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Development of the control system for Artificial Reproduction of Fish Populations Based on a Complex of Neroendocrinological research and Biotech Innovations

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Abstract

The participation of the hypothalamic-hypophysial neurosecretory system (HHNS) in fish reproduction using light, electron microscopy and immunocytochemistry was established by ecological-histophysiological studies for the first time in the world literature. At the beginning of passing fish migrations a violation of the HHNS basic osmo-regulative function, causing habitat change is revealed. Simultaneously the HHNS causes the neurotropic effect on the central nervous system (CNS) arousal in the form of a dominant state of "Migration impulse." At the beginning of spawning, HHNS also initiates spawning behavior and completes it by participating in the body's protective-adaptive reactions to natural physiological stress. The functional role of the HHNS in fish reproduction is to initiate energy-intensive (reproductive) processes of migratory and spawning behaviors and to complete spawning by suppressing the hyperactivity of target glands, which ensures the body's transition to energy-saving plastic exchange. The analysis of the key role of the HHNS in the integration of fish reproduction by the self-regulation principle has allowed the development of a constructive working scheme. New biotech methods of managing breeding time, rates of survival and growth of valuable fish species were developed on this basis. They are presented in the form of 10 inventions. To stimulate sex maturation, drugs of isolated anterior and posterior pituitary lobes were developed and introduced in sturgeon industry, increasing the extent of their fish-breeding use by 15% and saving the pituitary up to 40%. To delay puberty, a method of industrial fish breeders reservation in the critical salinity habitat of 4-8%, both in seawater and in table salt solutions has been developed. On this basis, a full-system method of sturgeon and salmon fish populations reproduction, covering all stages of farm biotechnology has been developed. It is carried out by mass breeders reservation in sea cages, obtaining their offspring and, after the river fish- farm growing young's to their readiness for migration, the final cultivation them in sea cages gardens till large life-resistant youngs. For the implementation of the proposed industrial biotechnology and year-round fish farming a new installation of recirculating aquaculture systems (RAS) hatcheries and farms was developed by means of the off-season underground conditioning of fish cultivation habitat and by the principle of natural-industrial engineering ecology. Due to the general aim – increasing the efficiency biotechnology of reproduction and logical relationships, all developments are integrated into the system of biotech management of fish population reproduction.

Keywords: *neuroendocrine regulation of fish reproduction, artificial reproduction of sturgeon and salmon fish, management system of fish-farm populations reproduction.*

Introduction

It was previously found that nonapeptide neurohormones (NP-NH: arginine-vasotocin and isotocin, in teleost fish), produced by nonapeptidergic neurosecretory cells (NSC) of the hypothalamic-pituitary neurosecretory system (HHNS) of fish, are involved in the regulation of the most important body's functions: water - osmotic metabolism, tone of smooth muscles of the gonads, spawning behavior and in the implementation of stress reactions of the body as a whole [15-17]. Therefore, it was assumed that HHNS is involved in the determination of migratory behavior associated with a change in the habitat of anadromous fish [1, 3]. However, in the world literature, specialized navigational processes of the effect of geomagnetic fields on the body's receptor systems [19, 21] and chemoreceptor processes of olfactory imprinting and homing, interconnected in the CNS, are usually considered as the only leading mechanism for fish migrations [20, 22]. There were no clear changes in the state of the HHNS during the spawning period of fish, probably due to the qualitative methods of its assessment, or the study of only the perikaryons of the NSC, as the center of the synthesis of NP-NH [23, 25].

For the first time, the participation of HHNS in fish reproduction was established by us due to the use of quantitative morphometric methods for assessing its functional state and comparative analysis of the results of the ecological-histophysiological direction of research [2]. Already a preliminary analysis of the results of the ecological-histophysiological study shows a fundamental similarity in the mechanisms of the participation of HHNS in the implementation of migrations and spawning and suggests that the functional role of HHNS is to initiate and complete reproduction in general. This makes it possible to present a simple working scheme for the neuroendocrine integration of fish reproduction according to the principle of self-regulation, which can be constructive enough for the development of new methods of control over reproduction biotechnology. An increase in its efficiency is necessary due to the extremely stressed state of populations of salmonid fish (Atlantic salmon, brown trout, char and Volkhov whitefish) in the Northwest region, the number of which is supported exclusively by hatchery reproduction [2, 4]. The decrease in their numbers (before the prohibitions on fishing) occurred due to the cessation of natural spawning and insufficient efficiency of hatchery reproduction, which harvests spawners in spawning grounds (to the detriment of natural spawning) and releases one-year-old juveniles of salmon (weighing up to 26 g) with a survival rate in nature of only 0.4 %, at a rate of 1.9% [2, 4].

Therefore, in order to increase the efficiency of artificial reproduction of populations, we carried out an ecological-histophysiological study, moreover, a full-system study, which should end with innovative biotechnological developments. The latter is the main task of this work.

Materials And Research Methods

For ecological and histophysiological studies, the morpho-functional state of the HHNS was studied in sexually mature spawners of valuable species of anadromous fish: spring-spawning Russian sturgeon *Acipenser gueldenstaedtii Brandt* (Linne, 1833) from the lower reaches of the Volga and autumn-spawning pink-salmon *Oncorhynchus gorbusha* (Walbaum, 1792) and chum salmon *O. keta* (Walb., 1792) in rivers Naiba and Umba (Southern Sakhalin, Kola Peninsula). Additionally, the states of HHNS were studied in breeders of beluga *Huso huso* (Linnaeus, 1758), stellate sturgeon *A. stellatus* (Pallas, 1771), sterlet *A. ruthenus* (L., 1758). For biotechnological developments, experimental and production material was collected at sturgeon fish hatcheries in the Lower Volga and Don, material on the Atlantic salmon *Salmo salar* (L., 1758) - at the Nevsky salmon fish hatchery (SFH) and the marine cage fish farm in the Finnish (Vyborg) Bay. Under laboratory conditions, experiments were carried out on the roach *Rutilus rutilus caspicus* (Jakowlew, 1870), the rainbow trout *Parasalmo mykiss* (= Oncorhynchus mykiss) (Walbaum, 1792) and the African clary catfish *Clarias gariepinus* (Burchell, 1822), as promising objects for aquaculture. The state of the HHNS was studied histomorphologically, electron microscopically and immunohistochemically on microimage analyzers "Morphoquant", "Videotest" and in electron microscopes JEM-100b and Tesla-500 [2]. Comparative results of production inspections were assessed according to the most important fish-breeding biological, morphometric and morphophysiological indicators. The novelty of technical solutions for the reproduction of fish populations was determined by the method of formalized comparative analysis, generally accepted in patent and inventive work. The results of quantitative morphometry were processed by the methods of variation statistics using the Microsoft Excel software package.

Results And Discussion

Nonapeptidergic NSCs, like HHNS in general, have the highest degree of functional plasticity in the central nervous system, which is ensured by their ability to functionally reverse [17]. On this elementary (structured) biomodel, it was possible to establish that they are organized according to the structural and functional principle of the "Triad of the balanced system". It is composed of two alternative morpho-functional states - the accumulation and extrusion of neurosecretory products (or material and energy resources) and the self-regulation center that controls the dynamics of their relationships. That is why the degree of plasticity of the HHNS turns out to be sufficient for its participation in the integration of fish reproduction. The idea is expressed that the key links of biological integration systems are organized according to this general structural and functional principle [2, 17].

The results obtained and the analysis of the results of the ecological-histophysiological study of HHNS using the methods of quantitative morphometry of light, electron microscopy and immunocytochemistry are consistent with this concept (Fig. 1, 2a).

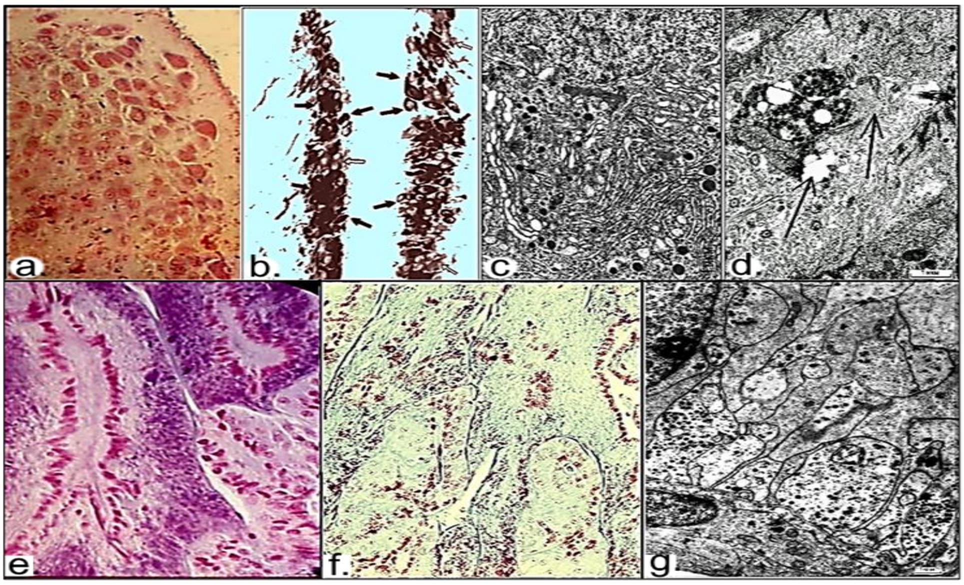

Fig 1: NSC in the preoptic nucleus (PN) of pink salmon, (a-c) and neurosecretory terminals in the neurohypophysis (NH) of the sturgeon (d-g) during spawning migration and spawning. a - functionally active light-colored NSCs predominate in the PN of pink salmon at the beginning of spawning migration; b - vasotocin-ergic NSCs are in massive contact with the cavity of the third ventricle of the brain (where neurohormones are excreted during migration and at the beginning of spawning from NH: f, g) (↑); c - mass formation of neurosecretory granules in the NSC Golgi complex reflects the active synthesis and transport of neurosecretory products; d - exit of a fine-grained neurosecretory product into the cavity of the pituitary bay of the sturgeon brain during the migration period; e - massive accumulation of neurosecretory material in the roots of sturgeon NG at the beginning of the spawning run; f, g - emptying of the NG from neurosecretory material, reflecting the activation of the sturgeon HHNS at the beginning and after spawning; a, b, e, f - light microscopy (approx. x10, volume x20), staining: paraldehyde-fuchsin (after Gomori-Gabe's method) and azan (after Heidenhain's method); b - vasotocin-ergic NSC, immunocytochemical reaction; c, d, g - electron microscopy.

The active synthesis of neurosecretory products in the PN of HHNS and their transport to the NH was established at the beginning of the spawning migrations of anadromous sturgeon and salmonids (Fig. 1 a-c). However, in NH, they are massively accumulated (Fig. 1e). Such a violation of the moderate activity of the HHNS, which provided a long-term adapted feeding (marine hypotonic) type of osmoregulation and is the main physiological stimulus for changing the habitat. At the same time, the excretion of neurosecretory products (containing nonapeptide neurohormones) into the cerebrospinal fluid of the third ventricle is observed from the bodies, dendrites of the NSC and neurosecretory terminals of the NH (Fig. 1 b, d). This causes their neurotropic effect in the behavioral centers of the CNS (limbic system of the brain) in the form of a dominant state of excitation - "Migration impulse".

The participation of HHNS in spawning was first established on the basis of observations of a strong depletion of spawned females (especially large-bodied fish species), clearly stressful in nature. The strongest activation of the HHNS was established at the beginning of spawning (V csr), followed by a decrease in its functional activity towards its end (Fig. 1 e-g; 2).

Such a reaction of the system corresponds to two phases of the course of stress (alarm and resistance) and reflects its participation in the protective-adaptive reactions of the body to natural physiological stress. Analysis of the results led to the conclusion that the functional role of HHNS in fish reproduction is to initiate the starting energy-consuming processes of migratory and spawning behavior and to complete spawning by suppressing the hyperactivity of the complex of visceral target organs, especially the reproductive system. The latter ensures the transition of the organism from the energetic type of metabolism to energy-saving plastic metabolism (in the form of “metabolic reversion”) [2, 17]. The final conclusion about the important key role of HHNS in the integration of fish reproduction (according to the

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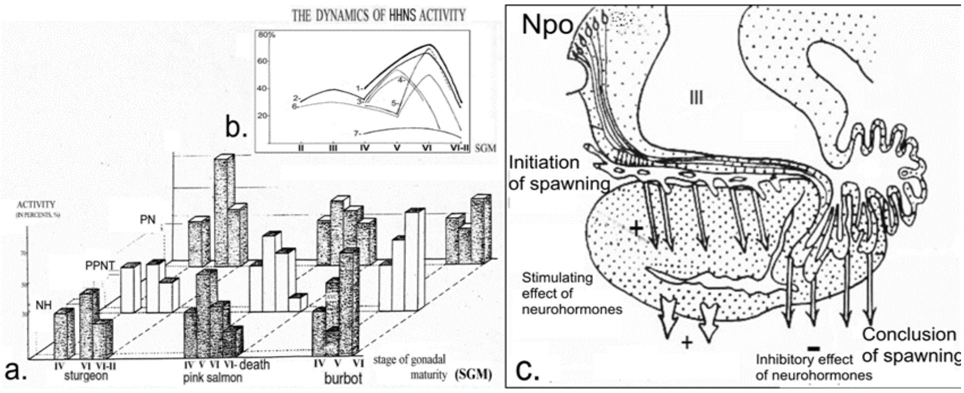

FIGURE 2. Changes in the functional activity of HHNS in commercial fish species with different spawning seasons and the basic principle of neuroendocrine integration of spawning. a. - activity of all parts of the HHNS at different stages of gonadal maturity (sgm, according to the morphometric results of complex light-optical and electron microscopic studies; b. - dynamics of changes in the functional activity of HHNS during spawning, proportional to the degree of stress intensity: 1 - beluga, 2 - sturgeon, 3 - pink salmon , 4 - chum salmon, 5 - burbot, 6 - sterlet, 7 - stellate sturgeon; c. - principle of HHNS participation in the integration of fish reproduction (NP-NH +: stimulating effect of nonapeptide neurohormones, NP-NH -: their inhibitory effect). Designations: PN - preoptic nucleus; (IV, V, VI - sgm); PPNT - preoptic-pituitary neurosecretory tract, NH – neurohypophysis.

principle of self-regulation) allows us to present a constructive working scheme (Fig. 2 c). And on its basis, to develop new methods for managing the biotechnology of fish hatchery reproduction in order to increase its efficiency. The methods consist in managing the timing, quality of reproduction and survival of breeders and broodstock, growth rates of juveniles and in effective biotechnology of reproduction of fish populations in general [2].

First of all, to accelerate the timing of offspring by stimulating the sexual maturation of breeders and increasing the degree of their fish farming use, a preparation of the isolated anterior pituitary gland was developed and used in industrial sturgeon breeding (Fig. 3 a,b: 1-3) [10].

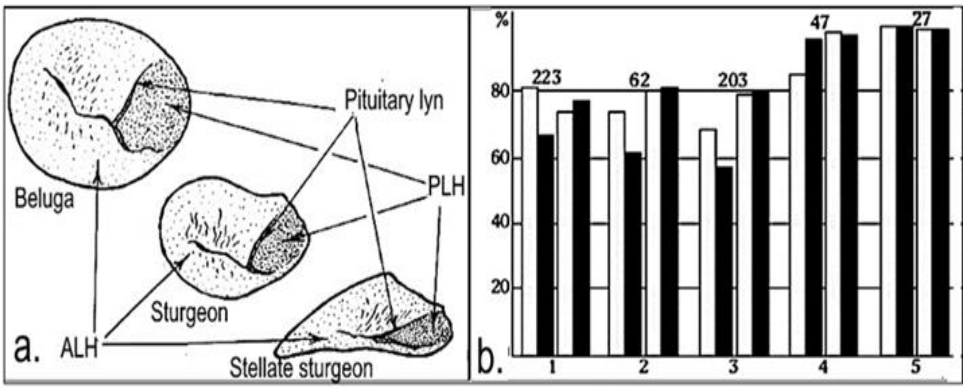

Fig 3: Division of the pituitary gland of sturgeons along the pituitary fissure (lyn) into anterior (glandular) and posterior (nervous) lobes of the pituitary gland (ALH, PLH) and a histogram of fish-breeding indicators of the effectiveness of their use: a. - structures of the pituitary gland of sturgeon fishes (on the medial section); b. - production trials of the effectiveness of the use of drugs for the isolated anterior lobe (IAL), whole pituitary gland (Hyp) and isolated posterior lobe (IPL). Series of experiments: 1. - comparison of the effectiveness of drugs IAL and Hyp. on females of spring sturgeon of the spring-passing, left pair of columns: percentage of fish breeding use: IAL (light columns), pituitary gland (Hyp, black, control), right pair of columns: % of prelarvae hatching; 2. - the same on females of winter sturgeon of the autumn-passing; 3. - the same on females of spring stellate sturgeon of the early spring-passing; 4. - efficiency of IPL and Hyp. on males of stellate sturgeon (left pair of columns: % of fish use, right pair: sperm activity); 5. - the same on male carp. Numbers above the columns: number of producers.

A method for stimulating maturity in male fish with an isolated posterior lobe of the pituitary gland (IPL) was developed for the same purpose, including the waste-free technology of using both drugs (Fig. 3 b: 4, 5) [13]. Long-term production tests of the effectiveness of the use of these preparations at sturgeon fish hatcheries in the lower Volga and Don rivers have shown an increase in the degree of fish breeding use of sturgeon producers by 15% on average and up to 40% savings in the biological material of the pituitary gland [2].

To extend the timing of offspring by delaying the breeders puberty, methods for their long-term industrial reservation in habitat of critical salinity 4–8 ‰ have been developed [11, 12]. Production checks have proven the highest survival rate, delayed puberty and the preservation of the high fish-breeding quality of broodstock in this environment during the production-required periods even at upper spawning temperatures (up to 260C). It is important that these effects have been established both in seawater and in solutions of table salt of the same concentration of

4-8 ‰, which is applicable for closed water supply systems (reciculating aquaculture systems, RAS) (Fig. 4).

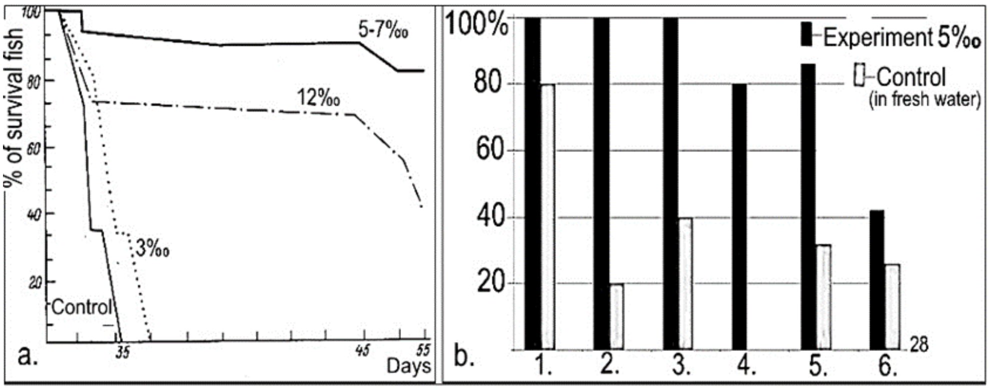

Fig 4: Fish-breeding biological effects of keeping fish producers in sodium chloride solutions: a. - survival of roach producers; b. - fish breeding quality of sevruga females: 1. - degree (%) of female survival, 2. - % of females in a state of physiological norm, 3. - % of maturation of females, 4. - % of fish breeding of females, 5. - % of fertilization of eggs, 6. - % of hatching of prelarvae.

With the aim of factory reproduction of fish populations with any spawning season, based on the above established effects, a biotechnology for controlling their reproduction was developed [7]. The main (ecological-physiological) management principle is to synchronize the factory breeding of various intrapopulation ecological forms (races, subpopulations, etc.) by reserving their producers in critical salinity, universal for different fish species, but under species-specific pre-spawning threshold conditions of "signal" factors: temperature and illumination (photoperiod). Subsequent production of offspring by stimulating the maturation of breeders (and further rearing of juveniles) is carried out by a smooth transition to a set of optimal habitat conditions (Fig. 5a).

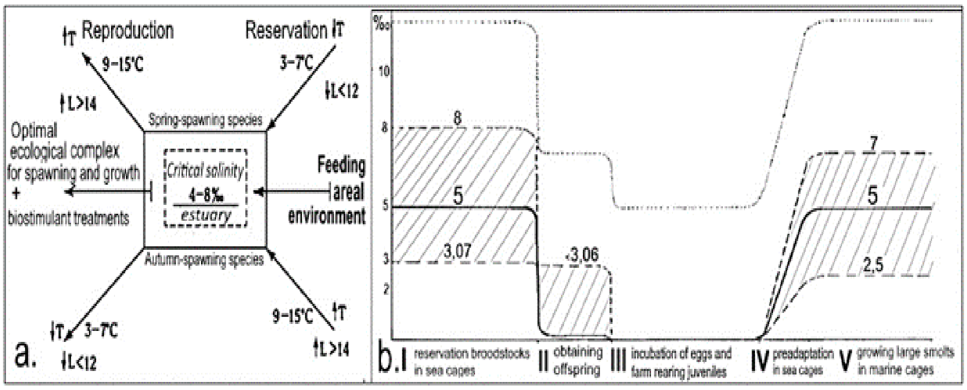

Fig 5: Basic biotechnological schemes for the reproduction of fish populations: a. - ecological and physiological principle of controlling the reproduction (and rearing) of anadromous fish by the triad of leading ecological factors: phylogenetic (‰) and signal (ToC, L - photoperiod) values, based on the leading mechanism of fish migration [7]; b. - dynamics of the change in salinity at all stages of reproduction biotechnology [8]. Legend: wide solid curve - optimal salinity regime, dashed curve: permissible salinity regimes (shaded sector - their range), dotted curve: expected upper (calculated) values.

However, all modern factory biotechnology of reproduction is based on the use of species phylogenetic adaptations of the river period of ontogenesis, when the degree of eurybionicity of an individual decreases as much as possible [1-3]. Therefore, on the basis of the additional (integrated) use of the systems of species phylogenetic adaptations of sea feeding, which ensure the highest productivity of populations, a new full-system method of their artificial reproduction has been developed (Fig. 5 b) [8]. By maximizing the manifestation and use of the adaptive species potencies of reproduction, survival and growth in a brackish marine environment of critical salinity, the method is aimed, first of all, at overcoming the main disadvantages of biotechnology for reproduction of salmonids - harvesting broodstock in spawning grounds to the detriment of natural reproduction and low survival rate in nature of hatchery one-year-old fry. [2, 4]. The method is carried out by means of mass harvesting of broodstock in marine fishing grounds, keeping them in cage in brackish sea water and obtaining offspring here (Fig. 5 b). The subsequent hatchery incubation of eggs in the river, the rearing of larvae and juveniles is carried out until signs of their readiness for migration (for example, salmon smoltification), after which the hatchery fry are grown in brackish seawater to a mass (from 40g), providing a survival rate of at least the normative - 2%.

As a result of long-term industrial tests of the method of breeding and rearing juveniles of Atlantic salmon from the Baltic population in critical salinity, fish breeding effects of increasing: 1) survival, 2) fish breeding quality of producers and 3) growth rates of juveniles were proved (Table 1).

Table 1: Comparative fish-biological results of rearing juvenile Baltic salmon at the Nevsky hatchery and in the sea cages of the Vyborg Bay.

A. Comparative characteristics of producers (annual average)

Indicators (Average values)	***General characteristics***		***out of them females***		***out of them males***	
	Nevsky hatchery	***Sea cages***	***Nevsky hatchery***	***Sea cages***	***Nevsky hatchery***	***Sea cages***
Number of separated fish	163	82	88	44	**75**	**32**
Body length to caudal peduncle (cm, limits)	74,9±0,71 (45-100)	71,6±0,2 (62,5-78,1)	82±0,53 (70-100)	74,3±0,25 (68,0-78,1)	66,1±0,9 (45-92)	63,2±0,04 (62,5-64,0)
Average mass	5,0±0,12 (0,9-10,6)	4,17±0,07 (1,5-5,7)	6,3±0,13 (3,2-10,6)	3,6±0,05 (3.1-5,1)	2,1±0,14 (0,9-8,6)	4,4±0,12 (1,5-5,7)
σ in length	9,166	2,6	5	1,683	7,833	0,25
σ by mass	1,616	0,7	1,233	0,333	1,283	0,7
Fulton's condition coefficient – Q (limits)	1,2 (0,8-3,02)	1,02 (0,6-1,4)	2,6 (2,3-3,02)	1,09 (0,9-1,4)	0,73 (0,98-1,10)	1,74 (0,61-2,17)
Fish use (% maturity)	84	92	82	95	96	97
Working fertility ♀ (ths. units)	-	-	4,7±0,03	2,4±0,1	**-**	**-**

B. Mass indices of juveniles of different age groups on the Nevsky hatchery, in the cages of the Vyborg Bay and according to the standards (g.)

	Nevsky hatchery	***cages of the Vyborg Bay***	***standards of the region***
underyearling 0+	11,3±1,84	15±1,07	5-7
one-year-old 1	26±3,23	160±7,35	9-18
two-year-old 1+	41,6±6,75	280,1±20,08	20-25

Comparative morphometric analysis showed that with the onset of smoltification, the development of one-year-old fry in marine cages is replaced by intensive growth, which corresponds to natural marine feeding [2]. However, it should be taken into account that the production interests (and the schedule) of the hatchery already from the first stage of the factory biotechnology are affected by the exclusion of the harvesting of producers (river fishing and during the spawning season) of salmon fish in the spawning grounds. Therefore, in order to apply compensatory measures as a feedback mechanism in this system of improved environmental management, we proposed to use a new development in the field of recreational aquaculture [14].

In order to expand the possibilities of using the method to off-season (year-round) fish farming in recirculating aquaculture systems (RAS) and based on the experience of two previous innovations [11, 12], the development of a new method of rearing fish fry in an artificially "modified biostimulating environment" was started [9]. Its essence lies in the rearing of juvenile fish in a solution of sodium chloride (its concentration is relatively close to the internal environment of the organism), in which an increase in its growth rate was first established (Fig. 6).

For industrial year-round use of the developed biotechnology, control of the fish growing environment and protection of products from external pollution, there were developed large-scale water supply systems (recycling aquaculture system: RAS) for fish hatcheries and farms, the device and principle of operation of which is based on underground (non-seasonal) conditioning of the growing environment for hydrobionts [5, 6]. They function on new natural and industrial principles: engineering ecology and

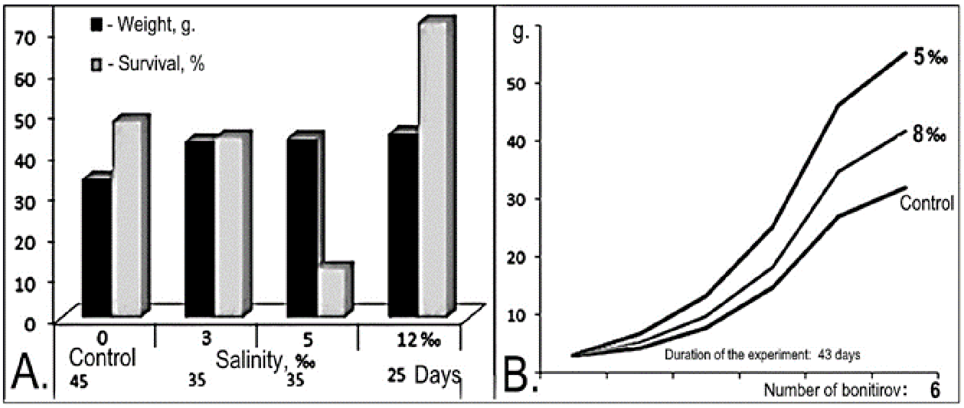

Fig 6: Indicators of body weight and survival of juvenile rainbow trout and catfish grown in solutions of sodium chloride of various concentrations and in control. **A**.- Indicators of trout under yearlings in 3, 5, 12 ‰ and in control (25 individuals in each group); **B**.- Dynamics of changes in the growth of under yearlings of the catfish in 5, 8 ‰ and in the control (150 individuals, 6 assessments, survival rate: 100% in each group.

biotechnology of reproduction management [2, 7]. Their essence lies in the fact that the water supply of ground-based fish-breeding tanks is additionally provided by systems of deep or semi-buried large-volume settling tanks in the ground, as reliable as possible due to the simplicity of the design (Fig. 7).

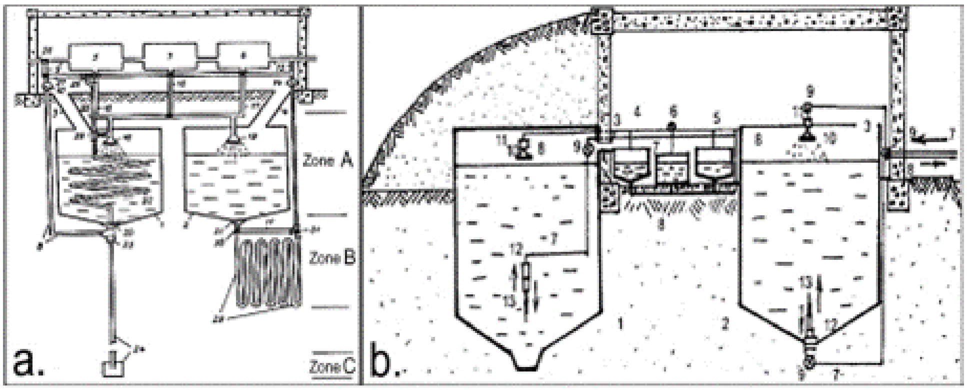

Fig 7: Water supply systems: **a**. - fish hatcheries and **b**. - fish farms of a combined type, with the aim of year-round joint reproduction of spring-spawning and autumn-spawning fish species. The systems contain underground reservoirs (1, 2) located below the layer of seasonal freezing (in zone A), each of which is connected with fish-breeding basins (5, 6) and with means of aeration and water purification.

Such fundamentally novel recirculating water systems make it possible to preserve and use natural seasonal hydropower resources all year round and for the first time to agree on alternative technical and economic volume-dependent problems: energy costs that need to reduce the volume of water in sedimentation tanks and its purification, which need to be increased. Technical and economic calculations proved that the gradient of heat transfer of water to the ground is <0.1oC/month is reliably provided with a reservoir volume already from 10.000 m3 and with its increase, this coefficient, as well as the specific cost of systems, proportionally decreases, and their productivity increases.

Conclusion

A full-system study of the mechanisms of neuroendocrine integration of fish reproduction has been carried out, as a result of which the basic principles and methods of management of reproduction biotechnology have been developed. To increase the efficiency of biotechnology for artificial reproduction of fish, methods have been developed to control the timing of offspring by stimulating and delaying sexual maturation of broodstock. By stimulating the maturation of sturgeon breeders, the effect of a (waste-free) increase in the degree of their fish farming use by 15% has been achieved. In seawater with a critical salinity of 4-8 ‰, the effects of long-term reservation of spawners, obtaining from them benign offspring, multiple acceleration of the growth of juvenile salmon, the highest (up to 7-fold) from one year of age, have been achieved. The indicated fish-breeding effects (but to a lesser extent) were also established when the fish were kept in solutions of table salt with an optimal concentration of 5 ‰, which is especially promising for use in RAS. The method of reproduction of fish populations, carried out by the multidirectional effect of a physiologically adequate complex (triad) of ecological factors, allows reproducing all elements of the intrapopulation structure by synchronizing the hatchery production of offspring from various ecological forms in a single fish-breeding season. The final full-system method of reproduction of populations of valuable fish species, covering all stages of factory biotechnology, opens up opportunities to stop the factory stocking of broodstock on spawning grounds, to increase the weight ("weight") and the volume of release of reared viable juveniles into nature. With the aim of year-round use of new biotechnology in aquaculture, large-scale systems of closed water supply to fish farms and farms have been developed based on the natural and industrial principles of engineering ecology.

All the presented developments, integrated into the control system for the reproduction of fish populations, are also proposed for saving the Ladoga population of the Atlantic sturgeon, an important task in the problem of preserving the biodiversity of natural resources of the North-West region [2 [. To securely obtain planting material, it is necessary to establish a sturgeon breeding farm in the Ladoga Lake basin, which is also proved by the successful experience of raising and releasing juveniles of the Atlantic sturgeon Acipenser oxyrhinchus (Mitchill, 1815) in Germany and Poland [18, 24]. We believe that on the basis of the interaction of nature conservation and fisheries cross-sectoral measures, this task can be successfully solved.

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