HEMATOLOGICAL PARAMETERS OF ONE YEAR OLD SEA TROUT (*SALMO TRUTTA*) IN SPRING

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ABSTRACT
Sea trout are fish which are most similar to salmon and that's why it is little studied. Despite included in one genus, salmon and sea trout are not as alike as it seems. Many points including physiological parameters might be and are different. Nowadays sea trout are reared in Latvian hatcheries. Ninety four sea trout blood samples were taken and examined and following parameters were identified – red blood cell count (RBC), hemoglobin, hematocrit and leukocyte formula. For sea trout smolts from natural watercourses RBC count was $1,22*10^{12}$/L, hemoglobin 7,77 g/L, hematocrit 29,40 %; for one year old sea trout from ponds RBC count was $1,01*10^{12}$/L, hemoglobin 10,13 g/L, hematocrit 39,75 %; for one year old sea trout reared in flow through system RBC count was $1.10 – 1.17 *10^{12}$/L, hemoglobin 8.97 – 10.32 g/L, hematocrit 29.21 – 36.94 %; for sea trout reared in recirculation system RBC count was $1.25 – 1.47*10^{12}$/L, hemoglobin 8,13 – 8,41 g/L and hematocrit 33.00 – 37.58 %. In sea trout leukocyte formula neutrophils, eosinophils, lymphocytes and monocytes were differentiated.

KEY WORDS: sea trout, hemoglobin, RBC, leukocyte formula.

INTRODUCTION
Knowledge about hematology of salmonids can be used as biomarker for establishing a state of the environment (Khan, 2010), for determination of physiological state of fish (Ruane et al., 2000; Stoskopf, 1993; Глаголева, 1989) and for diagnosis of a disease (Rehulka, 2003).

Fish blood consists of plasma and blood cells: erythrocytes, leukocytes and platelets. Count of fish erythrocytes usually is lower than in mammals and depends on the need for oxygen. The concentration of hemoglobin is lower than in mammals. More active fish which require more oxygen usually have higher hemoglobin level, smaller erythrocytes and low mean corpuscular volume (MCV). Mean corpuscular hemoglobin (MCH) differs between fish species because of different size of circulating erythrocytes. Mean corpuscular hemoglobin concentration (MCHC) in fish is lower than in mammals on account of nucleus which takes place in a cell. Young erythrocytes are bigger and have higher hemoglobin concentration resulting in higher MCV, MCH and MCHC than in older cells (Hrubec, Smith, 2010).

Hematocrit fluctuates from 21 – 28 % in flounder (Mahoney, McNulty, 1992), to 47,7% in herring (Medne, Balode, 2011). For rainbow trout it is 21 – 44 % (Miller et al., 1983) but there is no information about sea trout.

A count of leukocytes is variable, it increases just after feeding, whereas a significant decrease can be seen during a poisoning or using anesthetics. The most of leukocytes are lymphocytes. An amount of monocytes increases in winter and decreases in summer. Similar situation can be seen with neutrophils: an increase in winter and decrease in spring (Noga, 1996; Глаголева, 1989). Basophils are very rare (Hrubec, Smith, 2010).

The aim of the research was an analysis of one year old sea trout hematological parameters in spring.

MATERIAL AND METHODS

The study was carried out in the LUA Faculty of Veterinary Medicine, Institute of Food and Environmental Hygiene and in the Institute of Food Safety, Animal Health and Environment BIOR, laboratory of aquaculture and fish pathology.

There were 94 one year old sea trout used in the study. Fish were from different hatcheries and caught in river Salaca by using a research pot of fish migration.

A blood sample was taken from caudal vein immediately after catching fish. Blood was collected in tubes with anticoagulant (EDTA). Samples were transported to laboratory in ice-box within 1 - 2 hours.

A count of erythrocytes was determined by using a photoelectric colorimeter (FEC) MP-plus. Refractive index was determined with the wave length of 546 nm. To establish RBC count, some samples were examinated both with FEC and with Gorjajev chamber. Relevance between both parameters was calculated and expressed as a formula which was used for calculation of RBC count:

$$RBC(milj./mm^3) = 2{,}3409 \times RI - 0{,}1519, \quad (1)$$

where
RBC – RBC count;
RI – refractive index by FEC.

For clarifying a blood hemoglobin concentration, cyanmethemogtlobin method was used. An optical density was determined by using FEC with wave length of 546 nm, transformation solution was used as a benchmark. For comparision examination with Sahli's hemometer was carried out. Then a relevance between both parameters was calculated and expressed as a formula used for calculation of hemoglobin level:

$$Hgb(g/L) = 1{,}058 \cdot RI + 3{,}391, \quad (2)$$

where
Hgb – hemoglobin level;
RI – refractive index by FEC.

Hematocrit was determined by the microhematocrit method. Capillary tubes were centrifuged for 5 minutes at 6000 G, using centrifuge NF 048. Erythrocyte indices were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (Jemeļjanovs et al., 2007).

Blood smears were made to determine a leukocyte formula. Blood smears were dried and stained with JorVet stains DipQuick Stain Kit. Microscopic examination of blood smears was carried out using light microscope Leica DME at magnification 400x and counting 100 leukocytes (Stoskopf, 1993).

Average and standard deviation (SD) was calculated for the data obtained. To compare parameters of different fish rearing systems and in different living conditions T-test for comparision of two seperate samples was used (Sokal, Rohlf, 2000).

RESULTS AND DISCUSSION

When analysing results obtained in the spring of 2012 it can be seen that RBC count fluctuates from 1.10 to 1.47 x 10^{12}/L (table 1). In March and April RBC count in flow-through system is lower than ir recirculation system but there is no significant difference between months in none of the systems. Hemoglobin fluctuates from 8.13 to 10.32 g/dL, in flow-through system it decreases in April but in recirculation system it increases but there is no

significant difference between months. In April hematocrit significantly ($p < 0.05$) decreases in flow-through system and reaches 29.21 %. Hematocrit decreases in recirculation system too – from 37.58 % to 33.00 %. In March MCH is 61.77 pg in flow-through system and 36.10 pg in recirculation system but in April respectively 52.47 pg un 35.61 pg but the decrease is not significant ($p > 0.05$). MCHC significantly ($p < 0.05$) increases from March to April in flow-through system (from 12.99 to 18.64 g/L), in the recirculation system relevant changes are not seen. MCV in both rearing systems does not significantly change in March and April, in flow through system it decreases from 280.01 to 262.56 fL and in recirculation system from 303.46 to 224.63 fL.

Table 1

Hematological parameters of one year old sea trout reared in tanks

Parameters	Flow-through system		Recirculation system	
	March	April	March	April
RBC ± SD (10^{12}/L)	1.10 ± 0.19	1.17 ± 0.35	1.25 ± 0.15	1.47 ± 0.40
Hgb ± SD (g/dL)	10.32 ± 2,41	8.97 ± 1,95	8.13 ± 0,61	8.41 ± 1,39
PCV ± SD (%)	36.94 ± 4,71	29.21 ± 6,37*	37.58 ± 3,18	33.00 ± 13,75
MCH ± SD (pg)	61.77 ± 27.84	52.47 ± 35.02	36.10 ± 4.64	35.61 ± 5.60
MCHC ± SD (g/L)	12.99 ± 3.01	18.64 ± 7.75*	11.93 ± 1.37	10.15 ± 3.24
MCV ± SD (fL)	280.01 ± 28.22	262.56 ± 84.59	303.46 ± 30.07	224.63 ± 32.14
Lymphocytes ± SD (%)	67.77 ± 13.76	82.00 ± 9.37**	88.80 ± 2.77	92.32 ± 4.69
Neutrophils ± SD (%)	31.55 ± 13.45	17.89 ± 9.25**	10.60 ± 3.65	7.26 ± 4.20
Monocytes ± SD (%)	0.64 ± 0.79	0.11 ± 0.32**	0.60 ± 0.89	0.37 ± 0.68
Eosinophils ± SD (%)	0.05 ± 0.21	0.0 ± 0.0	0.00 ± 0.0	0.05 ± 0.23

* significant difference $p < 0.05$

** significant difference $p < 0.01$

In general it can be seen that all hematological parameters in fish from recirulation system are more settled and does not change significantly because rearing circumstances are more even than in flow- through system.

Table 2

Hematological parameters of one year old sea trout from ponds and natural watercourses

Parameters	Ponds	Natural watercourses
RBC ± SD (10^{12}/L)	1.01 ± 0.32	1.22 ± 0.25
Hgb ± SD (g/dL)	10.13 ± 0.72	7.77 ± 1.21**
PCV ± SD (%)	39.75 ± 5.73	29.40 ± 10.33
MCH ± SD (pg)	69.52 ± 26.40	33.81 ± 4.66
MCHC ± SD (g/L)	16.29 ± 2.08	15.35 ± 6.35
MCV ± SD (fL)	474.00 ± 159.01**	242.33 ± 72.20
Lymphocytes ± SD (%)	66.00 ± 9.35	78.40 ± 15.70
Neutrophils ± SD (%)	33.00 ± 9.10	21.00 ± 14.95
Monocytes ± SD (%)	1.00 ± 1.25	0.40 ± 0.89
Eosinophils ± SD (%)	0.0 ± 0.0	0.20 ± 0.45

* significant difference $p < 0.05$

** significant difference $p < 0.01$

Not only in tanks but also in ponds fish are reared in Latvia. When rearing fish in different systems, similar circumstances for fish are not provided. There can be differences in water quality, water pH, temperature, concentration of oxygen, feed base and other factors which can affect fish hematological parameters.

When comparising hematological parameters of fish from ponds and from natural watercourses (table 2), it can be seen that RBC count ranges from 1.01 to 1.22 x 10^{12}/L but there is no significant difference. Hemoglobin concentration is from 7.77 to 10.13 g/dL. It is significantly ($p<0.01$) lower for sea trout from natural watercourses. The low hemoglobin level might be as a result of poor feed base (Jemeļjanovs et al., 2007). In comparison with one year old sea trout from hatcheries, fish from natrual watercourses are not fed with commercially produced and balanced feed. They have to get along with feed resources found in nature.

Hematocrit ranges from 29.40 % to 39.75 % but there is no significant difference. MCH is 33.81 pg for sea trout from natural watercourses and 69.52 pg for sea trout from ponds, MCHC respectively 15.35 and 16.29 g/L but it doesn't differ significantly between groups. MCV is from 242.33 to 474.00 fL, significantly higher for sea trout from ponds. When looking at RBC count, hematocrit and MCV of sea trout reared in ponds, it can be concluded that the increase of hematocrit is connected with increase of a size of erythrocytes. Macrocytosis can be seen when the count of young, immattured erythrocytes rises in peripheral blood (Jemeļjanovs et al., 2007), it can be caused by stress during a lowering of the ponds and fish catches.

In the leukocyte formula lymphocytes, neutrophils, monocytes and eosinophils can be determined. Table 1 shows that in leukocyte formula in March and April the amount of lymphocytes rises from 67.8 % in March to 82.0 % in April but the amount of neutrophils decreases from 31.5 % to17.9 %. Monocytes compose 0.6 % in March and 0.1 % in April. The decrease of the amount of neutrophils and monocytes and the increase of the amount of lymphocytes is characteristic for salmonids when water temperature rises (Глаголева, 1989). In recirculation system leukocyte formula as other hematological parameters doesn't change sharp. In the flow-through system the count of eosinophils increases, it is connected with rising of water temperature. A low amount of monocytes in fish blood evidence of fish welfare – good living circumstances.

When looking at leukocyte formula of sea trout from ponds and natural watercourses, it can be seen that in both cases the most part of lymphocytes is formed by lymphocytes 66.0 – 78.4 %, which is followed by neutrophils 21.0 – 33.0 %. The amount of monocytes fluctuates from 0.4 to1.0 %, but eosinophils can be seen only for sea trout from natural watercourses and compose only 0.2 %. The high amount of neutrophils and the low amount of lymphocytes characterise a stress which affects fish during catches (Ruane et al., 2000). It could be that an amount of basophils in fish blood is too low or when using the regular method of fixation and staining with standard stains basophils are not fixed properly or can't be identified. Tavares - Dias (2006) have stated that the best fixation of basophils is by using copper subacetate and staining with acid toluidine blue stain.

CONCLUSIONS

1. There are 1.01 – 1.47 x 10^{12}/L erythrocytes, 7.77 – 10.32 g/dL hemoglobin level, hematocrit 29.21 – 39.5 % in one year old sea trout in spring.
2. Lymphocytes, neutrophils, eosinophils and monocytes can be differentiated in the leukocyte formula of sea trout.
3. When comparising the hematological parameters of one year old sea trout (March) and smolts (April) it can be seen that the RBC count and hemoglobin level doesn't differ

significantly but smolts have lower hematocrit. In leukocyte formula of smolts, the proportion of lymphocytes rises but the amount of neutrophils and monocytes decreases.

4. Hematological parameters of sea trout reared in recirculation system are more stable and don't change significantly because rearing conditions in this system is more stable than in flow-through system.
5. Sea trout from natural watercourses have significantly lower hemoglobin level than sea trout reared in ponds.

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