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Response of olfactory receptors and blood cells of Lake Baikal fish to phenol exposure*

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ABSTRACT

Exposure to phenol causes ultrastructural disorders in supporting and receptor cells in the peripheral segment of the olfactory organ of yellowfin (*Cottocomephorus grewingkii*) and in cells in peripheral blood of stone sculpins (*Paracottus knerii*), yellowfin and perch (*Perca fluviatilis*). Identical responses to phenol exposure in the blood cells were observed in endemic Lake Baikal fish and in nonendemic fish. Differences were found only in the rate and intensity of the recovery response. Fast activation of immune processes were found in the coastal nonendemic perch, but they were slow in the stone sculpin, a coastal endemic species.

INTRODUCTION

The olfactory epithelium of fish contacts directly with the environment. Pollutants may cause a rapid destruction of peripheral chemosensitive formations, as well as metabolic and homeostatic disorders, what can result in malfunctions of behavioural reactions of fish (Baartrup et al. 1990; Kasumyan and Morsi 1998; Klaprat et al. 1992). For the maintenance of homeostasis in multicellular organisms the immune system plays a key role (Galaktionov 2005). Therefore, studies of mechanisms of adaptive cell rearrangements of olfactory and limpho-myeloid systems of fish are of special interest.

All forms (natural and acquired) of immune response which are displayed by higher vertebrates are also present in fish. On the other hand, the antigene-identifying system in fish is imperfect and depends, to a great extent, on various external factors (Kondratyeva et al. 2001). In contrast to higher vertebrates, the same organs in fish possess haemopoietic as well as immune functions, therefore cells of the immune system enter directly the peripheral blood. It is known that immunocompetent cells in peripheral blood influence the immune response in fish (Kondratyeva et al. 2001). Among cell elements of peripheral blood, subpopulations of granulocytes and monocytes participate in the response of innate immunity, whereas lymphocytes play a main role in response of adaptive immunity (Doggertt and Harris 1989; Ellis 1976, 1977; Ferguson 1976, 1989; Galaktionov 2005; Imagawa et al. 1989; Kondratyeva et al. 2001). Increases or decreases in haematological parameters, changes of cells structure, and occurrence of atypical cells is the answer to ecological (including chemical) stressors (Heath 1995).

Different phenolic compounds are active metabolites and play a prominent role in the regulation of biosynthetic processes. Industrialization caused an increased use and consequently inflow of phenols of anthropogenic origin into the water bodies. Phenol and phenolic compounds are biologically active and exhibit a high water solubility as well as a good bioavailability. Many phenolic compounds are neuro-toxicants and affect cholinergic synapses, causing general intoxication influencing all systems of the organism including the immune system (Flerov 1973, 1989; Mikryakov 2001; Valedskaya 2005). Phenol induces toxic, genotoxic, carcinogenic and immunotoxic effects for fish health. On the contrary, phenol may act as free radical scavenger and prevents genetic damage caused by other agents. It has a high bioaccumulation rate along the food chain due to its

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lipophilicity. Thus phenol pollution presents a threat against natural environment and also to human health. When the phenol is present in the aquatic environment, fish feeding intensity, their mean weight and fertility are significantly reduced. For these reasons, phenol intoxication must be taken in consideration in the fish farming systems and also in natural aquatic habitat (Abdel-Hameid 2007).

The investigation of mechanisms and consequences of the effect of phenol is a key aspect for an assessment of potential contaminations of the ecosystems and this is of vital importance for the conservation of a unique environment such as Lake Baikal.

Phenol constitutes 3rd class of danger and its maximum permissible concentration (MPC) in fresh water fish management equals 0.001mg·l^{-1} (Filenko and Mikheyeva 2007). In the Lake Baikal deep drinking water MPC of phenol should not exceed 0.0005mg·l^{-1}. According to Frezenius Institute (Fresenius Consult GmbH) phenols are not found in potable water of Lake Baikal (Grachev 2002). In 2008 the water of the Irkutsk water basin and Baikal Lake was estimated as "conditionally pure" and "slightly polluted". In Angara River up to Bratsk water basin the concentration of phenols was up to 1.6 MPC, in many water basins and rivers of Russia it is 2-4 MPC, in Moscow River within the city it reaches 27 MPC. The occurrence of phenols is caused by the industrial pollution (Anon. 2009).

The objective of this work was to study characteristic features of the structure of olfactory receptor cells and cells of peripheral blood in some fish of Lake Baikal subjected to the phenol exposure under the controlled conditions.

MATERIAL AND METHODS

Haematological parameters and structural characteristics of blood cells were examined in the control group and under phenol exposure in non endemic coastal-shore perch (*Perca fluviatilis*) and endemic sculpins: stone sculpin (*Paracottus knerii*) from the coastal-slope zone at a depth of 0-200m and in yellowfin (*Cottocomephorus grewingkii*) from the pelagic zone at a depth of 0-200m (Table 1). The ultrastructure of the olfactory epithelium was analyzed in yellowfin. All perch and stone sculpins have been caught in a coastal zone, and yellowfin at the depth of 50-100m around southwest coast of Baikal Lake in spring 2008-2009.

Experiments were carried out at stable temperature and hydrochemical conditions of aquariums in Limnological Institute in Irkutsk. The water was constantly aerated with compressor pumps connected to internal filters, and the temperature was maintained at 14±1°C. Fish were fed earthworms and zooplankton two times a week. The group of fish in aquarium without phenol was considered as a control group. The water in each aquarium was renewed every 3 days; after water renewal the tested pollutant was added.

The solution of crystal phenol in concentration of 3mg·l^{-1} (variant I), 6mg·l^{-1} (variant II) and 12mg·l^{-1} (variant III) has been used. The choice of concentration of the phenol has been chosen in order to secure comparison of the obtained results to the similar experiments (Mikryakov 2001); another reason was the aim of studying the reaction of Lake Baikal fish organisms to high concentration of phenol.

Yellowfin and stone sculpin were kept for 1, 4 and 14 days in aquariums at variant concentrations I and II, perch – 1 day in variant I, 1 day and 14 days in variant II. Stone sculpin and yellowfin survived 0.3 and perch survived 0.6 day in variant III. All data obtained were compared with respective parameters of the control group of fish. The ultrastructure of the olfactory epithelium was analyzed in yellowfin only in variant II (5 fish in the experiment and 5 fish in the control).

Standard haematological (Blaxhall and Daisley 1973; Srivastava 1968, 1969) and ultrastructural (Uikly 1975) analyses were performed. Smears of peripheral blood stained with azure-eosine were examined under the microscope Axiovert 200 with a camera Pixera Penquin 600 CL x 100 (Zeiss, Germany).

Table 1. Morphological characters of investigated fish.

Character	*Cottocomephorus grewingkii*		*Paracottus knerii*		*Perca fluviatilis*	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Length, Smitt (mm*)	107.8±2.04	98.7±1.8	78.4±3.4	75.1±9.4	103.0±2.7	104.8±10.1
Weight (g)**	15.2±1.3	10.7±1.5	9.6±1.3	12.0±2.9	15.7±1.4	20.04±.8.2
Stage of sexual maturity	4	4-5	4-5	4-5	3-4	3-4

* - body length till the end of medium rays of caudal fin,
** - body weight of fish with internal organs,
number of the investigated fish are given in Table 2.

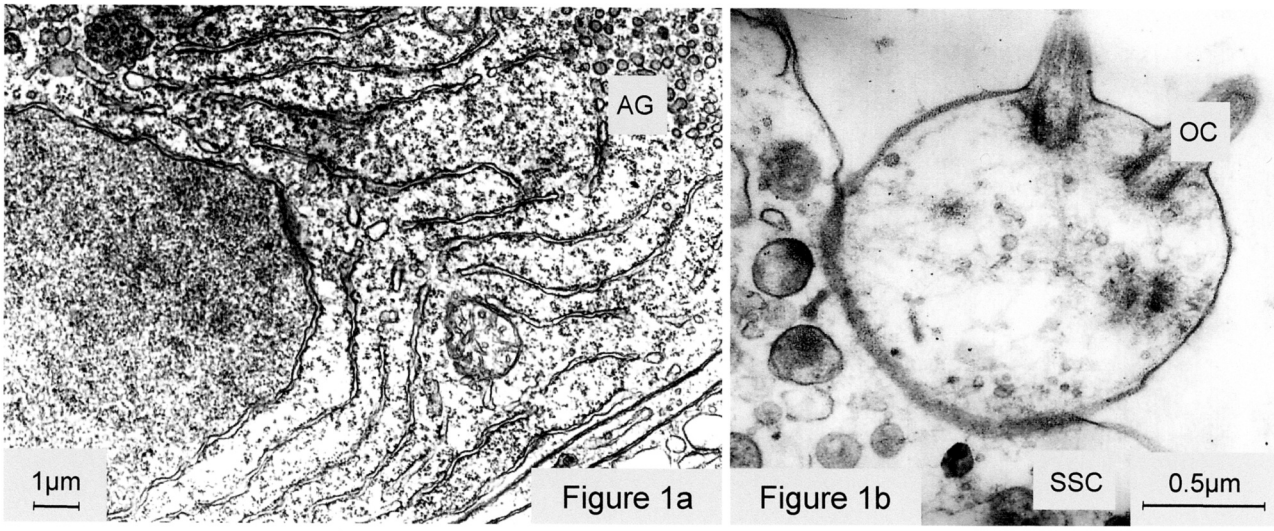


Figure 1. Normal structure of the olfactory epithelium of yellowfin. a) pericarion zone of olfactory cell with ordered channels of endoplasmic reticulum, b) olfactory bulb of receptors. AG – Golgi apparatus, OC – olfactory plait, SSC – secretory supporting cells.

For scanning electron microscopy preparations of blood and olfactory epithelium were fixed in 2.5% of gluteraldehyde in 0.1M phosphate buffer with a fixation in osmium tetroxide. After dehydration in ethanol at increasing concentrations and critical point drying in a CPD 0,30 (Balzers Union, UK) preparations were coated with gold in JEE-4B (Jeol Electron Optics Laboratory, Japan) and examined in a scanning electron microscope Philips 525M (Holland).

For transmission electron microscopy preparations fixed in gluteraldehyde were stained in 2% OsO_4 in 0.1M phosphate buffer for 12h. Then the material was dehydrated in ethanol at increasing concentrations and coated with resin. After polymerization, sections were made with ultramicrotome Ultracut R (Leica, Germany) which were analyzed in a transmission electron microscope LEO 906E (Leica, Germany).

Reliability of differences in haematological parameters was checked with Student's criteria (Urbakh 1964; Zhivotovsky 1991).

RESULTS

Response of olfactory system of yellowfin to phenol impact

A range of ultrastructural disorders in supporting and receptor cells occurred in the peripheral segment of the olfactory organ under phenol exposure (Figure 1a, b): volume increase of cysternal cavity of rough endoplasmic reticulum canals (Figure 2), vacuolization of different morpho-functional segments of Golgi apparatus (Figure 3a, b), as well as complete or partial mitochondrial swelling with destructive changes of cristae (Figure 4). Large (1.0-1.3μm) vacuole-like formations with light content were observed in the cytoplasm of sensitive neurons, both in the perikaryon (Figure 5) and in the peripheral appendix including its apical segment. In some parts of the epithelium, distal segments of supporting and receptor cells lost their integrity and discharged some of their structural elements (Figure 6a, b). Chemosensitive cilia of receptor and ciliary cells were damaged differently (Figure 7). In some cases the damage of their cytoskeleton was observed.

Response of peripheral blood to phenol impact

Unlike in fish from the control group (Figure 8) specimens under phenol exposure developed a vacuolization of cytoplasm in the erythrocytes and leucocytes (Figure 9a, b) and destruction was observed in case of erythrocytes (Figure 10), mitochondria cristae (Figure 11) and granules in eosinophils (Figure 12).

In the peripheral blood of phenol exposed yellowfin in groups I and II the percentage of young neutrophils and polymorphonuclear neutrophils has substantially increased during the first four days of the experiment, and in group III it has decreased within 0.3 days. After two weeks the quantity of polymorphonuclear neutrophils in the groups I and II and of young neutrophils in the group I did not differ from those in the control fish. The quantity of young neutrophils in the group II was substantially higher in comparison with control fish. After 4 days of the experiment in the peripheral blood of fish in the group I the polymorphonuclear neutrophils prevailed ($p<0.05$), and in the group II most abundant were young neutrophils ($p<0.05$). In the fish of the group II the eosinophils have been found after four days of the experiment (Table 2).

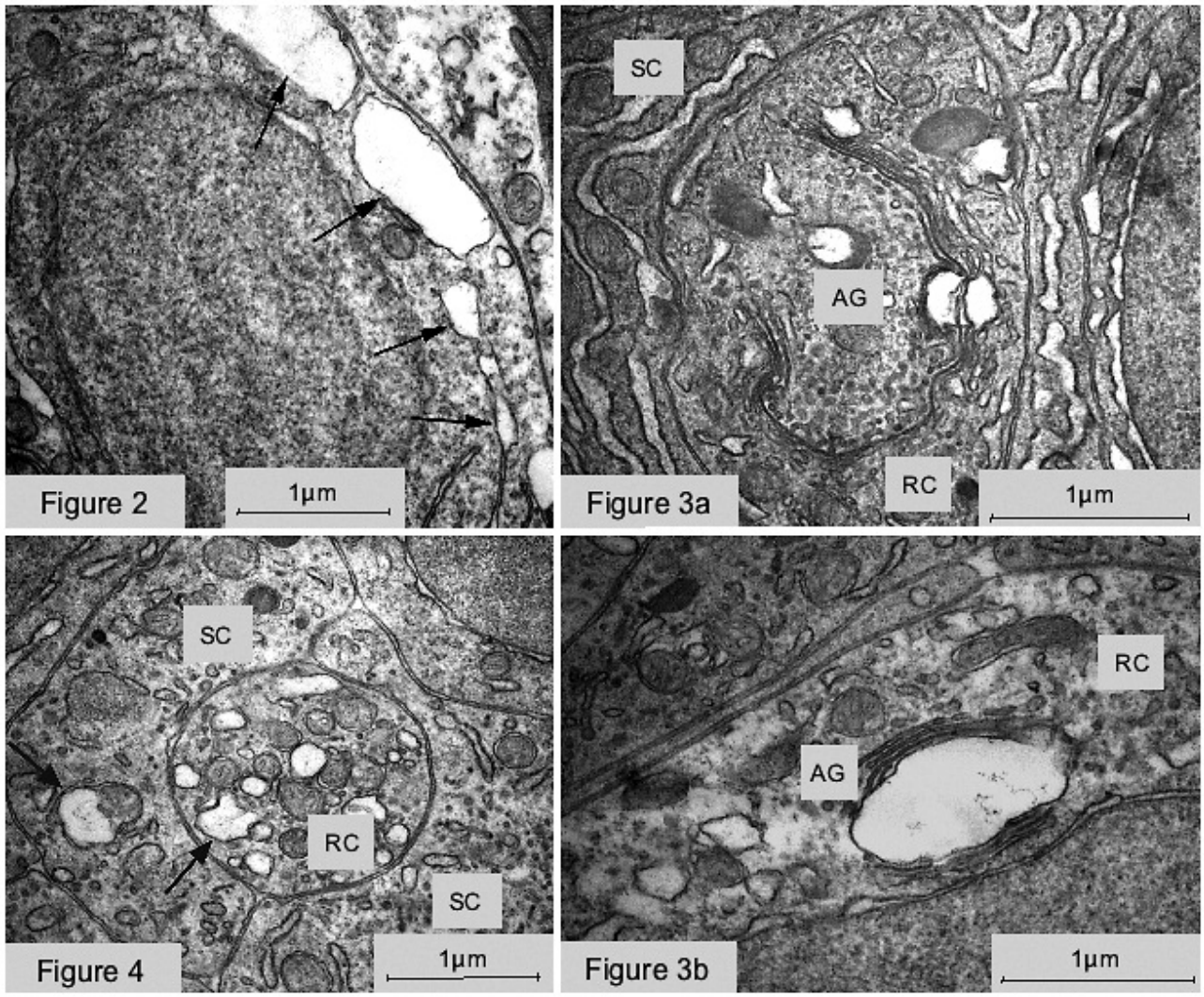


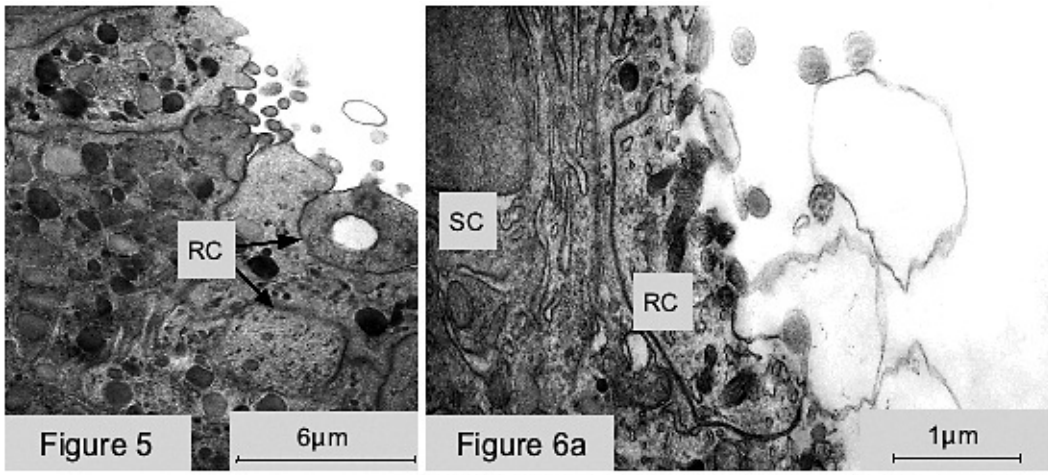
Figure 2. Fragmentation and increase in a gleam of channels of the rough endoplasmic reticulum (arrows) in a receptor cell following phenol exposure in yellowfin.

Figure 3 (a, b). Vacuolisation of Golgi apparatus in olfactory cells after phenol exposure in yellowfin. AG – Golgi apparatus, RC – receptor cells, SA – supporting cells.

Figure 4. Vacuolization of mitochondria (arrow) in receptor (RC) and supporting (SC) cells in phenol exposed yellowfin.

The change in the quantity of leucocytes in the peripheral blood of phenol exposed stone sculpin was smaller than that observed in yellowfin. The percentage of young neutrophils in stone sculpin has increased in the groups I (4 days) and II (1 day) in comparison with control fish. The percentage of polymorphonuclear neutrophils has decreased in the group I (1 day). After 0.3 days of the experiment in the group III the abundance of young and polymorphonuclear neutrophils has increased. The distinctions between the young and polymorphonuclear neutrophils was not clear in some variants of the experiment. The eosinophils were present in the experimental fish of the group II (1 and 4 days) (Table 2).

The percentage of young neutrophils in perch has increased in the first day in all variants of the experiment. The quantity of these cells has decreased to a normal state after two weeks in the group II. The substantial changes of the abundance of monocytes were revealed in perch and yellowfin during the first days of the experiment. Substantial changes in the quantity of monocytes and lymphocytes were revealed in perch and yellowfin in the first days of the experiment. The percent of lymphocytes has decreased at stone sculpin in the groups III (0.3 days) and I (4 days). In other variants of the experiment the abundance of these cells has changed slightly (Table 2).



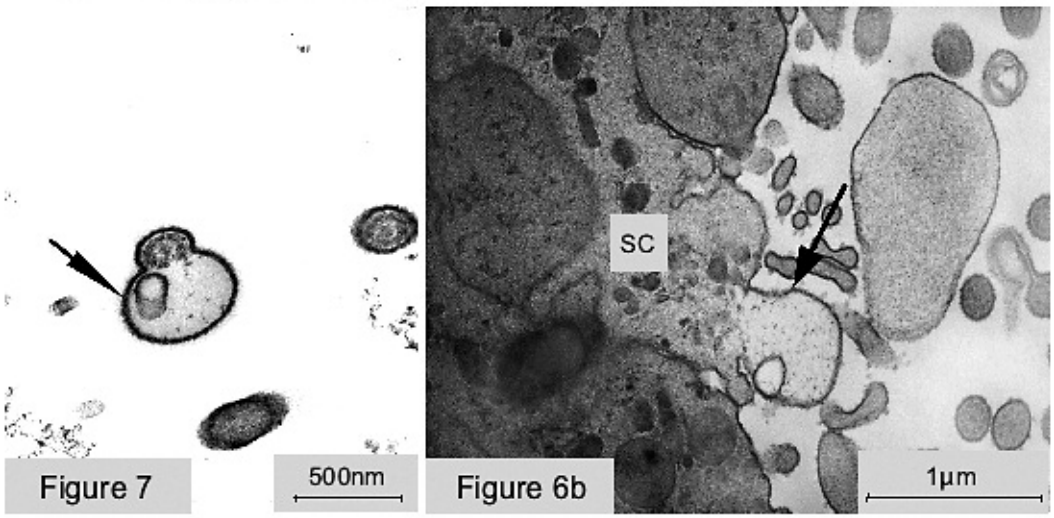


Figure 5. Localisation of vacuoles in the apical sector of receptor cell (RC) in phenol exposed yellowfin.

Figure 6. a) Disorder of apical sector of receptor cells (RC) and b) vacuole liberation site from apical part of supporting cell (SC) (arrow) in phenol exposed yellowfin.

Figure 7. Local vacuolisation of cilium (arrow) in phenol exposed yellowfin.

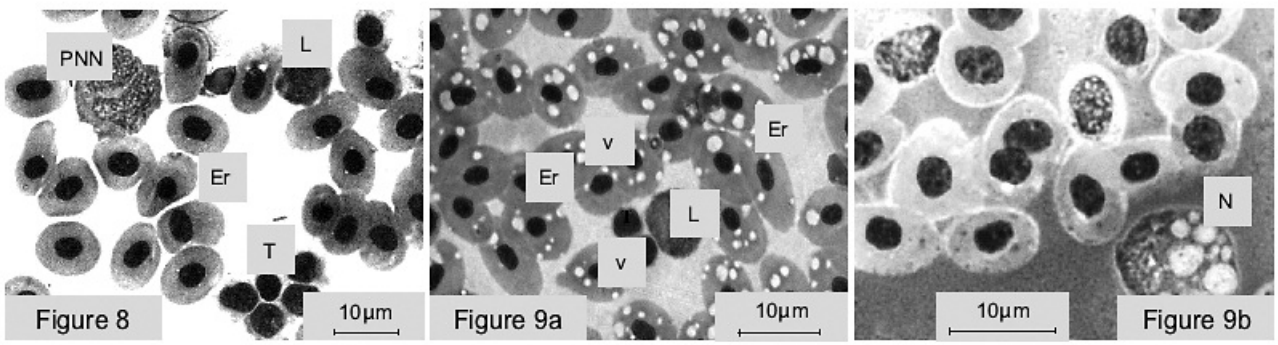
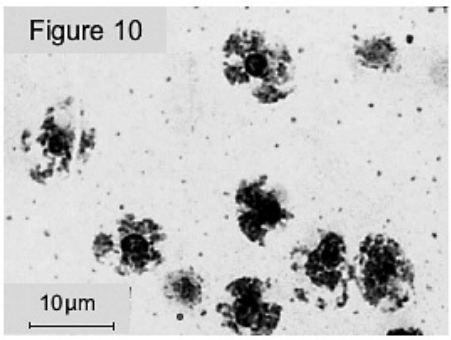
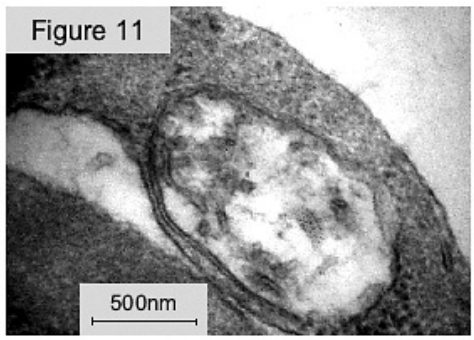


Figure 8. Stone sculpin peripheral blood in the control fish. Er – erythrocyte, L – leukocyte, PNN – polynuclear neutrophil, T – thromocyte, L – lymphocyte.

Figure 9. Vacuolisation of cytoplasm in a) erythrocytes in phenol exposed yellowfin and b) leucocytes in phenol exposed perch. Er – erythrocyte, L – lymphocyte, N – neutrophil, v – vacuole.





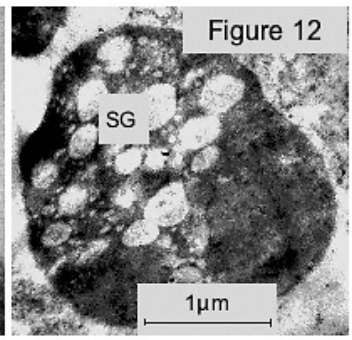


Figure 10. Destruction of erythrocytes in phenol exposed stone sculpin.

Figure 11. Destruction of mitochondria cristae in phenol exposed perch.

Figure 12. The eosinophils with destroyed granules in phenol exposed perch. N – nucleus, SG – secretion granules.

Table 2. Characteristics of leucocytes in peripheral blood of Lake Baikal fish exposed to phenol.

Leucocytes	Control	Phenol concentration [mg·l^{-1}] (experimental group)						
		3 (I)			6 (II)			12 (III)
		Days of exposition			Days of exposition			Days of exposition
		1	4	14	1	4	14	0.3
Cottocomephorus grewigkii								
Neutrophils	5.0±1.4[1] (26)	9.8±4.6 (7)*	17.6±2.5 (7)***	3.8±0.2 (6)	16.7±2.3 (14)***	40.0±11.0 (6)***	17.6±7.0 (5)***	30.9±18.1 1 (4)**
Polynuclear neutrophils	5.3±1.6	12.8±7.3	32.1±5.7***	3.6±0.1	16.7±4.2*	16.0±3.3**	8.3±2.1	27.9±2.2***
Monocytes	1.4±0.3	0.2±0.02***	3.4±0.4***	1.3±0.3	0***	2.0±0.9	0.9±0.1	1.7±0.2
Lymphocytes	88.3±1.7	77.3±9.9	47.0±15.1*	91.3±0.2	66.6±7.3	42.0±13.1	38.0±12.4	39.5±18.6**
Eosinophils	0	0	0	0	0	0	35.2±0.1	0
Paracottus knerii								
Neutrophils	9.2±3.4 (12)	14.4±2.4 (11)	23.2±4.5 (8)**	11.0±2.2 (4)	23.6±7.9 (12)**	11.3±2.5 (9)	18.3±2.9 (8)	22.0±2.2 (4)**
Polynuclear neutrophils	9.8±2.7	2.5±0.8*	20.3±3.1**	6.8±2.2	22.3±5.9	15.8±4.5	7.5±1.8	24.0±4.3***
Monocytes	1.1±0.7	2.5±0.7	2.9±0.9	4.1±1.2*	1.2±0.9	1.5±0.7	1.0±0.03	17.0±1.9
Lymphocytes	79.9±6.5	79.3±4.1	53.6±2.3***	78.11±16.7	50.7±10.3*	70.1±6.2	73.2±4.2	47.0±8.4*
Eosinophils	0	0	0	0	2.2±2.1	1.3±1.3	0	0
Perca fluviatilis								
Neutrophils	1.5±0.1 (6)	75.0±13.8 (8)***	- -	- -	58.3±5.4 (5)***	- -	4.1±0.2 (4)***	43.8±14.8 (3)**[2]
Polynuclear neutrophils	11.5±0.3	7.8±1.3	-	-	18.3±0.1	-	2.3±0.1***	8.9±5.3
Monocytes	0.9±0.1	1.6±0.9	-	-	1.7±1.7	-	2.3±0.3***	1.9±0.9
Lymphocytes	86.1±3.5	15.6±1.4***	-	-	21.7±3.6***	-	61.5±5.1***	45.4±15.4**
Eosinophils	0	0	-	-	2.2±2.1	-	0	0

[1] average value±standard deviation,
[2] 0.5 day of exposition,
– not measurable,
significance of differences in quantity of phagocytes in the blood of experimental fish in comparison to control fish *p<0.05, **p<0.01, ***p<0.001.

In case of several investigated fish the jaundice of their liver and its destruction was noted. .

DISCUSSION

Our study showed that an exposure to phenol results in a variety of ultrastructural disorders in all cell elements of the olfactory epithelium. The observed vacuolization of receptor neurons is interesting, as they predetermine chemosensitivity of olfactory system in animals. At present it is difficult to determine the content of these formations. Special cytochemical studies should be carried out to reveal their biochemical composition. Moreover, the potential of reversibility of ultrastructural changes in the cell elements of the epithelium remains uncertain: (i) are the receptor and supporting cells able to restore their initial morphology, (ii) are the structural changes of intracellular organelles irreversible, and (iii) do these changes cause cell apoptosis? It can be assumed that in case of irreversibility of these disorders the function of the olfactory system of animals can be changed significantly, which may impact their complex adaptive behaviour. An ultimate result could be unspecific anosmia which may lead to the situation that animals will lose their ability to differentiate between low concentrations of chemicals which would result in losing their avoidance behaviour.

The experiments conducted revealed an identical response to phenol of cell elements in Lake Baikal fish as they were observed in fish species from other water bodies (Mikryakov 2001; Valedskaya 2005; Wlasow 1985).

Phenol exposure during the first 4 days initiated an immune respose. The number of young granulocytes increased in peripheral blood of yellowfin by up to 40%. Synthesis of antibodies as a defence reaction of fish to antigene stimulants is the result of cell activity of the lymphoid macrophage system. It is related to the processes of identification, perception, and destruction of an antigene and differentiation of immunocompetent cells into antibody-forming cells. Myelopoiesis becomes initiated and the processes of granulocyte formation are intensified (Galaktionov 2005; Mikryakov 2001).

Disorders of lympho- and myelopoiesis were observed after chronic exposure to phenol. Neutrophilia and lymphopenia are caused by reducing processes occurring in fish organisms and by tissue necrosis. Neutrophils, due to the presence of proteolytic and other enzymes, take part in a variety of immunological functions, including phagocytosis and migration to localizations of pathogenic and toxic stimulants (Antonyak 1999; Heath 1995).

The percentage of segmentonuclear leucocytes also increased in peripheral blood of studied fish. It is known that under conditions of long-term exposure to high concentrations of phenol the specific immune response is inhibited. Both antigen-sensing and antigen-destructive structures are damaged under the influence of phenol. Neutrophil proteases destroy erythrocytes and other cells and suppress haemopoiesis under conditions of insufficient activity of inhibitors (Antonyak 1999; Mikryakov 2001). It is possible to assume that these were the causes of vacuolization of cytoplasm and of mitochondria, destruction of cristae in leucocytes, vacuolization of cytoplasm, segmentation of nuclei and destruction of erythrocytes. Destruction of erythrocytes and phenol-induced denaturation of hemoglobin (Bukowska et al. 2007) could be the reasons of a jaundice of the liver in the investigated fish. *Oreochromis niloticus* subjected to phenol accumulated this chemical in tissues of liver, muscles, and gills (Gad and Saad 2008), and the liver *O. aureus* showed high score of histopathological symptoms such as inflammation, necrosis and cell degeneration (Abdel-Hameid 2007). Similar symptoms of intoxication by phenol were observed in a coastal rainbow trout (Wlasow 1985).

This study suggests that the investigated Baikal Lake fish showed similar reaction of blood cells to phenol exposure as those observed in other, nonendemic fish species; some of the observed differences might be explained by ecological characteristics of the given species. Fast activation of immune processes was found in the coastal nonendemic perch, but it was slower in the coastal endemic yellowfin. Reaction of olfactory epitelium in yellowfin to phenol was identical to that observed in other species.

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REFERENCES

Abdel-Hameid, N.A.H. 2007. Physiological and histopathological alterations induced by phenol exposure in *Oreochromis aureus* juveniles. Turkish Journal of Fisheries and Aquatic Sciences 7: 131-138.

Anonymous. 2009. About a condition and about preservation of the environment of the Russian Federation in 2008. The State Report. Moskva, OOO "RPPR RusKonsaltingGrupp" by request of the Ministry Natural Resources and Ecology of the Russian Federation. 488 p.

Antonyak, G.L. 1999. A role of proteolytic enzymes in functional activity in neutrophil granulocytes. Uspekhi Sovremennoi Biologii 119: 476-486 (in Russian).

Baatrup, E., K.B. Doving, S. Winberg. 1990. Differential effects of mercurial compounds on the electroolfactogram (EOG) of salmon (*Salmo salar* L.). Ecotoxicology and Environmental Safety 20: 269-276.

Blaxhall, P.C., K.W. Daisley. 1973. Routine haematological methods for use with fish blood. Journal of Fish Biology 5: 771-781.

Bukowska, B., J. Michałowicz, A. Krokosz, P. Sicińska. 2007. Comparison of the effect of phenol and its derivatives on protein and free radical formation in human erythrocytes (in vitro). Blood Cells, Molecules and Diseases 39: 238-244.

Doggertt, T.A., J.E. Harris. 1989. Ultrastructure of the peripheral blood leucocytes of *Oreochromis mossambicus*. Journal of Fish Biology 33: 747-756.

Ellis, A.E. 1976. Leucocytes and related cells in the plaice *Pleuronectes platessa*. Journal of Fish Biology 8: 143-156.

Ellis, A.E. 1977. The leucocytes of fish: a review. Journal of Fish Biology 11: 453-491.

Ferguson, H.W. 1976. The ultrastructure of plaice (*Pleuronectes platessa*) leucocytes. Journal of Fish Biology 8: 139-412.

Ferguson, H.W. 1989. The cardiovascular system. In: Systemic Pathology of Fish, pp. 104-124. Iowa State University Press, Ames, IA.

Filenko, O.F., I.V. Mikheeva. 2007. Basics of Water Toxicology (ed. I.S. Shesterin, E.V. Ivanekha). 144 p. Izdatelstvo "Kolos" (in Russian).

Flerov, B.A. 1973. Experimental research of a phenolic poisoning of fishes. In: Phenol Influence on Hydrobionts (ed. M.M. Kamshilova), 195 p. Izdatelstvo "Nauka", Leningrad (in Russian).

Flerov, B.A. 1989. Ecological and Physiological Aspects of Toxicology of Freshwater Animals (ed. A.V. Monakov), 144 p. Izdatelstvo "Nauka", Leningrad (in Russian).

Gad, N.S., A.S. Saad. 2008. Effect of environmental pollution by phenol on some physiological parameters of *Oreochromis niloticus*. Global Veterinaria 2: 312-319.

Galaktionov, V.G. 2005. Evolutionary Immunology (ed. G.I. Abelev, A.A.Yarilin). 408 p. Izdatelstvo IKC "Akademkniga", Moskva (in Russian).

Grachev, M.A. 2002. About a Modern State of Ecological System of Lake Baikal (ed. D.N. Edzhington), 154 p. Izdatelstvo "Nauka", Novosibirsk (in Russian).

Heath, A.G. 1995. Water pollution and Fish Physiology. 373 p. CRC Press, Inc.

Imagawa, T., Y. Hashimoto, H. Kitagawa, Y. Kon, N. Kudo, M. Sugimura. 1989. Morphology of blood cells in carp (*Cyprinus carpio* L.). Japanese Journal of Veterinary Science 51: 1163-1172.

Kasumyan, A.O., A.M. Morsi. 1998. Influence of heavy metals on the food activity and flavouring behavioural answers of carp (*Cyprinus caprio*): Copper, cadmium, zinc and lead. Journal of Ichthyology 38: 393-409 (in Russian).

Klaprat, D.A., R.E. Evans, T.J. Hara. 1992. Environmental contaminants and chemoreception in fishes. In: Fish Chemoreception (ed. TJ. Hara), pp. 321–341. Chapman and Hall, London.

Kondratiyeva, I.A., A.A. Kitashova, M.A. Lange. 2001. The organisation of the immune systems of fishes. Vestnik Moskovskogo Gosudarstvennogo Universiteta. Biology 16: 11-20 (in Russian).

Mikryakov, V.P. 2001. Reaction of Fish Immune System to Acidification and Contamination of Water by Toxicants. 126 p. Izdatelstvo "Nauka", Moskva (in Russian).

Srivastava, A.K. 1968. Studies on the haematology of certain freshwater teleosts. IV. Leucocytes. Anatomischer Anzeiger 123: 520-533.

Srivastava, A.K. 1969. Studies on the haematology of certain freshwater teleosts. V. Thrombocytes and clotting of blood. Anatomischer Anzeiger 124: 520–533.

Uikly, V. 1975. Electron Microscopy for Beginners. 324 p. Izdatelstvo "Mir", Moskva (in Russian).

Urbakh, W.Yu. 1964. Biometry Methods (ed. N.N. Livshic), 295 p. Izdatelstvo "Nauka", Moskva (in Russian).

Valedskaya, O.M. 2005. The fish immunity condition of Volga River delta and features of its functioning at transformation of integuments (ed. R.I. Asfandiyarov, A.V. Boiko, A.I. Kulinarenko), 112 p. Izdatelstvo KaspNIIRKH, Astrakhan (in Russian).

Wlasow, T. 1985. The leucocyte system in rainbow trout *Salmo gairdneri* Rich affected by prolonged subacute phenol intoxication. Acta Ichthyologica et Piscatoria 15: 83-94.

Zhivotovsky, L.A. 1991. Population Biometry (ed. N.V. Glotov), 271 p. Izdatelstvo "Nauka", Moskva (in Russian).