fish in pulp mill effluents. *Dis. Aquat. Org.*, **18**: 81-93.
- Mallatt, J.**, 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Can. J. Fish Aquat. Sci.*, **42**: 630-648.
- Nikolic, V., P. Simonovic, and V. Poleksic**, 2003. Preference of Trichodinids (Ciliata, Peritrichia) occurring on fish-pond carp for particular organs and some morphological implications. *Acta Vete.*, **53**: 41-46.
- Nowak, B. F., D. Dawson, L. Basson, M. Deveney M and M. D. Powell**, 2004. Gill histopathology of wild marine fish in Tasmania: potential interactions with gill health of cultured Atlantic salmon, *Salmo salar* L. *J. Fish Dis.*, **27**: 709-717.
- Papoutsoglou, S. E.**, 1992. Impact of aquaculture on the aquatic environment in relation to applied production systems. In: N. De Pauw and J. Jozce (Editors) *International Conference Aquaculture Europe "Aquaculture and the Environment"*, Dublin, Ireland, 10-12 June 1991, pp. 71-78.
- Pillay, T. V. R.**, 2004. *Aquaculture and the Environment*. Blackwell Publishing, London, 208 pp.
- Piper, R. G., I. B. Mc Elwain, L. E. Orme, J. P. Mc Caren, L. G. Fowler and J. R. Leonard**, 1982. Fish hatchery management. *US Fish and Wildlife service*, Washington, D.C.
- Poleksic, V. and V. Mitrovic-Tutundzic**, 1994. Fish gills as a monitor of sublethal and chronic effects of pollution. In: R. Muller and R. Lloyd (editors). Sublethal and chronic effects of pollution on freshwater fish. *FAO Fishing News Books*, pp. 339-352.
- Poleksic, V. and V. Karan**, 1995. Pathohistological effects of some herbicides to carp. *Pesticidi*, **10**: 41-47.
- Poleksic, V., M. Vlahovic, V. Mitrovic-Tutundzic and Z. Markovic**, 1999. Effects of environmental conditions on gill morphology of carp from the "Dubica" farm during the 1998 rearing season. *Ichthyol.*, **31**: 43-52.
- Poleksic, V., Z. Dulic Stojanovic and Z. Markovic**, 2002. Gill structure of carp fingerlings from Baranda fish farm. *Ichthyol.*, **34**: 11-20.
- Poleksic, V., Z. Markovic, Z. Dulic Stojanovic and M. Vasiljevic**, 2004. Gill histology of fish from Zlatibor reservoir lake. In: I. Todorovic, S. Radulovic and J. Bloesch (editors). *The International Association for Danube Research Conference* (Proceedings of the 35th IAD Conference, 19-23 April 2004.), Novi Sad, Serbia and Montenegro, pp. 361-367.
- Roberts, R. J.**, 1989. Fish pathology. *Bailliere Tindall*, London, 467 pp.
- Svobodova, Z., R. Lloyd, J. Machova, and B. Vykusova**, 1993. Water quality and fish health. *Eifac Tech. Pap.* **54**. FAO, Rome, 59 pp.
- Svobodova Z., J. Machova, J. Drastichova, L. Groch, V. Luskova, G. Poleszczuk, J. Velisek and H. Kroupova**, 2005. Haematological and biochemical profiles of carp blood following nitrite exposure at different concentrations of chloride. *Aquacult. Res.*, **36**: 1177-1184.
- Takashima, F. and T. Hibiya**, 1995. An atlas of fish histology. Normal and pathological features. *Gustav Fischer Verlag*. Kodansha, Tokyo, 192 pp.