Factors for increasing the survival rate of catfish fertilized eggs and larvae

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Abstract. The improvement of the quality of eggs, decrease of developmental anomalies, increase of progeny survival rate are urgent problems of industrial fish breeding. We used biologically active agents – vitamins and adaptogens to solve the above problems. Adaptogen trekrezan (ethanolammonium salt of 2-methylphenoxyacetic acid) – a synthetic analog of ginseng – was applied in fish breeding. It does not demonstrate any gonadotoxic, embryotoxic, mutagenic, teratogenic, carcinogenic and allergenic action and is not accumulated in the organism. The agent has high immunotropic activity, stimulates cellular and humoral immune response, phagocytosis, demonstrates interferon inducer activity. We also used vitamin B1 – thiamine. The need for it is the highest in early ontogenesis. It is required for normal functioning of nervous, digestive and cardiovascular systems, hematopoietic system and is considered essential. The study of the influence of thiamine and trekrezan on egg fertilization rate showed that under the influence of these biologically active agents both individually and in a complex the fertilization increased by at least 14%. The study of the influence of thiamine and adaptogen on larvae hatching showed that they can increase the hatching by more than 20%. The study of the influence of thiamine and trekrezan on the level of developmental anomalies of African sharptooth catfish larvae showed that both components reduce this indicator. With the use of thiamine and trekrezan the level of developmental anomalies decreased in all groups by more than 10%. The study was supported by the Russian Foundation for Basic Research, grant No. 18-016-000127.

1. Introduction
One of the problems of modern fish breeding is high death rate of fish at early stages of ontogenesis [1, 2]. The reason of this phenomenon is directly connected with the quality of eggs, which is defined by a set of factors [3], including feed composition and their amount, temperature condition, hydrochemical composition and microbiological indicators of water [4, 5]. High level of fish stress during pre-spawning, hormonal injections and fish-breeding works are also important [6, 7]. It would be wrong to neglect the fact that the decrease of steroid hormones against the background of chronic stress may negatively affect the quality of gametial cells of male and female fish [8].

The biological assessment of eggs in the conditions of fish-breeding plants reveals low level of its qualitative characteristics [9]. The technologies of industrial aquaculture significantly change fish biology [10]. The African sharptooth catfish is a widespread object of aquaculture, which is losing its natural reproduction ability [11]. It is only possible to trigger the artificial spawning among the representatives of this species by hormonal inductors [10, 11].
In the conditions of industrial fish farming the eggs received via hormonal injections are characterized by deformation of oocytes and their covers, reduction of definitive sizes caused by protein decrease in a single oocyte. Larvae and whitebaits received from such eggs are characterized by high level of developmental anomalies. The study of such posterity shows developmental anomalies of a digestive tract, a respiratory system, olfactory organs, visual sensory system, barbels, etc. [12].

The search for possible options to improve the quality of fertilization eggs, decrease the level of developmental anomalies, and increase the survival rate of posterity are relevant problem of industrial fish breeding.

To increase the natural resistance and adaptation plasticity of farm animals different adaptogens are used. Trekrezan belongs to universal adaptogens. Trekrezan is ethanolammonium salt of 2-methylphenoxyacetic acid, a synthetic adaptogen and an immunomodulator. This new universal biological stimulant is synthesized and patented in Russia at Irkutsk Institute of Organic Chemistry. Trekrezan is a synthetic analog of natural adaptogens (ginseng, aralia, eleuterococcus, rhodiola rosea).

It represents a highly water soluble white powder with slight characteristic odor and sweetish-bitter flavor. The compound does not demonstrate any gonadotoxic, embryotoxic, mutagenic, teratogenic, carcinogenic and allergenic action and is not accumulated in the organism. The agent is not accumulated in products received through its application; it has high immunotropistic activity, stimulates all types of immune functions: cellular and humoral immune response, phagocytosis; demonstrates interferon inducer activity, in particular, stimulates gamma interferon products [13].

In our study we used trekrezan for treatment and subsequent cultivation of fertilized eggs of African sharptooth catfish to increase nonspecific resistance and adaptation plasticity of larvae.

Vitamins are also essential components increasing the general resilience of an organism to negative environmental impacts. They are successfully applied in medicine and livestock production. It is known that vitamins positively affect the reproduction stages of fish: quality of eggs, survival of embryos and larvae [14].

Thiamine (Vitamin B1) belongs to the group of water-soluble vitamins of heterocyclic row and is among irreplaceable vitamins not capable of independent generation by animals. Some ruminant species make an exception. Thiamine is synthesized by plants and microorganisms, and with food gets to an organism of animals. It is well soaked in digestive tract, accumulated in liver, heart, brain, kidneys, adrenal glands and skeletal muscles. Vitamin B1 is necessary for normal functioning of the nervous system, digestive and cardiovascular systems, hematopoietic system. Thiamine actively participates in protein and carbohydrate metabolism, in synthesis of thyroid hormones, regulates skin health and mucous membranes. Lack of Vitamin B1 affects the nervous system, is accompanied by muscle weakness, hypostases, heart rhythm disorder, tachycardia [15].

In the organism of fish B1 regulates carbohydrate metabolism, normalizes nervous, cardiovascular and digestive systems. The deficiency of vitamin B1 affects equilibrium, reduces feed consumption, causes growth and development depression, harms carbohydrate metabolism, water balance, leads to accumulation of peroxides and unsaturated fatty acids toxic for fish, entails dropsys and paralyses. The growth rate of sick fish slows down, muscles, dorsal and pectoral fins are damaged. In case of severe B1 hypovitaminosis the fish rejects feed and die in convulsions.

B1 hypovitaminosis is a widespread phenomenon, while hypervitaminosis is rare since thiamine does not exert any toxic action on the organism [15].

The need for vitamin B1 varies depending on age. As we know, at the initial stages of fish ontogenesis the need for vitamins is maximum, therefore in egg cultivation medium and in feed preparation for larvae and whitebaits we used high doses of vitamin B1.

The purpose of the study is to assess the influence of trekrezan adaptogen and vitamin B1 on fertilization rate, larvae yield, survival and developmental anomalies of African sharptooth catfish.

2. Materials and methods
The studies were conducted at the Laboratory of Experimental Biology and Aquaculture of the Faculty of Veterinary Medicine and Biotechnology of Ulyanovsk State Agricultural University. The object of the study included eggs, embryos, larvae of the African sharp-toothed catfish. The eggs were received from one-year-old females weighing about 1.5 kg grown in recirculating aquaculture systems at a temperature of 26°C. Hormonal stimulation for maturation of oocytes was carried out with surfagon at the rate of 1.0-1.5 ml/kg of fish weight. Surfagon is a synthetic analog of luteinizing hormone releasing hormone, a hormonal regulator stimulating secretion of pituitary gonadotropins of luteinizing hormone (LH) and follicle stimulating hormone [11]. In 11-12 hours after introduction of surfagon the females were ready to produce eggs. The eggs were taken into sterile ware and irrigated with the mixed sperm of two males at the rate of 4 ml of sperm per 200-250 g of eggs, then mixed within 1 minute and added 200-250 ml of water and mixed again within 5 minutes. Right after fertilization the eggs were washed within half an hour with trekrezan adaptogen solution at a dose of 1 mg/l and vitamin B1 – 30 m/l at a temperature of 28°C. The catfish fertilized eggs were incubated in Veisa device at a water temperature of 28°C. The incubation medium also contained adaptogen at a dose of 1 mg/l and vitamin B1 – 30 m/l. The oxygen content did not drop below 90%.

After yolk sac resorption the catfish larvae were fed with starter feeder fish – Artemia salina; it is a species of Crustacea from the order Branchiopoda. Both Artemia salina and starter feeder fish were enriched with adaptogen and vitamin B1 in similar concentration. For larvae trekrezan and vitamin B1 were also introduced at the same doses into starter feed. The control group and 3 experimental groups were created.

In the control experiment the treatment of eggs with adaptogen and vitamin was not carried out, agents were not added to eggs incubation medium and not included into the composition of starter feed. Concerning other parameters, the control group was not different from the experimental one.

In the first experimental group we assessed the effect of vitamin B1. In the second experimental group we assessed the effect of adaptogen trekrezan. In the third experimental group we assessed the total effect of vitamin B1 and adaptogen trekrezan in hatching. 500 species were considered in each group.

To assess the efficiency of thiamine (vitamin B1) and adaptogen trekrezan the following indicators were used: eggs fertilization rate (%), prelarvae (%), embryo development disorders. The share of eggs died in hatching was measured every two hours. The quality of fertilization was estimated as the ratio of normal eggs, not developing or false developing eggs. The morphological analysis of fertilized oocytes and embryos was carried out under binocular microscope Micromed-2 at 10x, 20x magnification. The accounting of larvae survival rate was carried out in the first week after hatching.

3. Results
The first stage of the study assessed the eggs fertilization rate in %. The obtained results demonstrate that in the control group where eggs were not treated with adaptogen trekrezan and vitamin B1 the fertilization rate made 71±3.3% (Figure 1). When eggs were treated with vitamin B1 (thiamine) the fertilization rate reached on average 85±1.3% (Figure 1). When eggs were treated with adaptogen trekrezan the fertilization rate was higher than the control group and above than when treated with thiamine and made 90±1.5% (Figure 1). Joint application of thiamine and trekrezan showed the best fertilization rate. When adaptogen and vitamin were used jointly the fertilization rate made 95±2.1% (Figure 1).

At the second stage we defined the generation of prelarvae from eggs, which was calculated in % of the number of fertilized oocytes. According to the study in the control group, which eggs were not treated with thiamine and trekrezan, the larvae made 40.7±4.6%. The addition vitamin B1 (thiamine) into the incubation medium of fertilized of increased the percentage of prelarvae up to 63±6.9% (Figure 2).
When fertilized eggs were treated with trekrezan in incubation the prelarvae was higher than in the control group and made 64±4.9% (Figure 2). The joint use of trekrezan and thiamine in incubation increased the percentage of prelarvae up to 65±5.1 (Figure 2).

At the next stage we studied the influence of adaptogen trekrezan and vitamin B1 (thiamine) on embryonic ontogenesis of the African sharptooth catfish. The level of developmental anomalies was used as the indicator value. The assessment of the level of developmental anomalies of larvae showed that in the control group it made 22±3.5% (Figure 3).

When vitamin B1 was introduced into the incubation medium the level of developmental anomalies was lower and made 7.1±1.72% (Figure 3). The use of adaptogen trekrezan in the incubation medium reduced the level of anomalies to 8.2±2.2% (Figure 3). The joint use of adaptogen trekrezan and vitamin B1 in the incubation medium allowed reducing the level of anomalies to 5.0±1.6% (Figure 3).
Figure 3. Impact of thiamine and trekrezan on the level of developmental anomalies in larvae.

Larvae received from eggs not treated with vitamin B1 and adaptogen trekrezan are characterized by a significant amount of developmental delays: in particular, 13% had spinal curvature, 7% – underdeveloped operculum, 2% – visual organ pathology.

4. Discussion
The study of the influence of thiamine and trekrezan showed the increase of eggs fertilization rate under individual and complex influence of these biologically active agents. Significant differences are observed in the comparison of fertilization results in the control and all experimental groups, as well as through the comparison of fertilization of eggs treated with vitamin B1 and trekrezan, vitamin B1 and B1 vitamin complex + trekrezan. The difference in fertilization rate in these groups was statistically valid.

Larvae release is an important indicator of eggs hatching. The study of the influence of thiamine and adaptogen on this process showed that these biologically active agents can increase larvae hatching from fertilized eggs. This regularity was typical for all experimental groups. In the control group the release of larvae made 40.7±4.6%. In experimental groups it made 20% more. When comparing experimental groups and the control group the difference in all cases was statistically valid. When comparing the results within experimental groups no statistically valid difference was observed.

The study of the influence of thiamine and trekrezan on the level of developmental anomalies of catfish larvae showed that both components reduce this indicator. The analysis confirms that when comparing experimental groups and the control group the difference in the amount of developmental anomalies in all cases was statistically valid. In the control group the level of developmental anomalies reached 22±3.5%. In all experimental groups the level of developmental anomalies did not exceed 8.2±2.2%. When comparing the efficiency of vitamin B1 and adaptogen trekrezan on the decrease of developmental anomalies no statistically valid difference was revealed. However, there are still certain trends. Joint use of thiamine and trekrezan had the major effect having reduced the level of developmental anomalies of larvae to 5.0±1.6%.

The decrease of the level of developmental anomalies in embryonic ontogenesis of African sharptooth catfish is caused by the increase in the general resistance at the stage of embryonal development under the influence of vitamin B1 and adaptogen trekrezan. Obviously, vitamin B1 gives additional biologically active agents to eggs thus mobilizing internal resources and increasing viability of larvae. In case of deficiency of vitamin B1 there is low ability of an organism to adapt to environmental conditions, especially at early stages of development, before transition to external feeding. The use of universal adaptogen trekrezan that increases cellular and humoral immunity,
participates in detoxication and provides homeostasis in combination with vitamin B1 can be useful to cultivate competent fish larvae resistant to adverse factors of the environment.

5. Conclusion
The problem of increasing the fertilization rate of eggs thus maintaining their viability during incubation and decreasing the level of developmental anomalies is quite relevant today. It is important to search for biologically active agents to solve this problem. The universal adaptogen trekrezan and vitamin B1 was used in the study.

The study showed that both separate and joint use of vitamin B1 and adaptogen trekrezan in fish breeding is quite promising. The treatment of eggs with adaptogen trekrezan and vitamin B1 raised their fertilization rate, reduced the level of embryonic mortality. It confirms higher release of larvae in comparison with the control group. The highest mortality of embryos was recorded during critical stages of development. It was 20% higher in the control group in comparison with experimental groups, which embryos were treated with biologically active agents.

Higher indicators in experimental groups in comparison with the control group confirmed that the separate or joint use of vitamin B1 and adaptogen trekrezan improves eggs fertilization rate, viability of embryos, positively influences their development during incubation, reduces the level of developmental anomalies and raises release and viability of larvae.

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References
[1] Zykov L A 2005 Bioecological and fishery aspects of the theory of natural mortality of fish (Federal Agency on Education, Astrakhan State University, Astrakhan) pp. 373
[2] Cherkashin S A, Nikiforov M V and Shelekhov V A 2004. Use of mortality indicators of sea fish prolarvae to assess the toxicity of zinc and lead. Marine Biology. 30(3) 247–252
[3] Lyutikov A A 2011 Incubation of eggs and cultivation of young nelma fish Stenodus leucichthys nelma (Pallas, 1773) under various temperature and light conditions. Bulletin of the State Polar Academy 1 48–49
[4] Shindavina N I, Nikandrov V Ya, Moiseeva E V and Yankovskiy V A 2013 Assessment of rainbow trout females on the quality of eggs: testing for split eggs Fishery 3 81–85
[5] Bully L I 1996 On the relation of the general biochemical structure of redlip mullet eggs to the quality of received posterity. Works of the Southern Res. Institute of Fishery and Oceanography 42 221–224
[6] Martemyanov V I 2014 Assessment of acute and chronic stress at freshwater fish according to indicators of water-salt metabolism. Achievements of Modern Biology. 134(6) 573–581
[7] Martemyanov V I 2002 Stress of fish: protective and damaging processes. Biology of Inland Waters. 4 3–13
[8] Berezhina D I 2017 Dynamics of cortisol level at the stress of fish. In the collection: Young researchers of agro-industrial and forest complexes to regions. II Int. Youth Sci. and Practical Conf. pp. 12–17
[9] Mikodina E V, Mikulin A E and Mikulina Yu A 2007 Abnormal eggs of Pacific salmons at fish-breeding plants of Sakhalin: biotechnical, technological and standard problems. Fishery 1 61–63
[10] Lyubomirova V N, Romanova E M, Romanov V V and Mukhitova M E 2018 Assessment of efficiency of gametogenesis inductors of the African sharptooth catfish. Bulletin of Ulyanovsk State Agricultural University 2(42) 148–154
[11] Romanova E M, Lyubomirova V N, Romanov V V, Mukhitova M E, Shlenkina T M, Shadyeva L A and Galushko I S 2018 Biology of reproduction of catfish (Clarias gariepinus, Burchell, 1822) in high-tech industrial aquaculture. J. of Fund. and Appl. Sci. 10 1116–1129
[12] Sadomov N A 2010 Embryonal development of sturgeon fish in the conditions of closed water supply: main anomalies and reasons. *Bulletin of Belarusian State Agricultural Academy*. 3 82–85

[13] Voronkov M G and Rasulov M M 2007 Trekrezan – ancestor of a new class of adaptogens and immunomodulators. *Chemical and Pharmaceutical J*. 41(1) 3–7

[14] Bakhareva A A and Grozesku Yu N 2013 Influence of vitamins on fish reproductive functions, *Natural Sci*. 3(44) 086–092

[15] Tkacheva I V and Tyshchenko N N 2011 Vitamins B in fish food. *Works of Kuban State Agricultural University* 28 140–142