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Research Article

The Ability to Identify and Exploit Growth and Survival Potentials of Valuable Fish Species in Artificial Biostimulating Medium

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ABSTRACT

Experimental-production studies effects of keeping in table salt solutions producers and juveniles of valuable fish species: Russian and stellate sturgeon, rainbow trout, catfish and roach were carried out in order to develop a new effective method of their cultivation in fully controlled environmental conditions. The results were evaluated according to fish-breeding-biological and physiological indicators − haemoglobin content, total protein, osmolality of blood serum, cavity fluid and urine. To assess the degree of stress, a cytomorphometric analysis of the hypothalamic-hyperphysical neurosecretory system state was carried out using light and electron microscopy methods. The enhance effects of fish survival, breeder's reproductive quality, young's growth rates were first established in "critical" salinity 4-8‰ medium. On breeders originally it was shown the possibility to change sea water on the table salt solution of the same concentration. With such a low concentration of salt a new bio stimulating effect of this artificial medium on the fish body was installed, due to the absence of its well-known toxicity. The highest degree of survival and delayed sex maturation of roach and stellate sturgeon were specifically installed in the same concentration of industrial salt solutions. The possibility of changing the sea water to industrial salt solution 5-7‰, allowed to developed "A method to reserve fish breeders", which however is not applied to the young fish cultivation. So the next stage in the development of this method was tested it already on juvenile fish to determine the possibility of its effective cultivation in this artificial bio stimulating medium. The results of the search experience on trout fingerlings showed the increased survival and growth in salt solutions, especially in 12‰ as compared to control. The first experimental verification of this method on the clarias catfish

fingerlings in the recycling aqua cultural system (RAS) showed the largest growth rates in 5‰ salt solution in full its survival in all variants. Fish received data and analysis bio stimulated effects impact on the body's critical salinity gives the opportunity to develop a fundamentally new way of cultivation of valuable fish species in an artificial bio stimulated habitat. This technique can allow manage quality of producers, obtaining offspring and the cultivation of young fish. It is aimed at improving the efficiency of breeding fish in aquaculture due to the availability of the application for any type of fish farms with the effects of increasing survival rate and young growth rate. It is expected that the change of the sea water on the cheap solution of table salt can be widely practiced at fish plants in various fields and at all stages of fish biotechnology works, especially RAS.

KEYWORDS

Fish, Salinity, Salt Solutions, Hypertonic Stress, Eustress, Distress, Aquaculture

INTRODUCTION

Long-term studies of fish toxic resistance to the effects of table salt solutions (subsequently: salts) of various concentrations (from 2 to 30‰), as one of the main types of pollution of water bodies, have shown that the absolute toxic effect of 100% fish mortality is achieved by a solution concentration of 25-30‰ [1]. Among the studied fish species at all stages of ontogeny, the highest toxic resistance to hypertonic salt solutions was shown by Salmonids fish species (coho salmon, steelhead salmon, rainbow trout), the lowest – Cyprinids (carp, grass carp) and the lowest –whitefish (peled, pelchir). At the same time, their most sensitive early stages of ontogenesis turned out to be especially indicative: the eggs and larvae of rainbow trout are the most resistant, carp are less stable, and peleds are the most vulnerable.

It is especially important that all the established indicators of the action of salt solutions: the boundaries of acute and chronic action, the values of their inactive (in terms of survival) concentrations (at the early stages of development of carp, rainbow trout and peled) are several orders of magnitude higher than all other test toxicants [2]. At the same time, in salt solutions with a concentration of up to 4‰, the quality of fertilization of eggs is equivalent (carp), or higher than in the control (trout, and peled). Thus, the regularities established in this work indicate the special nature and the most diverse and wide effect of salt solutions on fish among other basic test toxicants.

There is also a similar toxic bactericidal effect of hypertonic salt solutions with a concentration of 10, 12, 50‰, achieved by a relatively short-term effect on juveniles and adult freshwater fish [1,9]. Such "antiparasitic treatment", which causes a selective toxic effect on ectoparasites, is widely used in aquaculture - in pond, pool and aquarium fish farming for the prevention and treatment of mass fish diseases. There is also a method of short-term (up to several hours) transportation of juvenile fish in brackish solutions of sea water, or even salt (with a concentration of 12-15‰), for example, Pacific herring, mullet, etc., to increase their survival rate [5,6]. A multiple increase of the growth rate of juvenile salmonids (salmon, rainbow and brook trout) in Baltic sea water (with salinity from 2

to 20‰) was discovered as early as 1916 in Germany and later the degree of embryonic survival also [3,8]. In our long-term experiments on growing young's Baltic salmon in sea cages (up to 1,5 years old) the increase of survival and growth rates (by 3-7 times) were obtained also [8].

Despite the fact that these effects have long been known, we have not found a clear definition of the optimal salinity values for enhancing the survival of fish in seawater, growth juveniles and information on the biotechnology of growing and analysing these mechanisms in the most optimal medium in the literature [3,8,22].

However, we have established for the first time that the effects of increasing the degree of survival and preservation of the fish-breeding quality of producers, the growth rate of juveniles are most clearly manifested in the narrow range of "critical salinity - 4-8‰", which is the threshold for the maturation of gametes of marine and freshwater organisms. This marine medium determines the limits of their physiological stability and a number of important thresholds, boundaries and gradients of the relationship between the organism and the external medium [8, 10, 22]. On the basis of logical and experimental analysis of these ideas, a fundamentally new method of reproduction of valuable fish species has been developed [8, 13-16]. This method of biotechnics consists in settling producers from the marine fishery in sea cages, here to obtain offspring from them and, after growing factory juveniles (up to 1 year in the river), to accelerate their growth in brackish sea water [15]. Preliminary results of experiments on long-term reservation of fish breeders in this medium have already shown the possibility of replacing seawater with sodium chloride solutions − table salt (next: salt) of the same concentration of 4-8‰. With such a low salt concentration in these experiments, the well-known toxic effect characteristic of it hypertonic solutions did not manifest itself therefore, the use of this medium in aquaculture is very promising, especially in recirculating aquaculture systems (RAS) installations of continental fish farms. It is well known that the direction, nature and degree of severity of physiological effects primarily depend on the intensity and duration of the impact of medium factors [4,7]. Therefore, the first stage of the work was to identify the positive fishery effects of the influence of this medium on fish depending on the intensity of the impact, and specifically to find the optimal concentration of the salt solution for this.

The main goal of the work is to develop a new effective method of fish breeding in fully controlled medium conditions. The main task of the first stage of the work is the morph-physiological and fish-breeding biological analysis of the physiological effects of keeping producers and juveniles of the studied fish species in solutions of table salt of various concentrations.

MATERIALS AND METHODS

The objects of study in fish-breeding production (factory) conditions were especially valuable fish species: the Russian sturgeon Acipenser gueldenstaedtii (Brandt, 1833) and the Stellate-sturgeon Acipenser stellatus (Pallas, 1771), as promising objects of aquaculture were studied in laboratory (aquarial) conditions: rainbow trout Parasalmo mykiss (= Oncorhynchus mykiss) (Walbaum, 1792) and catfish Clarias gariepinus (Burchell, 1822),

as a widely available laboratory object - roach Rutilus rutilus caspicus (Jakowlew, 1870). The main fish-breeding-biological indicators of producers and juveniles were determined, and the weight of fish youngs was determined apiece and by the volumetric-weight method [8]. To assess physiological state of fish, content of hemoglobin and total protein in the blood, the osmolarity of blood serum, cavity (ovarian) fluid and urine were determined according to generally accepted methods.

For histomorphometric analysis of the hypothalamic-hypophysial neurosecretory system (HHNS) state, responsible for water-salt metabolism and the implementation of the body stress reactions, histological sections were stained with paraldehyde-fuchsine (according to Gomory-Gabe) and with azan (to Heidenhain) [17]. For HHNS electron microscopic study, the material was processed according to Sabatini and Caulfield, contrasted with tetroxide osmium and lead citrate according to Reynolds, and studied in the JEM-100B electron microscope.

RESULTS

For the first time, we have established the highest degree of survival and delayed sexual maturation in producers of roach and stellate-sturgeon, reserved 30 (stellate-sturgeon) and 55 (roach) days in salt solution of 5-7‰ concentration and at upper spawning threshold temperatures up to 26°C (Figure 1).

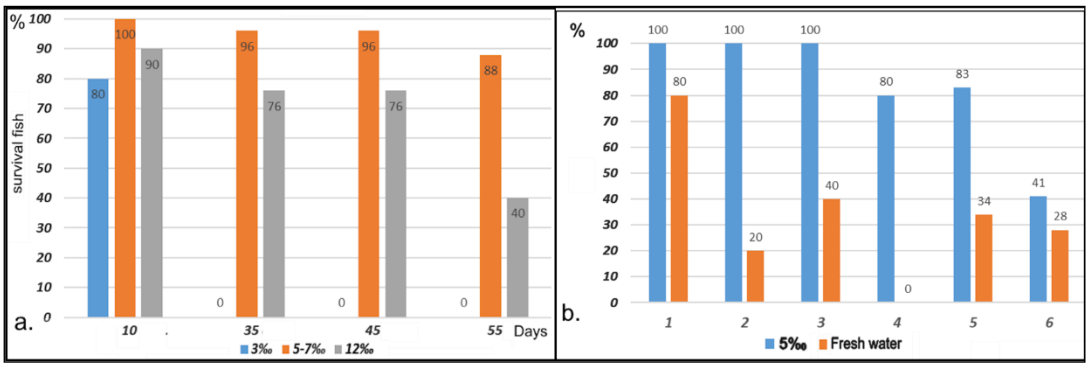

Figure 1: Fish-breeding-biological effects of keeping breeders in salt solutions: a. - survival of roach breeders (a total of 350 fish of both sexes, previously kept in fresh water during 14 days before the beginning of the experiments at the onset of the upper spawning temperatures 24-260C). And by the 10-th day of the experiment, all 50 fish in the control (fresh water) died; b. – fish-breeding quality of stellate-sturgeon females (both 10 individuals in experiment and control):

1. – degree (%) of survival

2. – % in a state of physiological norm, 3. – % in sex maturity, 4. – % of breeding, 5. – % of eggs fertilization, 6. – % of prelarvae hatching.

In this artificial medium, all stellate-sturgeon had benign offspring, and in the control (river water): total oocyte resorption was observed in all female roach and most stellate-sturgeon; and the degree of mortality − 100 and 20%, respectively. To analyse the mechanisms of the established effects manifestation, their morph-physiological state was studied and it was shown that it is most optimal in this medium, which is close to critical salinity, i.e. losses in the content of haemoglobin and protein in the blood serum are minimal (Table 1).

Histophysiological analysis of the state of the stellate-sturgeon HHNS showed a significantly lower degree of its activation in this medium (5‰) by the end of the experiment than in the control (Figure 2).

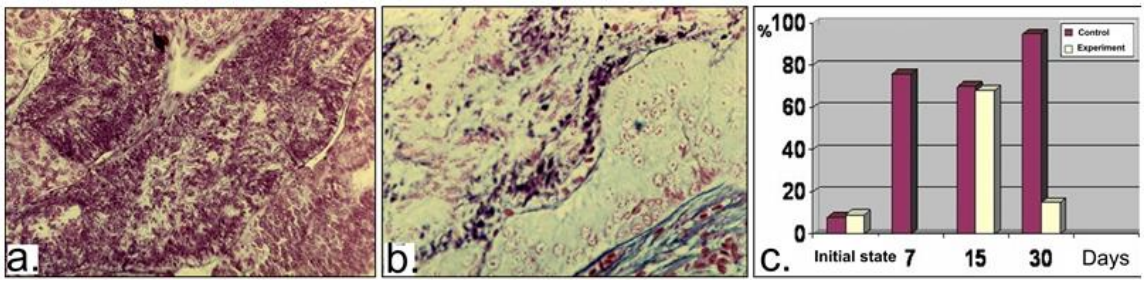

Figure 2: Histomorphological state of the neurohypophysis of female's stellate-sturgeon in experiment and control (light microscopy);
a. The neurohypophysis contains a large amount of neurosecretory material (5 points), which reflects the lack of excretion of neurohormones into the bloodstream – an inactive state of the HHNS (experiment 5‰, 30 days). Coloration: paraldehyde-fuchsin according to Gomory-Gabu with additional coloring of the azan according to Heidenhain. Magnification: Eyepiece: 10, Objective: 40.
b. The neurohypophysis is almost emptied of neurosecretory material (1.5 points), which reflects the massive excretion of neurohormones into the bloodstream – the active state of the HHNS (control, 30 days); Coloration and magnification are the same.
c. Histogram of the dynamics of changes in the degree of functional activity HHNS (in %, average) in stellate-sturgeon females during 7, 15, 30 days reservation in a salt solution of 5‰, compared to control in river water. The maximum activation of the HHNS is observed in the control by the end of reservation, moderate – in a 5‰ salt solution.

At the end of the experiment, the moderately active state of the HHNS in female stellate-sturgeon in a 5‰ salt solution clearly indicates a state of eustress – a state of weak or moderate tension of the body, and a high activation of the HHNS in the control – a state of chronic stress (Figure 2 c). In order to verify this conclusion and analyze the mechanisms of participation of the HHNS in the realization of all types of osmotic (and toxic) stress [8,11,12], acute experiments were carried out on the effect of hypertonic salt solutions of concentrations of 17‰, 22‰ and 32‰ on the HHNS state of stellate-sturgeon and sturgeon producers (Figure 3).

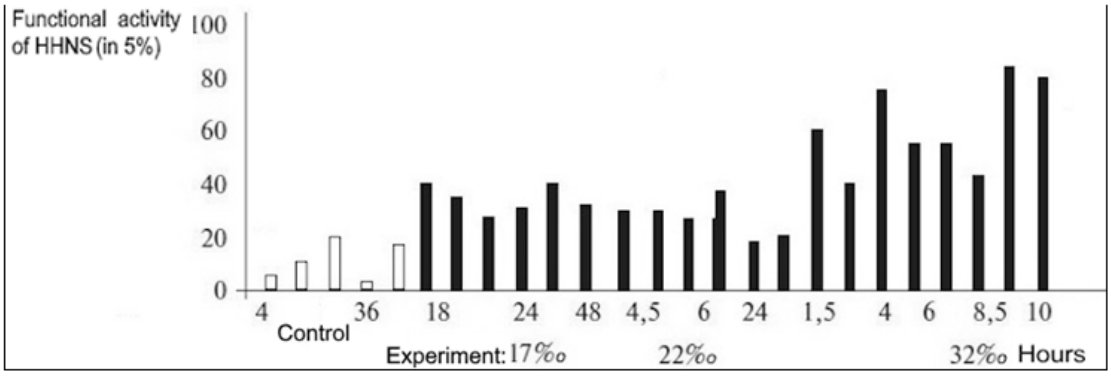

Figure 3: The degree of functional activity of stellate-sturgeon breeders HHNS after keeping in 17‰ (6 individuals for 18-48 hours), 22‰ (6 individuals: 4.5-24 hours), sturgeon breeders – in 32‰ (8 individuals: 1.5-10 hours) and in control (5 stellate-sturgeon in river water: 4-36 hours). (2+3); light columns).

Electron microscopic study of the ultrastructure of the neurosecretory terminals in neurohypophysis showed pictures of mass excretion of neurohormones from them into the sinusoid capillaries of the general blood flow in almost all individuals of stellate-sturgeon at 17 and 22‰ (Figure 4a).

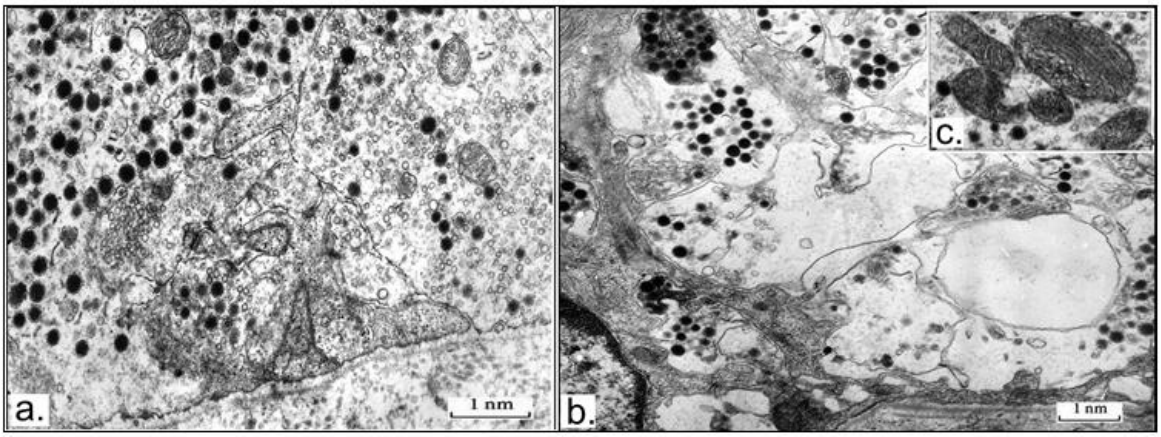

Figure 4: Ultrastructure of the neurohypophysis of stellate-sturgeon and sturgeon after keeping in moderate and high concentrations of salt solutions;

a. After 48 hours keeping stellate-sturgeon in salt solution of 17‰, many neurosecretory terminals are significantly emptied of neurosecretory granules, synaptic vesicles and swollen mitochondria are dominated there, which reflects the activation of HHNS in the form of mass excretion of neurohormones into the sinusoid capillary.

b. After 10 hours keeping sturgeon in salt solution of 32‰, neurosecretory terminals are partially destroyed, lamellar structures, lysosomes are observed in them, and in some places there are clusters of highly hypertrophic mitochondria of atypical structure with tubular-vesicular crists characteristic of steroidogenic cells (c.).

Morph functional analysis of the state of the structures and ultrastructures of the HHNS shows that at 17‰ the fish were in a state of acute hypertensive stress (Figure 3,4a). At the end of the experiment at 32‰ (Figure 4 b), the pictures of the greatest activation of the HHNS, functional exhaustion and intracellular pathologies in the neurohypophysis clearly reflect the state of distress of the body. These conditions are directly dependent on the intensity and duration of exposure. Their effects are alternative and therefore controllable, moving from bio stimulating to reversible stressor and, after reaching the limits of the species physiological load, to a toxic effect that leads fish to a state of distress and death. At optimal salinity (5-7‰), the levels of maximum retention of salts in the blood and cavity (ovarian) fluid and their minimum excretion in the urine indicate the preservation of the water-salt balance of the body (Table 1). This is achieved by optimizing the osmotic energy-saving gradient between the internal (близкой к 11-12‰) and external mediums (close to is tonicity), which ensures the preservation of water-salt and metabolic homeostasis of the body. This artificial medium has a bio stimulating effect that increases the body's resistance, which has led to the development of the "Method to Reserve Fish Producers" [16], which, however, was not applicate for the management of the rearing of juveniles.

Salinity of the medium (‰)	Duration of reservation (day)	Roach		Stellate-sturgeon females		
		Content		Osmolarity (average): mosM/l (salinity: ‰)		
		Hemoglobin g/l (limits)	Total protein g-% (limits)	Serum	Cavity fluid	Urine
3	15	6,6±1,1	1,93±0,38			
		(5,7-7,95)	(1,51-2,28)			
5	28			164.4(6.2 ‰)	196(7.7 ‰)	122(4.5 ‰)
	45	9,0±2,95(7,0-12,9)	2,32±0,21(2,18-2,61)			
12	45	6,3±1,5(4,9-7,9)	2,84±0,38(2,36-3,12)			
Control (river water)	34	6,7±0,7(5,6-7,0)	1,75±0,30(1,51-2,11)			
	28			153(5.8 ‰)	171(6.6 ‰)	155(5.9 ‰)

Table 1: The most important physiological indicators of roach and stellate-sturgeon producers in solutions of table salt of different salinity and in control.

Therefore, the next stage of the study was to test this effect on juvenile fish in order to establish the possibilities of its effective rearing [7,8]. Preliminary experiments were carried out on a mass, the most accessible object of industrial aquaculture − trout yearlings in laboratory of the Department of Aquatic Bioresources and Aquaculture of St. Petersburg State Agrarian University [18] at 11.3-13.50C (Table 2).

These results were confirmed experimentally in the Laboratory of Fish Toxicology in the State Scientific Research Institute of Fisheries (GosNIORKH) in order to study the effect of sub lethal concentrations of test substances (including NaCl) under long-term action for 30 days and a temperature of 120C on survival and growth rates of rainbow trout (Table 3).

It was found that the highest values of all final growth indicators of juveniles are observed in a solution of table salt 5‰. Comparative results given in Tables 2 & 3 show an increase in the growth rate of trout in table salt solutions of 5 and 12‰ in comparison with fish rearing in other variants of the experiment and especially in the control. It should be noted that trout is oxyphilic, i.e. highly sensitive to oxygen deficiency, temperature fluctuations and in general to housing conditions.

Final body weight (average, g)		Survival rate number of fish, (%)		Duration of the experiment (days)		Number of fish in the experiment	
experiment 1	experiment 2	experiment 1	experiment 2	experiment 1	experiment 2	experiment 1	experiment 2
Experiment: NaCl 3‰ solution*/							
43,0±4,15	-	11 (44)	-	35	-	25	-
Experiment: NaCl 5‰ solution							
43,8±4,48	112,8±5,4	3 (12)	25 (75)	35	98	25	33
Experiment: NaCl 12‰ solution							
45±3,20	115,9±7,1	18 (72)	23 (69)	25	98	20	33
Control (fresh water)							
34±3,94	100,6±6,3	12 (48)	27 (82)	45	98	25	33

*/ Water purification in experiments was difficult and violated several times.

Table 2: Survival and growth of trout yearlings in solutions of table salt various concentrations (experiment 1: initial average weight of 25.5 g; experiment 2: initial average weight of 10 g).

Test-substance	Concentration (‰)	Number of fish (шт.)	Initial Weight (g)	Final Weight (g)	Scrap Percentage	Weight gain (g)	Specific weight gain (%)
Table salt	2,5	10	52,0	82,0	0	30,0	58
	5	10	52,0	87,0	0	35,0	67
	10	10	48	71,0	0	23,0	47
Control (3 Repetition)	-	10	48,0	80,0	0	32,0	56
	-	10	48,0	78,0	0	30,0	52
	-	10	48,0	77,0	0	28,0	54

Table 3: Effect of sublethal concentrations of table salt solutions on survival and growth rates of rainbow trout juveniles.

The first pilot test of the new method development was carried out on juvenile catfish, a new promising object for breeding in aquaculture, in particular, for growing in recirculating aquaculture systems, where the experiment was carried out (Table 4) [18,19].

Dynamics of weight gain (average, g) based on the results of 6 gradations (1-5: at intervals of 10 days)						Survivability (%)	Duration of experience (days)	Number of fish in the experiment
1 Start	2	3	4	5	6 Ultimate			
Experiment: NaCl 5‰ solution								
2,92	6,89	13,37	25,28	46,38	55,24	100	43	150
Experiment: NaCl 8‰ solution								
2,92	5,28	9,80	18,22	34,73	41,70	100	43	150
Control (fresh water)								
2,92	4,21	7,77	14,69	26,96	32,11	100	43	150

Table 4: Survival and growth of juvenile catfish (yearlings) in solutions of table salt of various concentrations and in control.

The results show the greatest increase in the growth rate of juveniles in a solution of table salt of 5‰. Hydrochemical parameters in this medium at the beginning and at the end of the test period of the method correspond to the standard for breeding and growing this fish species, which is very resistant to a high level of nitrogen dioxide (NO2) in tap water of recirculating aquaculture (Table 5):

Timing of determinations	ToC	O2	pH	NH4	NO3	NO2	Fe
Start of experiment (1 day)	23-25	6,2	6,8	0,27	<0,1	17,8	0,24
End of experiment (43 days)	23-25	5,8	6,5	0,37	<0,1	20,8	0,40
Norm (optimum)	25-28	>1,7	6,0-8,0	<10	<100	<3,0	<0,5

Table 5: Hydrochemical parameters of isotonic solution in the experiment (mg/l)

CONCLUSION

As a result of the first stage of exploratory studies, the optimal concentration of table salt solutions of 5-7‰ was revealed, relatively close to the isotonic medium. Three positive fish-breeding effects of this medium on survival, preservation breeding-quality of producers and juvenile's growth rates of the studied fish species have been established, the latter of which, however, is less pronounced than in natural sea water [8,18]. The results of the performed morph physiological and fishery-biological analyses reflect the optimization of the fish physiological state in this medium, which indicates its bio stimulating effect on the body. However, we cannot exclude the possibility transition of the bio stimulating effect of this medium to toxic with a longer cultivation of fish in it and depending on age characteristics. Therefore, in order to find out the duration and stability of the bio stimulating effect of this medium on fish and, as a result, their commercial cultivation and reproduction, the next necessary stage of this development is the pilot testing with longer maintenance producers and rearing juveniles.

In the future, any, probably even alternative effects of salt solutions on the body (for example, toxic – bactericidal) are applicable and they provide an opportunity to develop a fundamentally new method of breeding valuable fish species in an artificial medium in order to manage fish maintenance cultivation, widely available for any type of continental fish farms and at all stages of biotechnology, especially in recirculating aquaculture [8,19]. In particular, for the industrial implementation of the proposed biotechnology and year-round fish breeding, we have developed new methods for keeping producers and growing juveniles in this artificial bio stimulating medium [13,14], as well as fundamentally new systems of closed water supply for fish hatcheries and fish farms by means of off-season underground medium hydro conditioning for growing fish youngs in aquaculture [20,21].

REFERENCES

1. Andrews K, Axell E, Carrington N (2007) Fish diseases. Prevention and treatment. Moscow, Aquarium Publ 208p.
2. Arshanitsa NM, Stekolnikov AA, Grebtsov MR (2022) Ichthyopathology. Toxicosis of fish. – 2nd edition, revised and supplemented (Textbook for universities). St. Petersburg: Lan 264p.
3. Boeuf GJ, Payan P (2001) How should salinity influence fish growth? Comp. Biochem. Physiol. C Toxicol Pharmacol 130(4): 411-423.
4. Bonga Wendelaar SE (1997) The stress response in fish Physiological Reviews 77(3): 591-625.
5. Chervinski J (1978) The use of coarce table salt in acclimatization of Liza aurata (Risso) and Chelon labrosus (Risso) (Mugil Chelo) to fresh water. – In: Fish and Aquacult. Res. Stat 30: 23-24.
6. Chittenden ME (1973) Transporting of American shad (Alosa sapidissima) in law salinity water. J. Fish. Res. Board Can 30(1) 38-45.
7. Eddie E, Deane NYS Woo (2009) Modulation of fish growth hormone levels by salinity, temperature, pollutants and aquaculture related stress: a review. Reviews in Fish Biology and Fisheries 19(1): 97-120.
8. Garlov PE, Nechaeva TA, Mosyagina MV (2018) Mechanisms of neuroendocrine regulation of fish reproduction and prospects for artificial reproduction of their populations. St. Petersburg, Prospekt Nauki Publ 336.
9. Golovina NA, Strelkov Yu.A, Voronin VN, Golovin PP, Evdokimova EB, Yukhimenko L.N. Ed. by N. A. Golovina, O. N. Bauer. Moscow, Mir Publ 2003448.
10. Khlebovich VV (2012) Critical Salinity-Homeostasis-Sustainable Development. Essays on the ecology of the individual. St. Petersburg, Zoological Institute of the Russian Academy of Sciences. Publ 143.
11. Krayushkina LS (2022) Functional evolution of osmoregulatory system in acipenserids (Acipenseridae). M.: Fizmatlit 320p.
12. Kültz D (2015) Physiological mechanisms used by fish to cope with salinity stress. J. Exper. Biol. 218(Pt 12): 1907-1914.
13. Method for growing juvenile fish in artificial biostimulating medium. Patent for invention No 2741648.
14. Method for keeping fish-breeders in artificial biostimulating medium. Patent for invention No 2726107.
15. Method of reproduction of stellate sturgeon and Baltic salmon populations. Patent for invention No 2582347.
16. Method to reserve fish produsers. Author's certificate No 965409.
17. Mikodina YeV, Sedova MA, Chmilevskiy DA Mikulin AYe, Pyanova SV, Poluektova OG (2009) Histology for ichthyologists. Experience and advice. VNIRO: 112.
18. Shinkarevich ED, Bugrimov BS, Garlov PE, Shutova GA (2018) On the development of a method of intensive fish cultivation in an artificial biostimulating environment. Proceedings of the National Scientific Conference of the Faculty of Staff and Postgraduate Students of SPbGAVM. St. Petersburg, SPbGAVM Publ 124-126.
19. Van Rijn J (1996) The potential for integrated biological treatment systems in recirculating fish culture. Aquaculture 139(3-4): 181-201.

20. Water supply system of fish farms. Patent for invention No 2400975.
21. Water supply system of fish hatcheries. Author's certificate No 982614.
22. Whitfield AK (2015) Why are there so few freshwater fish species in most estuaries?. J Fish Biol 86(4): 1227-1250.