

Resistant Capabilities of the Sterlet (*Acipenser Ruthenus*) In Modeling the Impact of Stress Factors in the form of Increasing the Temperature of the Aquatic Environment, Decreasing Oxygen in the Aquatic Environment and Crowding

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Abstract. During the formation of resistance processes under the influence of such stress factors as a decrease in oxygen concentration and an increase in the temperature of the aquatic environment, as well as crowding in sterlet (*Acipenser ruthenus*), a significant change in humoral resistance and a slight change in cellular resistance (phagocytic activity of blood leukocytes) are observed.

A decrease in the amount of oxygen in the aquatic environment to 3.0 mg/l decreases the protein level in the blood serum of sterlet to 15.29 ± 0.4 g/l, which is 37.7% ($p < 0.001$) lower than in the control group, where the amount of oxygen in the aquatic environment was 8.3 mg/l. We noted an increase of lysozyme activity in the blood serum up to $28.87 \pm 0.58\%$ which is 1.6 times ($p < 0.001$) higher than in the control group, where the amount of oxygen in the aqueous was 8.3 mg/l. It was found that in the group where the amount of oxygen in the aquatic environment was 3.0 mg/l, the index of phagocytic activity of leukocytes was $21.24 \pm 0.45\%$ ($p > 0.05$). The cortisol level was 223.33 ± 2.18 nmol/l, which is 3.4 times ($p < 0.001$) higher than in the control group, in which the amount of oxygen in the aqueous was 8.3 mg/l.

An increase in the temperature of the aquatic environment by more than 15 °C decreases the level of total serum protein after 3 hours to 17.5 ± 0.3 g/l, which is 30.5% ($p < 0.001$) less than in the control group, in which this indicator is 25.31 ± 0.38 g/l. After 12 h, this indicator in the experimental group remains consistently lower and is 19.44 ± 0.29 g/l (in the control group 23.42 ± 0.36 g/l). An increase in the temperature of the aqueous medium leads after 3 hours to an increase in the activity level of serum lysozyme by 2.3 times ($p < 0.001$) compared with the control group, in which this indicator is $16.65 \pm 0.51\%$. After 12 hours, this indicator decreases, but remains 1.5 times higher than in the control group ($17.31 \pm 0.46\%$). An increase in the temperature of the aqueous medium does not have a significant effect on the level of phagocytic activity of blood leukocytes. An increase in the temperature of the aquatic environment by more than 15 °C increases the level of cortisol in the blood serum of sterlet. In the experimental group, this indicator after 3 hours was 223.67 ± 2.22 nmol/l, which is 3.9 times ($p < 0.001$) higher than in the control group (58.33 ± 0.84 nmol/l). It remains at a high level after 12 hours and is 145.67 ± 2.16 nmol/l, in the control group 55.0 ± 0.77 nmol/l.

The study of crowding influence demonstrated that the level of total serum protein of sterlet in the group with a stocking density 25 fish/0.04 m³ by 39.6% ($p < 0.001$) less than that in the control group (25.37 ± 0.35 g/l). Lysozyme activity of blood serum was 1.9 times higher ($p < 0.001$) compared to the control group (15.32 ± 0.35). The results of experimental studies showed that the level of phagocytic activity of sterlet leukocytes in the group with a stocking density of 25 fish/0.04 m³ was $26.47 \pm 0.5\%$, in the group with a stocking density of 5 fish/0.04 m³ - $24.27 \pm 0.59\%$ ($p > 0.05$). The results of experimental studies showed that the serum cortisol index of sturgeon fish of the experimental group at a fish density of 25 fish/0.04 m³ was 213.8 ± 2.06 ($p < 0.001$) nmol/l, which is 3.1 times higher than in the control group. the group in which this indicator was 69.67 ± 1.13 nmol/l.

Key words: sterlet; cortisol; nonspecific resistance; temperature and oxygen of the aquatic environment; crowding; serum protein; serum lysozyme activity; phagocytic activity of blood leukocytes

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Sturgeon fish are important aquatic products not only for the conservation of genetic resources and the expansion of biological diversity, but also for breeding and consumption. They are especially important as freshwater aquaculture species with high nutritional value (Zhang et al. 2021).

The constant biotic and abiotic changes that fish species face in aquaculture are problems that cause physiological, endocrine and immunological reactions (Nordocci et al. 2014). In addition to poor water quality and physical disturbances, the social dominance of one fish over another can become a powerful environmental stressor (Pickering 1993). The stress response is initiated and controlled by two hormonal systems leading to the production of corticosteroids (mainly cortisol) and catecholamines (such as adrenaline and norepinephrine) and their precursor dopamine. Together, they regulate secondary stress response factors that alter the distribution of necessary resources, such as energy sources and oxygen to vital areas of the body, as well as disrupt hydromineral imbalance and the immune system. If fish can resist death due to a stressor, they recover to a similar or somewhat similar homeostatic norm. The long-term consequences of repeated or prolonged exposure to stress are maladaptive, since they negatively affect other necessary vital functions (growth, development, disease resistance, behavior and reproduction), largely due to the energy costs associated with an increased stress response (Schreck and Tort 2016). In response to various stressors, fish undergo a number of biochemical and physiological changes in an attempt to compensate for the negative impact of stress factors and thereby cope with stress (Basu et al. 2002). Fish under stress releases chemicals that increase cortisol index in individuals of their species (Barcellos et al. 2011). Stress hormones activate a number of metabolic pathways, which leads to changes in blood chemistry and hematology (Vijayan et al.).

Environmental changes expose fish to a complex set of interacting stress factors and have an important impact on the distribution and abundance of species (McBryan et al.). Stress response can be used as a measure of environmental quality (Pankhurst 2011). The categories of environmental, physical and biological stress factors help to group various possible stressors into several groups. Environmental stressors mainly include unfavorable water conditions. These are quality parameters such as dissolved oxygen, ammonia, hardness, pH, gas, partial pressure, and temperature. Physical stressors include those associated with handling, crowding, closing, transportation, or other forms of physical anxiety (Iwama 1998).

Despite the extensive study of cortisol indicators on the effects of stress factors, knowledge about humoral and cellular resistance when exposed to fish stressors is limited. This lack of information makes it difficult to predict the response of sturgeon resistance to environmental stressors.

Thus, the purpose of this study was aimed at establishing the effect of changes in temperature and oxygen in the aquatic environment and crowding on the level of stress hormone and indicators of nonspecific resistance (humoral and cellular resistance) in sterlet (*Acipenser ruthenus*).

Materials And Methods

Ethics Statement

All procedures with animals were carried out in accordance with the Guidelines for the maintenance and use of laboratory animals of the Institute of Fisheries of the National Academy of Sciences of Belarus. The procedures have been reviewed and approved by the Fisheries Institute's Laboratory Animal Welfare and Ethics Committee.

Experiment Protocol

The studies were carried out in the laboratory of fish diseases of the Institute of Fisheries. The object of the study was the sterlet *Acipenser ruthenus*.

3 stages of experimental studies were planned:

- determination of the influence of the temperature of the aquatic environment on the indices of resistance and the cortisol index of sturgeons;
- determination of the influence of the oxygen concentration of the aquatic environment on the indices of resistance and the cortisol index of sturgeons;
- determination of the effect of high fish stocking density (crowding) on resistance indices and cortisol index.

We carried out the determination of nonspecific factors of fish immunity according to Shoemaker, Klesius, Lim (2001) with modifications. We also looked at studies by Nardocci, Navarro, Cortés et al (2014) and Zhang, Fan, Wu et al (2021).

All analyzes were carried out in triplicate.

At the first stage of experimental studies, the effect of reducing the oxygen content in water to 3.0 mg/l on the indicators of humoral and cellular resistance and the cortisol index of fish was established.

At the second stage, the influence of water temperature increase on the indicators of humoral and cellular resistance and the cortisol index of fish was determined.

At the third stage, we studied the effect of increased density in sturgeons on the indices of humoral and cellular resistance and the cortisol index.

Experimental and control groups of sturgeons with an average weight of 53.4 ± 1.12 g were formed using random analogues.

At the first stage, blood samples were taken in the experimental groups (n=60) at an oxygen concentration of 3.0 mg/l. In the control groups (n=60), blood was taken at an oxygen concentration of 8.3 mg/L. The temperature was maintained at $12 \pm 2.0^{\circ}\text{C}$. The fish were housed in 24 aquariums. The volume of the aquarium is 60 liters.

At the second stage in the experimental groups (n=120) the temperature was raised to 28°C . In the control groups (n=120), the temperature was maintained at 12°C . The fish were housed in 48 aquariums. The volume of the aquarium is 60 liters. Aquariums are constantly aerated. Blood samples were taken at 3 and 12 hours.

At the third stage, the density of fish in the experimental groups (n=60) was 25 fish/0.04 m³, in the control groups (n=60) - 5 fish/0.04 m³. The temperature was within $12 \pm 2.0^{\circ}\text{C}$, oxygen - 8.2 ± 0.2 mg/l. The fish were housed in 24 aquariums. The volume of the aquarium is 60 liters, the volume of water is 40 liters.

The fish at all stages of the experiments were fed AllerAqua food for sturgeons (Denmark) at the rate of 1.5% by weight of the fish.

Sample collection and analysis

Blood was taken from fish immediately after euthanasia and collected with disposable 1 ml heparinized syringes through the tail vasculature of each fish, transferred to a disposable Eppendorf tube, centrifuged at 500–1000 g (4°C) for 15 min to obtain serum samples, and then Store immediately at -20°C until analysis.

Measuring cortisol

Cortisol parameters were determined by enzyme immunoassay using kits (Fish Cortisol ELISA Kit, USA). A calibration curve was generated by plotting the average absorbance data obtained for each reference standard. The corresponding cortisol concentration in nmol/l was determined from a standard curve using the average absorbance value for each sample.

The indicators of nonspecific resistance were determined: lysozyme activity of blood serum and phagocytic activity of leukocytes.

Measuring lysozyme activity of blood serum by nephelometric method

Plasma lysozyme activity was measured according to Eslamloo et al. (2012) with changes.

It is based on a method, the essence of which is the rapid lysis of a reference culture of *Misgurnus lysodeikticus* (Sigma-Aldrich, Bucharest, SRL, Romania) in the presence of lysozyme.

Equipment and reagents: daily culture of *M. lysodeikticus*, 0.5% sodium chloride solution; chemical test tubes, pipettes, glass rod, photometer, thermostat.

Measurement course: In experimental cuvettes (working width 10 mm), 2 ml of blood serum is diluted with 0.5% sodium chloride solution (pH 7.2) in a ratio of 1:20 (1.9 ml of 0.5% sodium chloride solution + 0.1 ml of blood serum) was added 2 ml of a suspension of a daily agar culture of micrococcus, prepared as follows: a daily culture of micrococcus was washed with 0.5% sodium chloride solution, the resulting suspension was standardized with a photometer to the content of 2 billion microbial bodies in 1 ml (extinction 0.320 at wavelength 400 nm).

A mixture was used as a control, in which 2 ml of the same (as in the experimental cuvettes) suspension of micrococci was added to 2 ml of a 0.5% sodium chloride solution. After that, the contents of the cuvettes were mixed with a thin glass rod and measured using a green light filter in cuvettes with a working width of 10 mm. Then the samples in test tubes were placed in a thermostat for 3 hours at a temperature of 26°C and measured again.

Calculation of % lysis of microbial bodies is carried out according to the formula:

$$L = \frac{(D_0 - D_1)}{D_0} \times 100 - \frac{(D_{k0} - D_k)}{D_{k0}} \times 100;$$

L - % of lysis;

D₀ - optical density of the contents of the experimental cuvettes before incubation;

D_k - optical density of the contents of the experimental cuvettes after incubation;

D_{k0} - optical density of the contents of the control cuvettes before incubation;

D_k - optical density of the contents of the control cells after incubation.

Measuring phagocytic activity of leukocytes

Equipment and reagents: sterile tubes, sterile pipettes 1.0 ml; sterile Pasteur pipettes; 0.65% sterile sodium chloride solution; 2% sterile sodium citrate solution; water bath adjusted to 60°C; the object of phagocytosis - 1x10⁹/cm³ suspension of a daily culture of the epizootic strain of *Aeromonas hydrophila* bacteria (Research Institute of Fisheries), inactivated at 60 °C for 30 minutes, prepared in 0.65% sterile sodium chloride solution; thermostat adjusted to 26 °C; glass slides; frosted glass; blood smear staining kit; methyl alcohol; working solution of azure-eosin dye; immersion oil; microscope.

The course of measurement: 0.1 ml of 2% sterile sodium citrate solution was added to the test tube; Added 0.2 ml of fresh frozen blood of the studied fish and 0.2 ml of the object of phagocytosis. The suspension was thoroughly but thoroughly mixed and placed in a thermostat at a temperature of 26°C. After the specified period, the mixture was centrifuged at 2000-3000 rpm until the liquid was separated into the upper - straw-yellow transparent layer of plasma, the lower - a layer of erythrocytes and the middle silver film between them - a layer of leukocytes. With a Pasteur pipette with a finely elongated capillary, the upper layer was first aspirated, then the middle layer was carefully removed, 3-5 smears were made from it, which were fixed for 5 minutes with methyl alcohol. Then the smears were stained for 20-40 minutes with the working solution of azure-eosin. After that, they were viewed under immersion (7x90). 100 leukocytes counted. The number of leukocytes captured by test microbes, to the total number counted, expressed as a percentage (percentage of phagocytosis), characterizes the capturing ability of leukocytes.

The total protein index was determined using a refractometer according to the instructions for use.

20 min before blood sampling, a 2% solution of novocaine at a dilution of 1:1000 was added to the injection to anesthetize the fish.

Statistical analysis

The obtained results were processed by the method of parametric statistics using the Microsoft Excel 2016 package. The significance of the differences in the results obtained was assessed using a t-test using a paired two-sample t-test with equal variances. t-Test: Two - Sample Assuming Equal Variances Differences were considered significant at α=0.05 and 0.05>p<0.001. Numerical data were presented as arithmetic mean (M) and standard error of the mean (±m).

RESULTS

Effect different concentrations of oxygen in the aquatic environment on index of blood serum total protein, lysozyme activity of blood serum, phagocytic activity of leukocytes, and cortisol

When studying the effect of the amount of oxygen in the aquatic environment on whey protein, it was found that in the group where the amount of oxygen in the aquatic environment was 3.0 mg/l, the level of whey protein was 15.29±0.4 g/l (G2), which is 37.7% (p <0.001) lower than in the control group (G1), where the amount of oxygen in the aquatic environment was 8.3 mg/l. When studying the effect of the amount of oxygen in the aquatic

environment on lysozyme activity, it was found that in the group (G4), where the amount of oxygen in the aquatic environment was 3.0 mg/l, the indicator of lysozyme activity in the blood serum was $28.87 \pm 0.58\%$, which is 1.6 times ($p < 0.001$) higher than in the control group (G3), where the amount of oxygen in the aquatic environment was 8.3 mg/l. When studying the effect of the amount of oxygen in the aquatic environment on the phagocytic activity of fish leukocytes, it was found that in the group (G6), where the amount of oxygen in the aquatic environment was 3.0 mg/l, the index of phagocytic activity of leukocytes was $21.24 \pm 0.45\%$ ($p > 0.05$), in the control group (G5) with the amount of oxygen in the aquatic environment 8.3 mg/l - $23.54 \pm 0.39\%$. The dependence of the cortisol index on the concentration of dissolved oxygen in the aquatic environment was revealed. The serum cortisol index in the group (G8), where the oxygen concentration in the aquatic environment was 3.0 mg/l, was 223.33 ± 2.18 nmol/l ($p < 0.001$), which is 3.4 times higher than in the control group (G7), in which the amount of oxygen in the aquatic environment was 8.3 mg/l (table 1).

Table 1. Indexes of starlet resistance at different water oxygen

Index	TP (g/l)		LA (%)		Pha (%)		C (nmol/l)	
Groups	G1	G2	G3	G4	G5	G6	G7	G8
M	24.54	15.29	18.76	28.87	23,54	21.24	223,33	66.33
SEM	0.45	0.4	0.36	0.58	0.39	0.45	2.18	1.37
Statistical significance		**		**		*		**

G1, G3, G5, G7 = control (oxygen concentrations = 8,3 mg/l); G2, G4, G6, G8 – oxygen concentrations = 3,0 mg/l; TP – total protein; LA = lysozyme activity; Pha = phagocytic activity of leukocytes; C = cortisol; SEM – standard error of mean

* $p < 0.05$; ** $p < 0.001$

Effect of temperature of the aquatic environment on the index of blood serum total protein, lysozyme activity of blood serum, phagocytic activity of leukocytes, and cortisol

An increase in the temperature of the aquatic environment by more than 15 oC reduces the total serum protein after 3 hours to 17.58 ± 0.31 g/l (G2), which is 30.5% ($p < 0.001$) less than in the control group (G1), in which this indicator is 25.31 ± 0.38 g/l. After 12 hours, the indicator of total serum protein at a temperature of 28 oC in the G4 group remains stably lower and amounts to 19.44 ± 0.29 g/l (in the control group G3 = 23.42 ± 0.36 g/l) (Table 2).

Table 2. Effect of water temperature on blood serum total protein

Index	Total protein (g/l)			
Interval	3 hours		12 hours	
Groups	G1	G2	G3	G4
M	25.31	17.58	23.48	19.44
SEM	0.38	0.31	0.36	0.29

Statistical significance		**		**
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G1, G3 = control (water temperature = 12 °C); G2, G4 – water temperature = 28 °C; SEM – standard error of mean

** $p < 0.001$

The activity of lysozyme reflects the natural resistance of fish organisms. The study of the effect of an increase in the temperature of the aquatic environment on the body of fish (G2) showed that after 3 hours there was an increase in the activity of serum lysozyme by 2.3 times ($p < 0.001$) compared with the control group (G1), in which this indicator was $16.65 \pm 0.51\%$. After 12 hours, this indicator decreases in the experimental group (G4), but remains 1.5 times higher than in the control group ($G3 = 17.31 \pm 0.46\%$) (Table 3).

Table 3. Effect of water temperature on lysozyme activity

Index	Lysozyme activity (%)			
Interval	3 hours		12 hours	
Groups	G1	G2	G3	G4
M	16.65	37.6	17.31	25.56
SEM	0.51	0.8	0.46	0.53
Statistical significance		**		**

G1, G3 = control (water temperature = 12 °C); G2, G4 - water temperature = 28 °C; SEM – standard error of mean

** $p < 0.001$

Cellular resistance includes the phagocytic activity of fish blood leukocytes. It was established that 3 hours after the increase in the temperature of the aquatic environment, the activity of phagocytes in the blood of fish from the experimental group (G2) was $22.47 \pm 1.03\%$, and the control (G1) $23.84 \pm 1.12\%$, and after 12 hours - $G4 = 26.35 \pm 1.1\%$ and $G3 = 25.45 \pm 1.12\%$, respectively (Table 4).

Table 4. Effect of water temperature on phagocytic activity of leukocytes

Index	Phagocytic activity of leukocytes (%)			
Interval	3 hours		12 hours	
Groups	G1	G2	G3	G4
M	23.84	22.47	25.45	26.35
SEM	1.17	1.04	1.12	1.1
Statistical significance		***		***

G1, G3 = control (water temperature = 12 °C); G2, G4 – water temperature = 28 °C; SEM – standard error of mean

*** $p > 0.05$

A significant increase in the cortisol index in the blood serum of fish was established with an increase in the temperature of the aquatic environment by more than 15 °C. In the experimental group (G2), this indicator was 223.67 ± 95.0 nmol/l after 3 hours, which is 3.9 times ($p < 0.001$) higher than in the control group $G1 = 58.33 \pm 0.84$ nmol / l. After 12 hours, this indicator in the experimental group (G4) remains stably high and amounts to 145.67 ± 2.16 nmol/l (in the control group $G5 = 55.0 \pm 0.77$ nmol/l) (Table 5).

Table 5. Effect of water temperature on cortisol

Index	Cortisol (nmol/l)			
Interval	3 hours		12 hours	
Groups	G1	G2	G3	G4
M	58.33	223.67	55.0	145.67
SEM	0.84	2.22	0.77	2.16
Statistical significance		**		**

G1, G3 = control (water temperature = 12 °C); G2, G4 = water temperature – 28 °C; SEM – standard error of mean

** $p < 0.001$

Effect of crowding on blood serum total protein, lysozyme activity of blood serum, phagocytic activity of leukocytes, and cortisol

The results of experimental studies of the effect of crowding on the total protein of blood serum, lysozyme activity of blood serum, phagocytic activity of leukocytes and cortisol are shown in Table 6. We found that the total protein of sterlet serum in the G2 group with a stocking density of 25 fish/0.04 m³ was lower by 39.6% ($p < 0.001$) compared to group G1, where the stocking density was 5 fish/0.04 m³, in which this figure is 25.37 ± 0.5 g/l. The indicator of lysozyme activity of blood serum of sturgeons in the group with a stocking density of 5 fish/0.04 m³ was 1.9 times higher ($p < 0.001$) compared to the control group, in which this indicator was $14.2 \pm 0.41\%$. The results of experimental studies of the influence of crowding showed that the index of phagocytic activity of leukocytes in the group with a stocking density of 25 fish/0.04 m³ was $26.47 \pm 0.5\%$, in the group with a stocking density of 5 fish/0.04 m³ (control) - $24.27 \pm 0.59\%$. The serum cortisol index of sturgeon fish of the experimental group at a fish density of 25 fish/0,04 m³ was higher. the indicator was 69.67 ± 1.13 nmol/l.

Table 6. Indexes of starlet resistance at the crowding

Index	TP (g/l)		LA (%)		Pha (%)		C (nmol/l)	
Groups	G1	G2	G3	G4	G5	G6	G7	G8
M	25.37	15.32	14.2	26.67	26.47	24.27	69.67	213.8
SEM	0.5	0.35	0.41	0.72	0.5	0.59	1.13	2.06
Statistical significance		**		**		***		**

G1, G3, G5, G7 = control (5 fish/0.04 m³); G2, G4, G6, G8 – stocking density of fish is 25 fish/0.04 m³; TP – total protein; LA = lysozyme activity; Pha= phagocytic activity of leukocytes; C = cortisol; SEM – standard error of mean

** $p < 0.001$; *** $p > 0.05$

Discussion

Environmental stressors play an important role both in the wild and in aquaculture. Wassink (2020) has shown that environmental stressors faced by wild animals can have profound effects on behavior and physiology, which can have implications for survival. At the same time, individuals of lake sturgeon grown at 18°C have a higher level of swimming activity and a higher survival rate. Physiological and behavioral phenotypes associated with early stress may be adaptive later in life under conditions of high stress. In the wild, many migratory fish species are under increasing threat due to exploitation, pollution, habitat destruction and require protection, sustainable use and adaptive management (Tamario et al. 2019).

In aquaculture research, stress factors also play an important role. In this case, a positive or negative role will depend on acute or chronic stress. A low level of stress can have a positive effect, while a higher level of stress has a negative effect on fish (Shrek, 2010). The results of our studies have shown that stocking density and hypoxia lead to a significant increase in cortisol levels in sterlet, i.e. have a stressful effect. These data are consistent with those of Ni et al. (2014), who showed that cortisol, glucose, and hematological parameters significantly increase in juvenile Amur sturgeon under conditions of increased stocking density and hypoxia. They noted the adaptive ability to survive in conditions of stable crowding, but without changes in immune parameters. Our studies have shown the presence of a response from nonspecific resistance (an increase in the lysozyme activity of blood serum, a slight increase in the phagocytic activity of blood leukocytes, as well as a decrease in the total protein of blood serum). Thus, stressors affect fish differently depending on the nature and severity of the stressors.

The study of Alfonso et al. shows that the physiology of stress affects the main factor in the fitness of fish to cope with environmental changes. (2021). However, the possibility of adaptive changes partly depends on the nature of correlations between traits associated with tolerance. For example, negative genetic correlations between temperature tolerance and hypoxia may limit the potential for adaptation to combined stressors, while positive genetic correlations may be beneficial. The limited data currently available indicate that tolerance to hypoxia and high temperature is positively correlated in some fish species, which indicates the possibility of adaptive evolution of these traits in response to environmental changes (McBryan et al., 2013). However, variability in the corticosteroid index arises from a number of factors other than stress, including gender and maturity, time of day or after feeding, and season. These factors need to be understood in order to reasonably assess the response to stress in fish. The impact of stress on fish is difficult to assess due to the difficulty of sampling in contrast to terrestrial ecosystems (Pankhurst 2011).

Modeling body processes under rapidly changing environmental conditions is a challenging task (Guenard et al. 2020). However, it is possible to use the stress response as a measure to assess the impact of environmental quality, but only if the underlying response to environmental stress is well understood first.

In written sources, there is not enough information about the influence of environmental factors on nonspecific resistance in sterlet against the background of stress in nature and aquaculture. The results of a controlled laboratory study of the effect of temperature increase, oxygen content changes and crowding on non-specific resistance in sterlet give an idea of what can be expected in the wild and in aquaculture. An increase in the temperature of the aquatic environment, a decrease in the concentration of oxygen in the aquatic environment, crowding, is a physiological load, causes a stress reaction in the sterlet and affects nonspecific resistance. This may compromise their fitness and in certain situations may be of ecological importance.

Conclusion

The results of our own research confirmed that a decrease in the amount of oxygen in the habitat, an increase in the temperature of the aquatic environment, an increase in the population density of sturgeon fish can be attributed to damaging stress factors that also lead to a decrease in the resistance of sterlet (*Acipenser ruthenus*), namely nonspecific resistance, which determine the degree of damaging factors and play an important role in the formation of adaptation processes.

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 - [19] **Contributions** All authors contributed to the concept and design of the study. Material preparation, experiments, data collection and analysis were performed by A. Kurbanov, J. Nomonov, N. Titova, S. Polaz, S. Dziahtsiaryk and Y. Maksimyuk. The first draft of the manuscript was written by S. Polaz. All authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.
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 - [21] **Conflict of interest** The authors declare no competing interests.