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DYNAMICS OF SOME CELLULAR AND HUMORAL NON-SPECIFIC IMMUNE PARAMETERS IN SIBERIAN STURGEON (*Acipenser baeri* Brandt) REARED IN A WATER RECIRCULATION SYSTEM

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ABSTRACT. The present study deals with the dynamics of selected cellular and humoral non-specific immune parameters in Siberian sturgeon (*Acipenser baeri* Brandt) fry during three years of intensive rearing under controlled conditions. It was observed that NBT reduction ability, lysozyme index, γ -globulin level, and total plasma protein content were closely related to fish body mass. Lysozyme activity and γ -globulin level were temperature-dependent. The observed relationships were described with the regression equations. Ceruloplasmin activity in older fry was significantly higher than in younger fish. Changes in phagocytic ability of leucocytes were also noted, accompanied by high variation of lymphocyte counts and lysozyme activity.

Key words: SIBERIAN STURGEON, INTENSIVE REARING, IMMUNOLOGY, CELLULAR AND HUMORAL NON-SPECIFIC IMMUNE RESPONSES

INTRODUCTION

Adverse anthropogenic influences resulted in considerable reduction of the environments appropriate for sturgeons, so many species of these fish became endangered (Pavlov et al. 1994, Kolman R. 1996a). Their extinction could be stopped closing the entire rearing cycle in a controlled environment (Gershanovich, Burtsev 1993) using the already available and efficient biotechnologies of sturgeon pre-rearing and rearing (Kolman R. 1993 a, b, 1996 b, Kolman R. et al. 1994, 1995, 1996 a, b). It is, however, necessary to develop effective immunoprophylactic methods, as has been done in the case of teleost fish (Siwicki, Studnicka 1986, Siwicki 1987, 1990, Anderson, Siwicki 1989, Siwicki et al. 1989, Siwicki, Cossarini-Dunier 1990, Siwicki, Anderson 1993 a, b, c, Vikhman 1996).

Data on cellular immune mechanisms in sturgeons are lacking and on humoral mechanisms – incomplete. The aim of the present study was an identification of these mechanisms, this being necessary to undertake further studies on the development of immunoprophylactic methods for sturgeons reared under intensive conditions.

MATERIAL AND METHODS

The study was carried out on Siberian sturgeon (*Acipenser baeri* Brandt) fry. The fish were reared in tanks in a water recirculation system, under optimum conditions for their development and growth (Klashtorin 1976, 1981, Kolman R. 1992): ammonia level was maintained below 0.02 mg/l, nitrite – 0.15 mg/l, and dissolved oxygen concentration in the outflowing water did not drop below 60% of saturation (Kolman R. 1996 b, Kolman H. 1995, Szczepkowski 1995). The fish were in good condition and healthy.

The study on the effect of body mass upon selected immunological indices was performed on five groups of Siberian sturgeons of average body weight: 40, 108, 471, 1820, and 4060 g, in three age groups: 0⁺, 1⁺, and 2⁺. The fish were kept under uniform conditions in a closed water recirculation system, at the temperature 20±3°C, and fed with the same pelleted feeds in quantities appropriate for the body mass, according to the „feeding curves“ (Kolman R. 1996 b).

The study on the effect of temperature on lysozyme activity, and the level of γ -globulin was performed on the groups of pre-reared Siberian sturgeon fry of initial body mass 150 g. Water temperature changed according to the following pattern: first week - 18 °C, second week - 20°C, third week 24°C, fourth week - 22°C, fifth week - 20°C.

Blood was sampled from 10 fish in each group. To eliminate manipulation stress, the fish were anesthetized with Propiscin.

The following parameters were evaluated: total leucocyte count, leucogram, metabolic and phagocytic activity of leucocytes, lysozyme activity (LZM), lysozyme index, ceruloplasmin activity Cp, level of total plasma protein, and γ -globulin fraction. Blood plasma was isolated by centrifugation of the blood for 10 min., at 5000 rpm, and stored at -20°C until analysis. Leucograms were evaluated in the blood smears stained using May-Grunwald and Giemsa method (Kalashnikova 1976, Ivanova 1983, Krzemińska-Ławkowicz, Maj 1993).

Metabolic activity of PMN cells was evaluated using NBT reduction test, and spectrophotometry (Studnicka et al. 1985, Siwicki, Anderson 1993 b). 0.2% NBT solution (Sigma) was used. Percentage of polymorphonuclear cells (PMN) was estimated using cytochemical method described by Szczylik et al. (1979). Samples were fixed with alcohol and stained with safranin, according to Van Oss et al. (1973). NBT

index was calculated using the method by Siwicki et al. (1985). Phagocytic activity of leucocytes (IF) was determined with the method described by Avtalion, Shahrabani (1975), and O'Neill (1985), and expressed as phagocytic index (IF). *Staphylococcus aureus* 209 P suspension was used.

Plasma lysozyme activity (LZM) was evaluated using turbidimetric method (Studnicka et al. 1986). *Micrococcus lysodeikticus* (Sigma) suspension in phosphate buffer was used. Extinction was measured with the spectrophotometer Eskalal – Smith Kline Instruments, USA. Lysozyme of chicken egg (Sigma) was used as the standard. LZM index was calculated according to Siwicki, Studnicka (1987). Ceruloplasmin activity (Cp), level of total plasma protein and γ -globulin fraction were measured with the micromethods of Siwicki and Anderson (1993 a).

RESULTS

Average body mass of the fish increased over 100-fold during the experiment. Among leucocytes, lymphocytes predominated (Tab. I). Granulocytes considerably prevailed over monocytes, but this domination decreased from over 14-fold at the beginning of the experiment to 7-fold in 0+ age groups, 3.5-fold in 1+, and to 2.25 at the end of rearing (age 2+).

In the first year of the study, the fish mass increased almost 12 times – from 40 to 471 g (Tab. I). At the same time, initial increase followed by a decrease of leucocyte counts in the circulating blood was observed (Tab. I). Percentage of NBT-positive PMN cells increased with the increase of the average body mass of fry from 13.1 ± 5.2 to $42.3 \pm 11\%$, and NBT reduction ability dropped from 2.2 ± 0.3 to 0.9 ± 0.1 mg/ml (Tab. I).

During the next two years of rearing, average body mass of Siberian sturgeon increased from 471 to 4040 g. Age groups 0+ (471 g), 1+, and 2+ were sampled in November – every 12 months. Comparison of these groups revealed that the content of neutrophils in fish blood significantly decreased with age, from 7 to about 2 thousand/ mm^3 , and monocyte and eosinophil counts increased from 0.8 to 1.3 thousand/ mm^3 , and from 0.8 to 1.6 thousand/ mm^3 respectively (Tab. I). Fluctuations of lymphocyte counts in all age groups were accompanied by similar changes of IF, lysozyme activity, and γ -globulin fraction level (Tab. I). NBT reduction ability stabilized at 0.9 mg/ml with the fish age, but NBT and lysozyme index significantly

TABLE I

Values of immunophysiological indices in Siberian sturgeon (*Acipenser baeri* Brandt) blood during intensive rearing

Average body weight g	Age years	Total numbers of leucocytes th./mm ³	Numbers of neutrophils th./mm ³	Number of eosinophils th./mm ³	Number of monocytes th./mm ³	Number of lymphocytes th./mm ³	% MN NBT+	NBT reduction ability mg/ml	NBT index	IF	LZM activity in serum μg/ml	LZM index	Cp activity in serum mg%	γ-globulin in serum g/l	Total protein in serum g/l
40.0	0+	36.0±4	7.6±1.8	0.6±1.8	0.5±0.18	28.1±2.1	13.1±5.2	2.2±0.3	0.3±0.04	-	9.1±4.4	1.2±0.3	9.3±3.3	-	-
108.0	0+	55.0±5	10±2.7	5.5±2.2	1.1±0.5	37.9±3.3	26.1±5.0	1.0±0.1	0.17±0.1	-	5.4±1.4	0.7±0.16	-0	3.8±1.1	24±7.1
471.0	0+	41.0±5	7.0±2.2	0.8±0.4	0.8±0.4	29.2±4.1	42.4±11	0.9±0.02	0.09±0.02	7.0±2.2	54±5.1	5.2±0.45	25±0.2	8.5±3.0	49±17.4
1820	1+	50.0±1	2.5±0.5	1.0±0.5	1.0±0.5	43.5±4.0	17.4±6.5	0.9±0.07	0.11±0.02	10.0±3.0	71±13.7	8.8±1.6	21.0±5.0	15±3.1	44±9.1
4060	2+	33.0±4.7	2.0±0.4	1.6±0.9	1.6±0.9	25.7±2.0	47.8±10	0.84±0.04	0.18±0.04	5.0±1.7	46.5±9.0	10.3±1.6	21.0±4.5	14±2.5	43±7.9

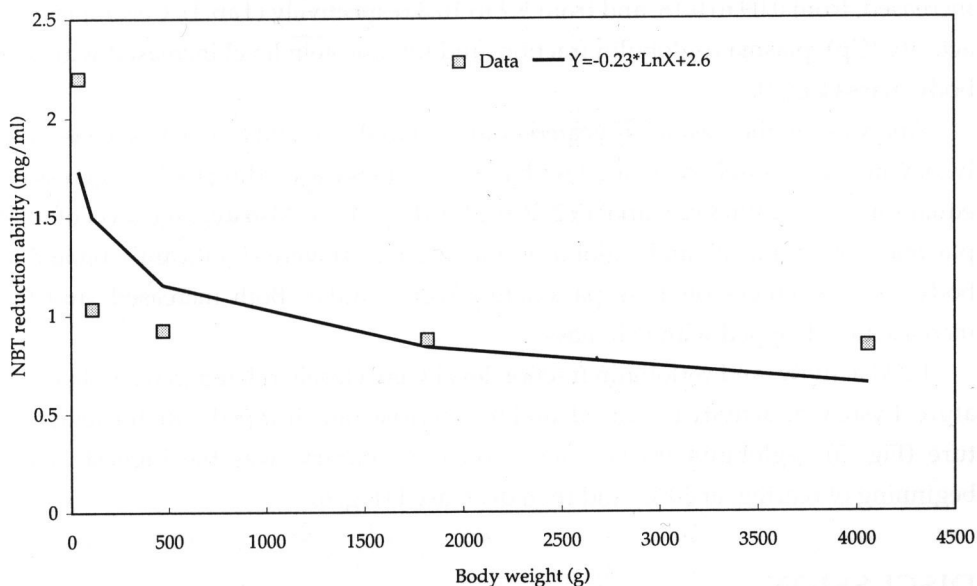


Fig. 1. Changes of NBT reduction ability of PMN cells in Siberian sturgeon (*Acipenser baeri* Brandt) plotted against body mass

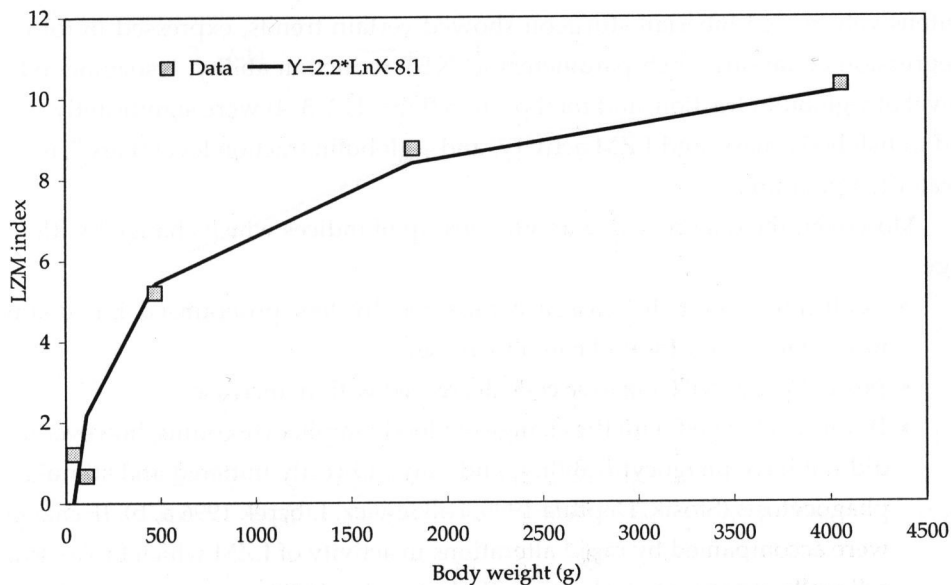


Fig. 2. Relation between lysozyme index and body mass in Siberian sturgeon (*Acipenser baeri* Brandt)

increased, from 0.09 to 0.18, and from 5.2 to 10.3 respectively (Tab. I). Ceruloplasmin activity (Cp), plasma α -globulin fraction, and total protein level increased with fish body mass (Tab. I).

Analysis of the results revealed that metabolic activity of PMN cells, and lysozyme index closely correlated with fish body mass, according to the regression equations: $Y = -0.23 \cdot \ln X + 2.6$ and $Y = 2.2 \cdot \ln X - 8.1$ (Figs. 1, 2). Also average level of total plasma protein (Fig. 3), and γ -globulin fraction (Fig. 4) were significantly related to body mass. Changes of these parameters were similar. Both increased, and the increase rate dropped with fish mass.

LZM activity, and γ -globulin fraction level were closely related to water temperature. Lysozyme activity increased, and the increase rate dropped with the temperature (Fig. 5). γ -globulin fraction level, on the contrary, was the highest at the beginning of rearing, at 20°C, and then decreased (Fig. 6).

DISCUSSION

The study revealed that changes of the majority of parameters under study in intensively reared Siberian sturgeon showed certain trends, expressed in form of regression equations. Such parameters as NBT reduction ability, lysozyme index, level of γ -globulin fraction, and total protein (Figs. 1, 2, 3, 4) were significantly related to fish body mass, and LZM activity, and γ -globulin fraction level (Figs 5, 6) – to water temperature.

Moreover, the data revealed another group of indices which changed with fish age:

- neutrophil count decrease accompanied by less pronounced but distinct monocyte and eosinophil count increase,
- percentage of NBT-positive cells decreased with IF increase,
- IF values changed with the change of blood lymphocyte counts, however they did not have phagocytic ability, and only indirectly initiated and stimulated phagocytosis (Stosik, Deptuła 1995, Falkiewicz, Liberek 1996 a, b). IF changes were accompanied by rapid alterations in activity of LZM which broke down cell walls of saprophytic bacteria (Lukyanenko, 1989).

The youngest Siberian sturgeon fry had the highest NBT values compared to other sturgeon species (Kolman H. 1996). Neutrophils predominated over

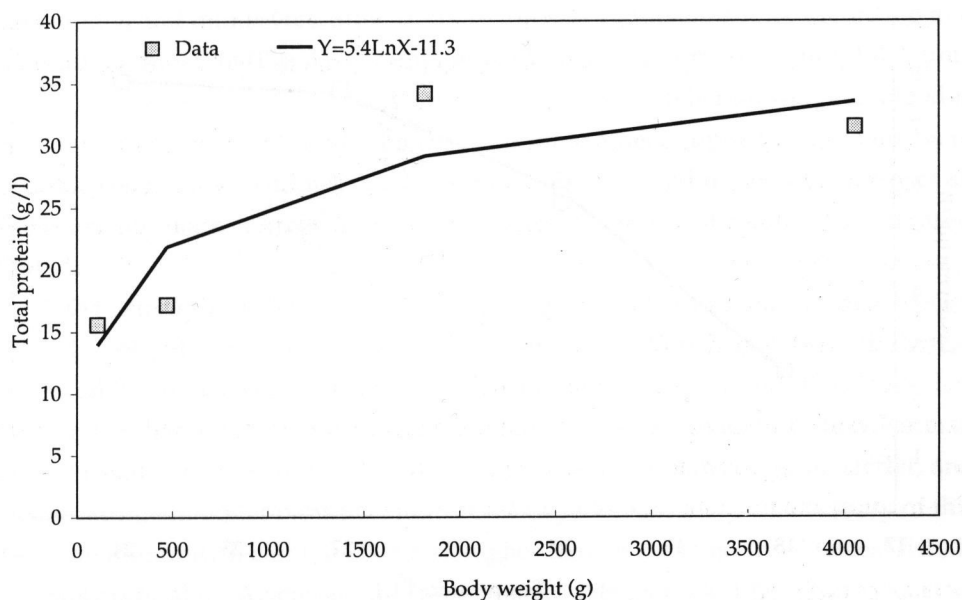


Fig. 3. Relation between total plasma protein content and body mass in Siberian sturgeon (*Acipenser baeri* Brandt)

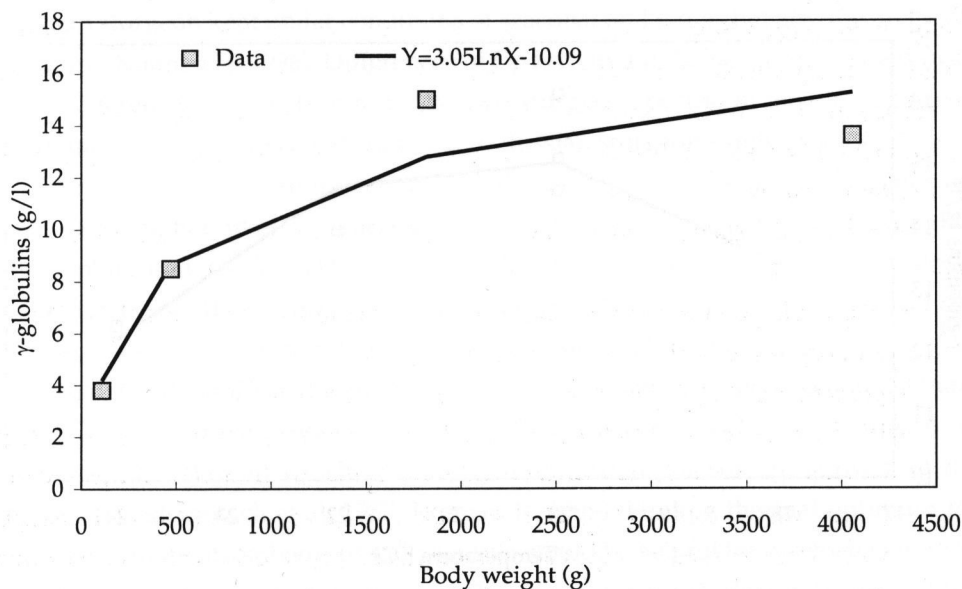


Fig. 4. The increase of plasma γ -globulin level in Siberian sturgeon (*Acipenser baeri* Brandt)

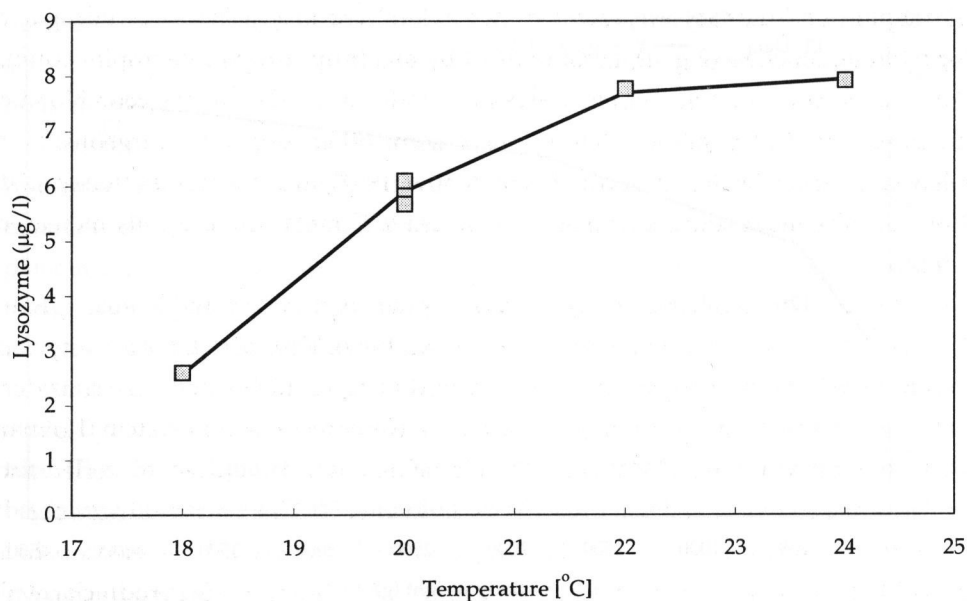


Fig. 5. Relation between plasma lysozyme activity in Siberian sturgeon (*Acipenser baeri* Brandt) and water temperature

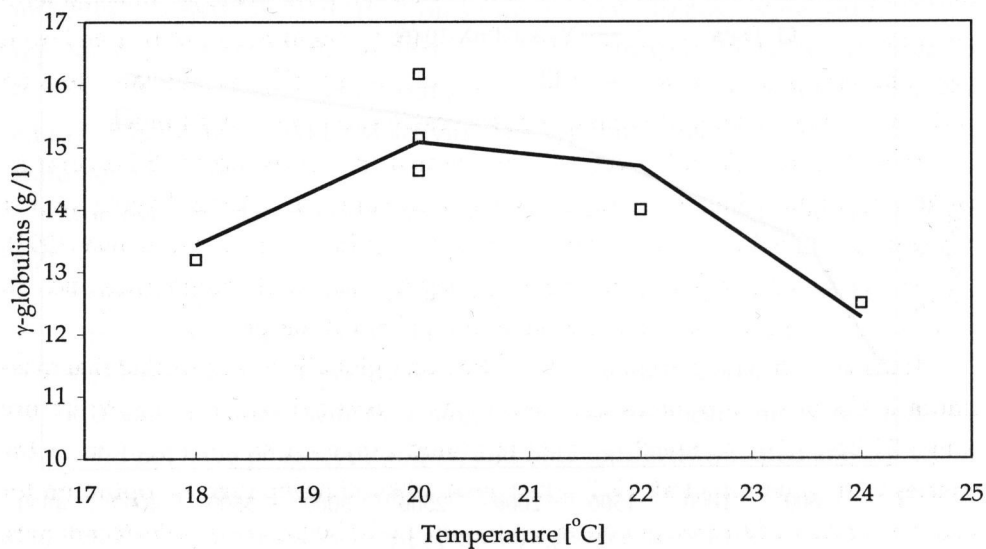


Fig. 6. Relation between plasma γ -globulin level in Siberian sturgeon (*Acipenser baeri* Brandt) and water temperature

eosinophils and monocytes. Slow decrease of NBT reduction ability according to a logarithmic function (Fig. 1), accompanied by an abrupt drop of neutrophil counts (3.5 fold), suggest that the source of oxidative radicals in Siberian sturgeon blood is not limited to neutrophils and there must be some other supply. In sturgeons, as in teleosts, eosinophils (Rudnickaya, Zagretdenova 1997), and monocytes (Jeney et al. 1994) are the main sources of oxidative systems. Counts of these cells increased (Tab. I).

LZM activity in Siberian sturgeon was higher than in cultured teleost species (Siwicki 1990, Studnicka et al. 1986, Lukyanenko 1989). Very high LZM activity was observed in Russian sturgeon and bester reared in cages, the values were considerably higher than in Siberian sturgeon reared in a water recirculation system (Kolman H. 1996, Kolman H. et al. 1997), and than in beluga, Russian sturgeon, sterlet, and stellate sturgeon under natural conditions (Lukyanenko 1989). High activity of this humoral immunity factor (10-100 times higher than in the majority of teleost fish) is probably typical for Acipenseridae (Lukyanenko 1989, Siwicki 1990, Studnicka et al. 1993).

The increase of Cp activity in the first year of rearing was much higher than in Russian sturgeon kept under conditions of seasonal and daily changes of water temperature (Kolman H. 1996). Differences in Cp activity might have also resulted from high differences of growth rate in the two sturgeon species: in Siberian sturgeon body mass increased over 10 times, and in Russian sturgeon – only 4 times.

Percentage of γ -globulin fraction in total plasma protein content in Siberian sturgeon was higher than in sturgeons reared in cages, the latter having higher eosinophil counts (Kolman H. 1996). According to Rudnickaya, Zagretdenova (1997), main function of these leucocytes, besides phagocytosis of microorganisms and cell debris, is also ingestion and digestion of immunological complexes.

Analysis of the effect of water temperature on γ -globulin level revealed that maximum levels of these proteins in *A. baeri* plasma occurred within the temperature range 20-22°C (Fig. 6) which was an optimum range for somatic growth of the species (Gershanovich et al. 1987, Kolman R. 1996). Finding thermal optimum for anabolic activity of antibody-producing cells would be helpful for *in vitro* techniques.

Studies performed on Siberian and Russian sturgeons (Kolman H. 1996) added data on immunological indices to the earlier conclusion about the relation between morphotic and dissolved blood constituents, and growth indices and environmental

factors (Korzhuev 1964, Kudryavcev et al. 1969, Alakrinskaya, Dolgova 1984, Gershanovich et al. 1987, Lukyanenko 1971, 1989, Gershanovich, Kiselev 1993). Siberian sturgeon showed very high growth rate, much higher than the data presented by other authors (Gershanovich et al. 1987, Androsov et al. 1990), and this affected dynamics of the analysed parameters. Blood is a physiological system participating in various metabolic processes, and its composition reflects all changes caused by internal and external factors. In the first year of Siberian sturgeon life, counts of white blood cells considerably fluctuated. This might have resulted from adaptative reactions to changing environmental conditions. Also the effect of various feeds applied in this period of rearing, containing various microorganisms of different immunogenic properties, should not be neglected. Decrease of neutrophil counts, and increase of γ -globulin level, accompanied by fluctuations of lymphocyte counts, IF, and lysozyme activity (Tab. I) indicate directions of adaptation to environmental conditions.

Lability of the analysed indices during fish ontogenesis and related to environmental factors show their diagnostic value in sturgeons, similar as in teleost fishes (Siwicki 1990, Siwicki et al. 1993, Studnicka et al. 1993, Prost et al. 1995). Moreover, monitoring of these indices may be helpful in optimizing the biotechnology of sturgeon rearing (Vikhman 1996, Kryuchkov 1997).

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STRESZCZENIE

KSZTAŁTOWANIE SIĘ WYBRANYCH NIESWOISTYCH KOMÓRKOWYCH I HUMORALNYCH WSKAŹNIKÓW ODPORNOŚCI U JESIOTRA SYBERYJSKIEGO (*Acipenser baeri* Brandt) PODCHOWYWANEGO W OBIEGU ZAMKNIĘTYM WODY

W pracy przedstawiono wyniki badań nad kształtowaniem się wybranych immunologicznych wskaźników nieswoistych, komórkowych i humoralnych, u narybku jesiotra syberyjskiego (*Acipenser baeri* Brandt) w trakcie trzech lat intensywnego chowu w warunkach kontrolowanych. W tym okresie u badanych ryb zdolność redukcji NBT przez krew, a także indeks lizozymu, poziom γ -globulin i zawartość białka całkowitego w osoczu krwi były ściśle zależne od masy ciała, natomiast aktywność lizozymu oraz poziom γ -globulin - od temperatury wody. Stwierdzone zależności opisano równaniami regresji. Aktywność ceruloplazminy u narybku starszego istotnie była wyższa niż u młodszego. Wykazano zmiany zdolności fagocytarnej leukocytów w czasie dramatycznych zmian liczebności limfocytów i aktywności lizozymu.

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