HEMATOLOGICAL EFFECTS OF CROWDING STRESS IN EUROPEAN CHUB *LEUCISCUS CEPHALUS* L. AND COMMON CARP *CYPRINUS CARPIO* L.

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Abstract. Hematological changes following short-time confinement in two cyprinid fish species: European chub and common carp were compared. Juvenile chub *Leuciscus cephalus* and carp *Cyprinus carpio* were subjected to a 30 min. confinement. Hematological parameters were evaluated 2 weeks before, immediately after, and one week post treatment. Within a month most chub died, while all carp survived. Both species developed stress reaction that was indicated by hyperglycemia observed immediately post treatment. In chub, it was accompanied by reduction of oxidative metabolic activity of phagocytes and thrombocytopenia, while in carp a decrease in erythroblast frequency was observed. In one week post confinement chub showed anemia (reduced hematocrit and hemoglobin levels accompanied by erythroblastosis), and oxidative metabolic activity of phagocytes remained decreased. In carp, leukopenia and thrombocytopenia occurred, and frequency of erythroblasts remained reduced. No changes in differential leukocyte count were observed in any species. The results indicate higher sensitivity of chub to confinement stress compared to carp.

Keywords: European chub, common carp, crowding stress, hematological parameters

Introduction. During aquaculture or experimental procedures fish often experience stress that may alter many physiological parameters, and thus affect rearing success or research results. Harvesting, handling, sorting, holding, and transporting are routine practices that can have significant effects on fish physiology and survival (Benfey and Biron, 2000; Portz et al., 2006). Aquaculture, restocking and experimental procedures involve confinement and crowding of fish, e.g. during transportation, transfer to another tank or exchange of water.

Stress responses in teleost fishes are manifested as primary, secondary, and in some cases tertiary reactions (Wendelaar Bonga, 1997; Barton, 2002). The primary response to stress involves the activation of two major systems: the sympathetico-chromaffin (SC) system and the hypothalamic-pituitary-interrenal (HPI) axis. Stimulation of the SC system results in increased circulating levels of adrenaline, while stimulation of the HPI axis causes an increase in circulating levels of cortisol (Frisch and Anderson, 2000).

The primary stress response triggers sequential secondary responses that are manifested as changes in a range of biochemical, physiological, hematological and immunological parameters (e.g. increases in plasma glucose, hematocrit, lactate, heart rate, gill blood flow, potassium, liver glycogen, muscle protein (Barton and Iwama, 1991; Svoboda, 2001; Barton, 2002). According to Wendelaar Bonga (1997) and Svoboda (2001), catecholamine release during stress causes an increase in blood oxygen carrying capacity, while cortisol mediates the inhibitory effects of stressors on fish immune response. If the stress is severe or prolonged, tertiary responses follow. These include reduced growth rate end metabolic scope for activity, decreased disease resistance and reproductive capacity, as well as altered behaviour (Barton and Iwama, 1991). Increase in mortality induced by bacterial infection in *Danio rerio* subjected to crowding and handling was reported by Ramsay et al. (2009).

European chub (*Leuciscus cephalus*) is a cyprinid fish that occurs in freshwaters of almost all Europe, South Caucasus and parts of Asia Minor, except for Italy and Adriatic basin (Hliwa et al., 2009). Chub has no economic importance but it is valued as a game fish. Although it is one of the most widely distributed freshwater fish in Europe, in recent years it has disappeared from many habitats and has become an endangered species (Penczak and Kruk, 2000; Krejszeff et al., 2008). Therefore, fish farmers try to produce chub stocking material to restock natural waters (Krejszeff et al., 2008). Common carp (*Cyprinus carpio*) is a domesticated cyprinid species for centuries popular in Central Europe aquaculture and very often also used as experimental object in fish biology research.

Hematological values and blood picture of chub are poorly known (Hlavova, 1993; Lamkova et al., 2007). The results obtained by Bau et al. (2000) and Bracewell et al. (2004) showed that handling, net capture and electrofishing resulted in stress in chub. Pottinger et al. (2000), Pottinger (2010) reported that European chub showed considerably higher basal and confinement-induced cortisol levels compared to the other fish species, including common carp. However, other symptoms of stress in chub such as glucose level were similar to those reported for other species.

The present study was undertaken to evaluate and compare the changes in peripheral blood parameters and blood picture in two cyprinid fish species: European chub and common carp following confinement stress.

Materials and Methods

Chub juveniles (89.8 ± 2.6 g) were obtained from the hatchery and common carp juveniles (35.9±5.0 g) from the rearing pond of the Inland Fisheries Institute in Żabieniec. The fish were transported in plastic bags with

water and pure oxygen. Then they were transferred to the constantly aerated 300 dm^3 flow-through plastic tanks and left for 4 months. They were fed once a day carp pellets Aller Aqua Master 4.5 mm *ad libitum*. Water quality parameters (temperature, pH, dissolved oxygen concentration, concentrations of NH_4^+ and NO_2^-) were measured every 3 days. Temperature and DO levels were measured using oxygen meter Hanna Instruments USA, pH with the pH-meter N5123 Elwro Poland, and nitrogenous metabolites using colorimetric Visocolor kits (Visocolor Eco Ammonium 3, and Visocolor Eco Nitrite) by Machery Nagel, Germany. The results of water quality measurements are shown in Table 1. All the values were safe for the fish. Blood was sampled at the beginning of the experiment from 12 fishes of each species to provide control values (experimental groups named LC and CC for chub and carp, respectively) and then these fishes were transferred to the separate tanks. The fishes were left to recover for two weeks after initial blood sampling and then they were subjected to a confinement stress: all fish harvested from the tank using a net and was placed for 3 hours in a plastic baskets of 30 dm^3 suspended in the same tanks so as the each fish group confined in about 20 dm^3 of water. Immediately after this time, blood was collected from all fish (LS1 and CS1) and afterwards they were released back into the tanks. Subsequent blood sampling took place in one (LS2, CS2) week post confinement.

Table 1. **The values** (arithmetic means and standard deviations, for pH – minimum and maximum) **of water quality parameters in experimental tanks**

Parameter	Chub	Carp
O_2 [mg/L]	8.6±0.2	8.6±0.3
NH_4^+ [mg/L]	0.03±0.1	0.03±0.1
NO_2^- [mg/L]	0.002±0.01	0.005±0.01
Temperature [°C]	16.4±0.9	16.4±0.9
pH	7.1-7.2	7.1-7.2

Blood was sampled from the fish by heart puncture using heparinized chilled needles into heparinized chilled Eppendorf tubes, without anesthesia. Blood sampling from each fish lasted about 30 s and no more than 100 mm^3 of blood was collected each time. Immediately after sampling, blood was subjected to standard hematological analysis (Svobodova et al., 1991). Following parameters were evaluated: hematocrit (Ht), hemoglobin concentration (Hb), red and white blood cell count (RBC, WBC), and oxidative metabolic activity of phagocytes (NBT). Derived erythrocyte parameters were calculated: mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Blood smears were also made and stained May-Grunwald and Giemsa solutions for evaluation red and white blood cell picture, and estimation of thrombocyte count (PLT). Hematocrit was measured using microhematocrit method – blood was placed in heparinized glass capillary hematocrit tubes and centrifuged in a microhematocrit centrifuge for 3 min. at 12000 rpm. Hemoglobin concentration was measured using cyanmethemoglobin method. Blood (10 mm^3) was mixed with 1 cm^3 of Drabkin solution, left for 15 min. and then absorbance was read at 540 nm wavelength. Concentration of hemoglobin was calculated using the equation of relationship between absorbance and concentrations of hemoglobin standard solutions. Red and white blood cell counts were done in blood diluted 1:100 with Hayem solution, using Burker hemocytometer. Other red blood cell parameters: MCV, MCH and MCHC were calculated according to the formulas: MCV = Ht×10/RBC, MCH = Hb/RBC, MCHC = Hb×100/Ht. Oxidative metabolic activity of phagocytes was measured using nitrotetrazolium blue (NBT) reduction method adapted for fish blood (according to Studnicka et al., 1985). Blood (50 mm^3) was mixed with equal amount of dye solution and incubated for 1 h at 28°C. Then, 2 cm^3 of dimethyloformamide (DMF) was added to each sample, the samples were shaken for 3 min. to disrupt cell membranes and release formazan (the product of NBT reduction), and centrifuged for 10 min. at 3000 rpm for sedimentation of cell debris. Absorbance was read at 546 nm wavelength and concentration of formazan was calculated using the equation of relationship between absorbance and concentrations of formazan standard solutions. Blood smears were viewed at ×1000 magnification using Nikon Eclipse E600 microscope. Differential red and white blood cell counts were calculated (in percent) based on cytological evaluation of 300 erythrocytes and 100 leukocytes in each smear. Thrombocytes accompanying 100 leukocytes were also counted, and the thrombocyte count was estimated using WBC value. Plasma glucose concentrations were also measured using Accu-Check Go glucometer.

The obtained results were subjected to statistical analysis using Statistica 9.1 software. Arithmetic means and standard deviations of all parameters for each sampling time were calculated, and the significance of differences was evaluated with Kruskal-Wallis test at $p \leq 0.05$.

Results

During confinement, behaviour of two fish species was different: chub remained almost immobile, while carp were agitated during first 5–10 minutes, and then swam normally. Within a month post experiment 7 out of 12 chub died, while all carp survived. The control values of most hematological parameters differed between fish species. Chub showed significantly higher Hb, MCV, MCH and MCHC values compared to the common carp, while in the latter RBC, percentage of erythroblasts, WBC and oxidative activity of phagocytes (NBT) were significantly higher than in chub. The Ht values of both species were very similar, and so did PLT and glucose levels.

Immediately after confinement (LS0) chub showed a significant hyperglycemia, decrease in oxidative metabolic activity of phagocytes and thrombocytopenia (Tables 2 and 3). A week later (LS1) Ht and Hb values significantly decreased compared to the control, which

was accompanied by a decrease in MCH and MCHC. Oxidative metabolic activity of phagocytes remained reduced, while thrombocyte count and glucose level no more significantly differed from the control level. The fish developed also erythroblastosis. The chub subjected to crowding showed higher average percentage of neutrophils compared to the control but the differences were insignificant due to high individual variability.

The only significant hematological changes in carp after confinement (CS0) included hyperglycemia and reduction of erythroblast frequency (Table 2). WBC value decreased and average neutrophil frequency was higher compared to the control but the differences were not significant due to high variability within the group. In a week post treatment common carp developed leukopenia and thrombocytopenia, and erythroblast level remained reduced.

Table 2. **The effects of crowding stress on glucose levels and red blood parameters of *Leuciscus cephalus*** (L) **and *Cyprinus carpio*** (C)

Groups (n)	Glucose [mg/100 cm^3]	Ht [%]	Hb [g/dm^3]	RBC [10^6/mm^3]	MCV [fl]	MCH [pg]	MCHC [g/dm^3]	Erythro-blasts [%]
LC (12)	51.4±8.5 ^{a}	26.3±1.4 ^{ab}	85.6±10.0 ^{a}	1.25±0.33 ^{a}	227±70 ^{a}	72.4±18.1 ^{a}	330±36 ^{a}	2.9±1.0 ^{a}
LS0 (12)	76.3±24.3 ^{b}	27.9±3.3 ^{a}	74.9±13.7 ^{a}	1.16±0.39 ^{a}	273±82 ^{a}	70.7±22.7 ^{a}	267±46 ^{ab}	3.3±1.3 ^{a}
LS1 (12)	63.6±15.7 ^{a}	22.3±4.7 ^{b}	41.5±20.8 ^{b}	1.02±0.29 ^{a}	230±34 ^{a}	43.1±21.3 ^{b}	167±61 ^{b}	8.1±3.1 ^{b}
CC (12)	48.8±9.2 ^{a}	26.1±2.2 ^{a}	52.0±11.6 ^{a*}	1.56±0.39 ^{a*}	174±35 ^{a*}	35.0±11.3 ^{a*}	200±46 ^{a*}	9.9±2.5 ^{a*}
CS0 (12)	92.0±21.0 ^{b}	26.0±3.1 ^{a}	58.9±17.7 ^{a}	1.30±0.15 ^{a}	201±24 ^{a}	45.1±12.6 ^{a}	228±63 ^{a}	0.7±1.2 ^{b}
CS1 (12)	38.8±7.6 ^{a}	25.7±3.7 ^{a}	62.0±14.3 ^{a}	1.31±0.17 ^{a}	197±26 ^{a}	47.8±11.8 ^{a}	240±37 ^{a}	0.3±0.8 ^{b}
LC, CC – control, LS0, CS0 – immediately after confinement, LS1, CS1 – 1 week post confinement, different letter superscripts indicate statistically significant differences among groups for each species, * - differences between LC and CC, Kruskal-Wallis test, $p \leq 0.05$)								

Table 3. **The effects of crowding stress on white blood cell parameters and thrombocyte count of *Leuciscus cephalus*** (L) **and *Cyprinus carpio*** (C)

Groups (n)	WBC [10^3/mm^3]	NBT [g/dm^3]	Lymphocytes [%]	Neutrophils [%]	Monocytes [%]	PLT [10^3/mm^3]
LC (12)	38.6±12.7 ^{a}	0.927±0.254 ^{a}	95.3±3.2 ^{a}	1.5±1.0 ^{a}	3.3±3.4 ^{a}	16.9±5.7 ^{a}
LS0 (12)	33.3±14.6 ^{a}	0.314±0.133 ^{b}	93.5±3.5 ^{a}	3.0±2.4 ^{a}	3.5±3.8 ^{a}	4.7±3.1 ^{b}
LS1 (12)	34.3±8.3 ^{a}	0.499±0.299 ^{b}	92.6±4.3 ^{a}	4.3±4.1 ^{a}	3.1±2.9 ^{a}	11.1±5.8 ^{ab}
CC (12)	107.5±40.8 ^{a*}	1.684±0.419 ^{a*}	96.6±2.5 ^{a}	1.8±2.3 ^{a}	1.6±0.8 ^{a}	14.0±7.3 ^{ab}
CS0 (12)	54.6±11.9 ^{ab}	1.353±0.355 ^{a}	93.1±6.6 ^{a}	4.7±4.3 ^{a}	2.2±2.6 ^{a}	15.2±8.0 ^{a}
CS1 (12)	36.5±12.0 ^{b}	1.156±0.395 ^{a}	95.3±4.1 ^{a}	1.8±2.3 ^{a}	2.4±2.1 ^{a}	6.4±4.9 ^{b}
LC, CC – control, LS0, CS0 – immediately after confinement, LS1, CS1 – 1 week post confinement, different letter superscripts indicate statistically significant differences among groups for each species, * - differences between LC and CC, Kruskal-Wallis test, $p \leq 0.05$)						

Discussion

Hematological values of chub from natural waters show high individual and seasonal variability (Hlavova, 1993; Lamkova et al., 2007 and Aras et al. 2008). Generally, the values observed in the present study in the control group fit within the range reported by these authors, except for hematocrit, which was lower compared to the values obtained by Hlavova (1993) and Lamkova et al. (2007).

Comparison of the control hematological values of chub and carp revealed many significant differences. Chub showed significantly higher values of most red blood parameters (except for RBC and erythroblast level), lower leukocyte count and oxidative metabolic activity of phagocytes compared to carp.

The increase in glucose levels in chub and carp immediately following confinement show that stress reaction developed in fish. Hyperglycemia in chub was accompanied by a drop in oxidative activity of phagocytes and thrombocytopenia.

In a week, post confinement glucose levels differed no more from the basal levels in both species but the fish developed other hematological changes: in chub anemia and erythroblastosis was observed and oxidative activity of phagocytes remained significantly reduced, while carp showed leukopenia and thrombocytopenia.

Increase in blood glucose concentration following acute and chronic confinement stress in chub was reported by Pottinger et al. (2000) but in both cases the difference between glucose level in stressed and non-stressed fish was much higher than in the present study. Pottinger (2010) observed the difference between basal and confinement-induced glucose levels of chub and carp, the latter showing higher initial and lower stress-induced values. In the present study no significant difference occurred between the control glucose levels of both

species, while the increase caused by confinement was higher in carp (88.5 %) compared to chub (48.4 %). Increase in glucose level because of crowding was also reported by Rotllant et al. (1997) in *Pagrus pagrus*, by Ortuno et al. (2001) in *Sparus aurata* and by Basrur et al. (2010) in *Salmo salar*.

Significant anemia was observed by Pankhurst et al. (1992) in *Scorpis violaceus* because of confinement and by Pages et al. (1995) in *Sparus aurata* subjected to daily pursuing. In the present study anemia in chub was partially compensated by increased release of erythroblasts and only slight decrease in RBC occurred but Hb level was reduced approximately twice compared to the control. A decrease in Ht without significant changes in RBC was also reported by Sampaio de Abreu et al. (2008) in *Brycon amazonicus* after experimental transport and crowding. Marcalo et al. (2006) observed gradual reduction in Ht and Hb values in *Sardina pilchardus* stressed by seine fishing. On the other hand, Montero et al. (1999) observed a significant increase in Ht, Hb and RBC of crowded *Sparus aurata*, while Trenzado et al. (2009) reported an increase in Ht, Hb, RBC and MCHC in *Oncorhynchus mykiss*.

The most pronounced immune alterations induced by crowding included reduction of phagocyte oxidative activity in chub and leukopenia in carp. However, carp did not develop any disease and all fish survived, while most chub died within 4 weeks post experiment. It is noteworthy that initial WBC and NBT in carp were significantly higher than in chub.

Immunosuppression is an important secondary effect of stress in fish (Ortuno et al., 2001; Svoboda, 2001; Portz et al. 2006). Leukopenia is a commonly observed result of stress in fish (Wendelaar Bonga, 1997; Svoboda 2001). Benfey and Biron (2000) observed that acute stress was followed by marked leukopenia in *Oncorhynchus mykiss* and *Salvelinus fontinalis*.

Fish rely mainly on the nonspecific immune response and phagocytosis is their most important immune mechanism. Reduction of phagocyte respiratory burst in fish subjected to confinement was also observed by other authors: in *Dicentrarchus labrax*, *Sparus aurata* and *Oreochromis mossambicus* (Barton and Iwama, 1991; Montero et al., 1999; Vazzana et al., 2002; Binuramesh et al., 2005). Suppression of phagocytosis in crowded *Sparus aurata* was reported by Ortuno et al. (2001). A decrease in phagocytic activity and other non-specific immune parameters (lysozyme and complement activities) were also observed in *Cyprinus carpio* subjected to chronic (30 days) crowding (Yin et al., 1995). It is noteworthy that the decrease in enzyme activities developed already in one day of confinement, while the response of phagocytes took place after 7 days.

In the present study thrombocytopenia was observed in both species of fish following confinement (in chub immediately after application of stressor, in carp a week later). The effects of stress on thrombocyte count in fish are poorly known, and little data are available in the literature. According to Casillas and Smith (1977), and Frisch and Anderson (2000), the count of thrombocytes may increase, and blood clotting is usually accelerated proportionally to the degree of stress (Ruis and Bayne, 1997).

The obtained results show that brief confinement resulted in a stress reaction in both species of fish but the strength of the response was different. Anemia, thrombocytopenia and persistent reduction of oxidative metabolic activity of phagocytes in chub followed by death of most fish post experiment indicate that this species is highly sensitive to confinement and handling compared to common carp that experienced only leukopenia and thrombocytopenia. High sensitivity of chub to stress and resulting immunosuppression make the fish susceptible to diseases.

References

1. Aras M., Bayir A., Sirkecioglu A. N., Polat H. and Bayir M. Seasonal variations in serum lipids, lipoproteins and some haematological parameters of chub (*Leuciscus cephalus*). Italian Journal of Animal Sciences. 2008. 7. P. 439–448.

2. Barton B. A. and Iwama G. K. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases. 1991. 1. P. 3–26.

3. Barton B. A. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology. 2002. 42. P. 517–525.

4. Basrur T. V., Longland R. and Wilkinson R. J. Effects of repeated crowding on the stress response and growth performance in Atlantic salmon (*Salmo salar*). Fish Physiology and Biochemistry. 2010. 36. P. 445–450.

5. Bau F., Ferroni-Claverie N. and Parent J. P. Reponses physiologiques de sept especes de poissons lacustres a un stress de capture (filet maillant et epuisette). Bulletin Francais de Peche et Pisciculture. 2000. 357/358. P. 157–168.

6. Benfey T. J. and Biron M. Acute stress response in triploid rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Aquaculture. 2000. 184. P. 167–176.

7. Binuramesh C., Prabakaran M., Steinhagen D. and Dinakaran Michael R. Effects of chronic confinement stress on the immune responses in different sex ratio groups of *Oreochromis mossambicus* (Peters). Aquaculture. 2005. 250. P. 47–59.

8. Bracewell P., Cowx I.G. and Uglow R.F. Effects of handling and electrofishing on plasma glucose and whole blood lactate of *Leuciscus cephalus*. Journal of Fish Biology. 2004. 64. P. 65–71.

9. Casillas E. and Smith L. S. Effects of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). Journal of Fish Biology. 1977. 10. P. 481–491.

10. Frisch A. J. and Anderson T. A. The response of coral trout (*Plectropomus leopardus*) to capture, handling and transport and shallow water stress. Fish Physiology and Biochemistry. 2000. 23. P. 23–24.

11. Hliwa P., Żyła A. and Król J. Gonadogenesis in chub *Squalius* (*Leuciscus*) *cephalus* (L. 1758) Folia biologica. (Kraków). 2009. 57. P. 115–120.

12. Krejszeff S., Kucharczyk D., Kupren K., Targońska K., Mamcarz A., Kujawa R., Kaczkowski Z. and Ratajski S. Reproduction of chub, *Leuciscus cephalus* L., under controlled conditions. Aquaculture Research. 2008. 39. P. 907–912.

13. Lamkova K., Simkova A., Palíkova M., Jurajda P. and Lojek, A. Seasonal changes of immunocompetence and parasitism in chub (*Leuciscus cephalus*), a freshwater cyprinid fish

14. Parasitology Research. 2007. 101. P. 775–789.

15. Marcalo A., Mateus L., Duarte Correia J. H., Serra P., Fryer R. and Stratoudakis Y. Sardine (*Sardina pilchardus*) stress reactions to purse seine fishing. Marine Biology. 2006. 149. P. 1509–1518.

16. Montero, D., Izquierdo, M. S., Tort L., Robaina L. and Vergara J. M. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. Fish Physiology and Biochemistry. 1999. 20. P. 53–60.

17. Ortuno J., Esteban M. A. and Maseguer J. Effect of short-term crowding stress on the gilthead seabream (*Sparus aurata* L.) innate immune response. Fish & Shellfish Immunology. 2001 11. P. 187–197.

18. Pages T., Gomez E., Suner O., Viscor G. and Tort L. Effects of daily management stress on haematology and blood rheology of the gilthead seabream. Journal of Fish Biology. 1995. 46. P. 775–786.

19. Penczak T. and Kruk A. Threatened obligatory riverine fishes in human-modified Polish rivers. Ecology of Freshwater Fish. 2000. 9. P. 109–117.

20. Portz D. E., Woodley C.M., Cech J. J. Stress-associated impacts of short-term holding on fishes. Reviews in Fish Biology and Fisheries. 2006. 16. P. 125–170.

21. Pottinger T. G., Carrick T. R., Appleby A. and Yeomans W. E. High blood cortisol levels and low cortisol receptor affinity: is the chub, *Leuciscus cephalus*, a cortisol-resistant teleost? General and Comparative Endocrinology. 2000. 120. P. 108–117.

22. Pottinger T. G. 2010. A multivariate comparison of the stress response in three salmonid and three cyprinid species: evidence for inter-family differences. Journal of Fish Biology 76: 601-621. doi: 10.1111/j.1095-8649.02516.x

23. Rotllant J., Pavlidis M., Kentouri M., Bad M. E. and Tort L. Non-specific immune responses in the red porgy *Pagrus pagrus* after crowding stress. Aquaculture. 1997. 156. P. 279–290.

24. Ramsay J. M., Watral V., Schreck C. B. and Kent M. L. Husbandry stress exacerbates mycobacterial infections in adult zebrafish, *Danio rerio* (Hamilton). Journal of Fish Diseases. 2009. 32. P. 931–941.

25. Ruis M. A. W. and Bayne C. J. Effects of acute stress on blood clotting and yeast killing by phagocytes of rainbow trout. Journal of Aquatic Animal Health. 1997. 9. P. 190–195.

26. Sampaio de Abreu J., Sanabria-Ochoa A. I., Goncalves F. D. and Criscuolo Urbinati E. Stress response of juvenile matrinxa (*Brycon amazonicus*) after transport in a closed system under different loading densities. Ciencia Rural Santa Maria. 2008. 38. P. 1413–1417.

27. Studnicka M., Siwicki A. K. and Ryka B. Phagocytic ability of neutrophils in carp (*Cyprinus carpio* L.), The Israeli Journal of Aquaculture – Bamidgeh. 1985. 37. P. 123–128.

28. Svoboda M. Stress in fishes (a review). Bulletin VURH Vodnany. 2001. 37 P. 169–191.

29. Svobodova Z., Pravda D. and Palackova J. Unified methods of haematological examination of fish. Research Institute of Fish Culture and Hydrobiology, Vodnany. 1991. Methods No 20, 31 p.

30. Trenzado C. E., Morales A. E., Palma J. M. and de la Higuera M. Blood antioxidant defences and hematological adjustments in crowded/uncrowded rainbow trout (*Oncorhynchus mykiss*) fed diets with different levels of antioxidant vitamins and HUFA. Comparative Biochemistry and Physiology. Part C. 2009. 149. P. 440–447.

31. Vazzana M., Cammarata M., Cooper E. L. and Parrinello, N. Confinement stress in sea bass (*Dicentrarchus labrax*) depresses peritoneal leukocyte cytotoxicity. Aquaculture. 2002. 210. P. 231–243.

32. Wendelaar Bonga S. E. The stress response in fish. Physiological Reviews. 1997. 77. P. 591–625.

33. Yin Z., Lam, T. J. and Sin Y. M. The effects of crowding stress on the non-specific immune response in fancy carp (*Cyprinus carpio* L.). Fish & Shellfish Immunology. 1995. 5. P. 519–529.

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