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## Contents

OXIDATIVE STRESS AND ITS PREVENTION AT RAISING YOUNG STURGEONS IN RECIRCULATING AQUACULTURE SYSTEMS .....	6
THE MAIN TRENDS IN THE DEVELOPMENT OF AQUACULTURE TECHNOLOGIES. WATER RECIRCULATION SYSTEM AS A BASIS FOR INDUSTRIAL COMPLEXES TO PRODUCE HYDROBIONTS.....	7
DETECTION OF TOXIN PRODUCING CYANOBACTERIA IN WATER RESERVOIRS OF BELARUS BY POLYMERASE CHAIN REACTION	9
EXTERNAL SEXSPECIFIC SIGNS IN THE STRUCTURE OF DERIVATIVES OF STERLET CORIUM .....	11
SURVIVAL OF EMBRYOS AND LARVAE OF THE RAINBOW TROUT UNDER INFLUENCE OF OPTICAL RADIATION AT VARIOUS TEMPERATURE REGIMES .....	13
COMPREHENSIVE IMPACT OF RIVER WATER QUALITY INDICATORS ON FISH HOMEOSTASIS CHARACTERISTICS .....	14
POSSIBILITY OF <i>ACUTODESMUS DIMORPHUS</i> (TURPIN) TSARENKO USING AS A FEED ORGANISM IN <i>DAPHNIA</i> CULTIVATION .....	16
THE OXIDATIVE PROCESSES UNDER TEMPERATURE CHANGES IN JUVENILE FISH COMMON ROACH ( <i>RUTILUS RUTILUS</i> L.).....	18
SPECIFIC FEATURES OF FISH FARMING EXPLOITATION OF THE INTEGRATED SYSTEM “POND – RAS – BASIN – POND” .....	19
SPERM CRYOPRESERVATION AS A TOOL FOR CONSERVATION AND SELECTION IN AQUACULTURE .....	23
NUTRIENT COMPOSITION AND HYDROLYTIC ACTIVITY OF ARTEMIA NAUPLII SATURATED WITH $\omega$ -3 FATTY ACIDS.....	24
IMPACT OF $\omega$ -3 PUFA BIOENCAPSULATION TECHNOLOGY ON THE GROWTH AND SURVIVAL RATE OF <i>ARTEMIA</i> NAUPLII .....	26
COLLABORATION ON ATLANTIC STURGEON RESTORATION IN THE NEMUNAS RIVER BASIN .....	28
A TECHNOLOGICAL SOLUTION FOR THE REMOVAL OF PHOSPHORUS COMPOUNDS FROM THE CIRCULATING WATER OF RECIRCULATING AQUACULTURE SYSTEMS .....	30

EXPERIENCE OF CULTIVATION OF AUSTRALIAN CRAYFISH (CHERAX QUADRICARINATUS) IN THE CONDITIONS OF THE SOUTH OF UKRAINE.....	31
USE OF RECIRCULATING AQUACULTURE SYSTEM IN SALMON AND SEA TROUT RESTOCKING IN LITHUANIA.....	33
ASSESSMENT OF VITAL CAPACITY AND ECOLOGICAL FUNCTIONS OF ALIEN HYDROBIONTS OF WATERS IN-STEPPE UKRAINE .....	34
ESTIMATION OF AQUACULTURE PRODUCTION OF UKRAINE ...	36
FRACTIONATION OF SUSPENDED PARTICLES AS A WAY TO STUDY THE DISTRIBUTION OF HEAVY METALS BETWEEN THEIR CHEMICAL FORMS IN WATER.....	38
PHYTOREMEDIATION AS PERSPECTIVE ENVIRONMENTAL TECHNOLOGY FOR CONTROL OF HUMAN IMPACT ON AQUATIC ECOSYSTEMS .....	40
FIRST RESULTS OF WWF PROJECT «LIFE FOR DANUBE STURGEONS» IN UKRAINE.....	41
EXPERIENCE OF USING GASTROPODS FOR THE TRANSFORMATION OF ORGANIC POLLUTANTS OF RECIRCULATING AQUACULTURE SYSTEMS .....	43
RECREATIONAL FISHING IN UKRAINE AND ITS ECONOMIC AND ENVIRONMENTAL VALUES IN REGIONAL SUSTAINABLE DEVELOPMENT .....	44
ACCLIMATIZATION AND PROSPECTS OF CULTIVATION SHRIMP <i>MACROBRANCHIUM NIPPONENSE</i> IN THE WATERS OF UKRAINE	46
A NEW STRATEGY OF A PASTURE MARICULTURE IN THE LIMANS OF THE NORTH-WESTERN BLACK SEA REGION .....	48
EXPERIENCE IN GROWING OF PIKE-PERCH ( <i>SANDER LUCIOPERCA</i> L.) LARVAE ON LIVE FOOD TO VIABLE STAGES...	50
EXPERT ESTIMATION OF PRODUCTION CHARACTERISTICS OF ECOSYSTEMS OF SMALL LAKES WITH THE HELP OF REGRESSION MODELS.....	51
THE COMPACT RECIRCULATING AQUACULTURE SYSTEMS (RAS) FOR FISH FARMING .....	53

QUICK-TEST METHOD FOR EVALUATING PHYSIOLOGICAL STATE OF STURGEONS UNDER THE AQUACULTURE CONDITIONS .....	54
PROSPECTS OF USING THE RECIRCULATING AQUACULTURE SYSTEMS (RAS) IN THE AQUACULTURE OF EGYPT .....	56
SPECIFIC FEATURES OF BREEDING STERLETS TO MATURE STATE IN THE RECIRCULATING AQUACULTURE SYSTEM .....	58
CURRENT STATE OF COMMERCIAL FISH FAUNA OF THE URAL RIVER WITHIN THE BOUNDARIES OF THE WEST KAZAKHSTAN REGION .....	60
THE DYNAMICS OF GENETIC STRUCTURE OF ROUN GOBY <i>NEOGOBIOUS MELANOSTOMUS</i> (PALLAS) GROUPINGS IN THE ODESSA BAY OG THE BLACK SEA UTILIZING BIOCHEMICAL MARKER LOCI .....	62
CONSERVATION HERPETOCULTURE OF <i>BOMBINA BOMBINA</i> IN RECIRCULATING AQUACULTURE SYSTEM (RAS) IN LATVIA: LARVAE KEEPING TECHNOLOGY .....	63
CONSERVATION HERPETOCULTURE OF <i>EMYS ORBICULARIS</i> IN RECIRCULATING AQUACULTURE SYSTEM (RAS) IN LATVIA: KEEPING TECHNOLOGY IN FIRST YEAR AFTER HATCHLING ....	66
RECIRCULATING AQUACULTURE SYSTEMS (RAS) IN SMALL ZOO EXHIBITION: APPROACH OF NATURE-FRIENDLY LATGALES ZOO, DAUGAVPILS, LATVIA .....	68
VETERINARY MEDICATION ANTHELMINTIC OF ACTION "DIPLOCIDUM" FOR FIGHT AGAINST DIPLOSTOMUM OF FISHS	70
REARING OF LARVAE OF COMMON CARP <i>CYPRINUS CARPIO</i> ON DIFFERENT DIETS IN CLOSED RECIRCULATORY SYSTEM ON DIFFERENT FEEDING REGIMES .....	72
ROLE OF EPIGENETICS IN AQUACULTEURE .....	73

## **OXIDATIVE STRESS AND ITS PREVENTION AT RAISING YOUNG STURGEONS IN RECIRCULATING AQUACULTURE SYSTEMS**

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Improvement of existing and development of new fish-breeding recirculation systems is a very urgent problem, as it allows moving to a more reliable and less vulnerable production of fish. At present, there is industrial equipment for the cultivation of hydrobionts both in our country and abroad that uses different methods for the purification of circulating water: mechanical, adsorption, and biological. In existing installations of closed water supply (RAS), even a combination of all cleaning methods often does not provide them with a stable and optimal hydrochemical regime. The main reason for this is the accumulation in the system of inorganic nitrogen, including ammonium ions. This is aggravated by high stocking densities, feeding by concentrated mixed feeds and the accumulation of fish's own metabolites, which are the main stress factors in raising fish.

As a result of studies of the influence of stress factors on juvenile sturgeons, there was revealed a change in the direction of bioenergetic and biosynthetic processes related to the degradation of free radical oxidation in the body, promoting the activation of lipid peroxidation (LPO) processes.

According to our studies, activation of LPO is accompanied by a decrease in the intensity of food intake, growth rate, fatness, violation of the enzyme transport conveyor and related diseases.

Thus, when lipid peroxidation processes are activated in young sturgeon, an increase in the number of hydroperoxides in the body of fish by more than 50% is observed with a simultaneous decrease in the activity of antioxidants (superoxide dismutase - SOD,  $\alpha$ -tocopherol, vitamins A and C) by more than 20%. The low content of natural

antioxidants in the body can be the reason of the imbalanced processes of free radical oxidation, which in its turn, disrupts the processes of cellular and tissue respiration. As a consequence, the balance of activity between succinate dehydrogenase and lactate dehydrogenase changes, the ratio between which decreases by a factor of 1.5-1.7. As a result, the reactions of glycolysis and gluconeogenesis, and the synthesis of fatty acids are violated, which is confirmed by our data. Thus, when the LPO activity increases, in the juvenile we observe a decrease in the content of fatty acids, especially of polyunsaturated  $\omega$ 3 series, such as C18: 3, C20: 5, C22: 5 and C22: 6, the level of which decreases from 1.5 to 5 times. Moreover, the ratio between membrane and reserved lipids changes towards reduction of membrane lipids, which characterizes the involvement of the latter in energy metabolism, that is, unproductive energy costs. These changes give evidence of some metabolic disorders with a predominance of destruction over the synthesis of structural lipids, an imbalance between the rate of utilization of fatty acids and antioxidants from feeds and the rate of their mobilization to ensure the growth and health of fish.

To restore the disturbed balance in metabolic processes of young sturgeon, it is necessary to enrich mixed feeds with lipids with an increased content of polyunsaturated fatty acids of the linolenic series and  $\beta$ -carotene as an active antioxidant and a precursor of vitamin A. Such an approach to feeding contributed to the improvement of physiological status of young sturgeon, which was confirmed during cultivation in RAS and flowing-water tanks.

## **THE MAIN TRENDS IN THE DEVELOPMENT OF AQUACULTURE TECHNOLOGIES. WATER RECIRCULATION SYSTEM AS A BASIS FOR INDUSTRIAL COMPLEXES TO PRODUCE HYDROBIONTS**

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25 years of experience in creating industrial complexes to produce hydrobionts.

1. Analysis of the experience of creating industrial manufacturing in the field of aquaculture, diverse types of constructions and intensification levels of production.

2. The main trends in the development of aquaculture technologies. Types of system constructions for production complexes.

3. Comparative efficiency of productions with diverse types of structures, intensification levels and their combination.

4. New technological solutions used to create industrial complexes for production of hydrobionts.

5. The main distinctive construction features, when designing modern enterprises based on recycling water supply systems:

5.1. Assembly of production equipment.

5.2. Technical and biological safety of industrial production complex.

5.3. The modular water purification complex from metabolic products of hydrobionts (water regeneration).

5.3.1. Mechanical filtration, separation.

5.3.2. Biological purification of water from metabolic products of hydrobionts. Efficiency of biofilters. Regulated bioreactors. Innovative technologies in the development of phytofilters.

5.3.3. Biofilter filler, its efficiency. Hydrodynamic features of the bioreactor filler elements.

5.3.4. UV disinfection system.

5.3.5. Systems of water saturation with oxygen without excess pressure. Combination of diverse types of oxygenation (oxygen dispersion in water, water spraying in oxygen) in one oxygenation system construction with no-pressure.

6. Adaptation of new species of hydrobionts to industrial technological complexes.



7. In the report proposed further scientific researches in accordance with the main trends in the development of aquaculture technologies. Research programs are proposed by an international group of scientists under the auspices of NACEE. These studies were previously conducted by a group of researchers on the base of following enterprises: Экоресурс, Биос (Astrakhan, Russia), Silkraсти, Mottra, Hibitech Piebalga, SIA Zinātniskas pētniecības centrs Hidrobioteh, Daugavpils University (Latvia).

## **DETECTION OF TOXIN PRODUCING CYANOBACTERIA IN WATER RESERVOIRS OF BELARUS BY POLYMERASE CHAIN REACTION**

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Cyanobacteria appear to be an integral component of aquatic ecosystems worldwide and in Belarus in particular. Among the large number of secondary metabolites synthesized by cyanobacteria, toxins are of particular interest. During the algal nuisance in reservoirs a significant amount of produced microcystins with hepatotoxic activity shows danger to the health of animals and humans. That fact makes the revealing of potential toxin producing cyanobacteria in water an important task. In that case using the molecular genetic methods provided a powerful tool for detection [1].

The aim of present work was to perform PCR screening of water samples to identify the aminotransferase domain sequence of *mcyE* gene which is involved in the non-ribosomal synthesis of microcystin and nodularine in cyanobacteria of *Microcystis*, *Anabaena*, *Planktothrix*, *Nodularia* and *Nostoc* genera [2].

Water samples were collected from natural water reservoirs and fish farm ponds during 2012-2016 years. Total DNA was isolated from the samples and was amplified with specific primers as previously been described [3]. We analyzed 3 groups of samples, according to the year of collection - 2012-2013, 2014-2015, respectively. Each group

contains samples from 24 different water reservoirs: ponds of fish farms "Selets", "Izobelino", "Krasnaya Sloboda", "Beloe", "Vilejka", "Luban", "Novinki", "Krasnaya Zorka", "Shemetovo", lakes Bolshie Shvakshty, Svir, reservoirs Drozdi, Zaslavskoe and some other.

PCR approach was applied successfully. It allow to detect potentially toxic cyanobacteria in water samples. Positive samples have been revealed in several fish farm ponds and natural reservoirs. 50% of ponds were positive in 2012-2013. 31% and 61% in 2014-2015 and 2016, respectively. No correlation between geographical distribution of positive ponds and climatic conditions of the region was revealed. Also no year to year tendency was shown. It should be mentioned that using of PCR gives us qualitative results and allow to reveal the presence of potentially toxic cyanobacteria. For objective toxicological assessment of water quality and health risk estimation quantitative analytical research should be carried out. In that case methods of HPLC are perspective. Using of PCR gives us fast tool for preliminary identification of potentially dangerous areas and should be carried on initial steps of research.

This study was partially supported by the Belarus Republican Foundation for Fundamental Research.

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# EXTERNAL SEXSPECIFIC SIGNS IN THE STRUCTURE OF DERIVATIVES OF STERLET CORIUM

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We found for the first time that the spinal scutes of the sterlet *Acipenser ruthenus* have structural features related to the sex, which makes it possible to develop a method for the lifelong identification of sex of sterlet and other sturgeon.

The aim of the work is to study the morphological structure of sterlet dorsal scutes of different ages and to reveal the dependence of their structure on the sex.

The research was carried out from 2012 to 2016 at the Department of Ichthyology and Pisciculture and ‘fish farm complex’ of the Belarusian State Agricultural Academy, the Vasilek farm (Minsk region) and the fish farm “Selets” (Brest region). In the studies, the age-old sterlet of the Volga population was used: adult specimens (age 3 years, average length  $61.2 \pm 1.3$  cm); Young (1 year old, average length  $24.8 \pm 1.5$  cm), larva (age 3 months, average length  $70.3 \pm 3.6$  mm). There were no statistically significant differences between the lengths of the studied sterlet.

For the study of adult sterlet, specimens with gonads were selected in the second stage of maturity according to Trusov's classification.

Observation of sterlet was carried out from three months of age, with further supervision at the age of 1 year and with confirmation of sex at the age of 2 years. Observed individuals were labeled individually from the age of 3 months which allowed later, after confirming the sex in 2 years, to establish sex at the age of 3 months and 1 year.

To determine the sex of sterlet, the ultrasound-diagnostic method was used on the portable veterinary scanner MindrayDP-6600, followed by a visual check of the gonads in the killed individuals. The killing of fish was carried out in accordance with the principles of humane treatment of animals. All the mortified specimens, a layer of dorsal scutes was cut from the head to the dorsal fin. After cutting, the dorsal scutes were cooked, cleaned, washed and photographed on a Canon

EOS 500D camera in macro mode. The resulting images were measured in the ImageJ program.

Our results show that in the males of all ages (adult individuals, juveniles, and larvae) the general poly specific regularities in the morphological structure of the dorsal scutes (mainly on the first five scutes) remain: in comparison with the females, the dorsal scutes of males are more elongated in width, in relation to the length. Due to the smaller size of the blades, the dorsal scutes plates in males appear more flattened and narrower than in females. Based on the results of the filling factor, we observed that the dorsal scutes in females look more rounded or oval than in males. The bright distinctive feature of all male scutes examined is the presence of longer, thin and pointed teeth, which are distinguished from the width of the scutes, and their number is larger than in females.

We have established for the first time that the dorsal scutes of the sexually mature sterlet have significant morphological differences, depending on the sex. To assess the morphological structure of dorsal scutes, it is proposed to determine two groups of indices characterizing the shape of the plate, as well as the structure of their teeth. In male sterlets the spinal bugs are more elongated in width, have a more flattened shape and also have longer and more pointed teeth, the number of which is larger than in females. Based on the analysis of spinal scutes by grouping (qualitative) criteria, we developed an expanded and then optimized sex determination system. In the final version, within the framework of an optimized flooring system, it is suggested to visually assess the spine scutes by their shape as well as the sharpness of the teeth, with the enrollment of the corresponding scores.

# **SURVIVAL OF EMBRYOS AND LARVAE OF THE RAINBOW TROUT UNDER INFLUENCE OF OPTICAL RADIATION AT VARIOUS TEMPERATURE REGIMES**

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As our long-term studies have shown laser radiation as well as the radiation of light-emitting diode (LED), has a stimulating effect on fish and their sexual products (caviar and sperm), as well as on the development of *Artemia salina*. However, our studies were based on optical radiation on bio objects within the same temperature. The question is raised about the most favorable temperature regimes, in which the maximum effect of optical radiation on aquaculture objects is manifested.

The purpose of our research is the effect of optical radiation on the embryonic and postembryonic development of rainbow trout under *in vitro* conditions of different temperature regimes.

The object of research were unisex embryos (fertilized eggs at the eye) of female rainbow trout, which during the study passed to the stage of a free embryo and then to the stage of exogenous nutrition. Source of optical radiation is a semiconductor laser (LD) of the phototherapeutic device "Lotos" (red spectral region  $\lambda = 650$  nm) and a matrix of LED sources of the "Strong" optical device (red spectral range  $\lambda = 630 \pm 10$  nm).

Effects of radiation on embryos were carried out for 5 days for 20 minutes at a power density of  $3.0 \text{ mW} / \text{cm}^2$ . Five so-called "Temperature" study groups including control and test groups (LD and LED) in triplicate for each temperature: 8, 9, 10, 11, 12 °C was formed. For the statistical processing of the results, the software environment R was used, including the packages R Commander, PMCMR, MASS, etc.

As a result of the study of the ten-day dynamics of the average survival rate during the experiment, when constructing the logit regression line with regard to the slope coefficient for each study group, we observed significant differences in the study groups. The best results were

obtained with a water temperature of 8 ° C. So the slope coefficient in the control group was 3.04, whereas in the experimental groups (LED, LD) it was 5.11 and 4.78, respectively. Thus, in the groups studied, the rate of increase of the effect was higher, as evidenced by the steeper logit regression lines. It should be noted that LD50 values in the test groups were also higher than in the control group. As shown by the deviance analysis, the established differences were reliable. We have established that optical radiation has a stimulating effect on the lifespan of 2/3 of embryos and larvae of rainbow trout under study in the absence of feeding at a temperature of 10, 9, and 8 ° C. For a more detailed study of this survival factor, we studied the individual lifetime of larvae and embryos during the experiment with the construction of the Kaplan-Mayer curves and using the Weibull regression. As the results showed, the optical emission of the red region of the spectrum has a significant effect on the individual lifetime of embryos and larvae of rainbow trout in vitro in the absence of feeding. The best results were obtained at a temperature of 8 ° C.

As studies have shown, the temperature regime of growing aquaculture objects, even in the ranges of optimal values, is able to exert an effect on the magnitude of the stimulating effect of optical radiation. The obtained results create prospects for more effective use of low-intensity optical radiation in aquaculture technology of valuable fish species.

## **COMPREHENSIVE IMPACT OF RIVER WATER QUALITY INDICATORS ON FISH HOMEOSTASIS CHARACTERISTICS**

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The aim of our research was to analyse the impact of the set of water quality indicators of the rivers of Rivne Oblast (Ukraine) upon the fish homeostasis, in order to determine hydro-ecosystem response to the impact of human activity. Therefore, the paper envisaged to use a

combination of representative and relatively simple (rapid) methods that are able to reflect the effects of the combined effects of hydro-ecosystems' pollution and give integrated response regarding their health.

Thus, the main reasons that contribute to the water quality deterioration in regional rivers include the flow of inadequately treated wastewater, as well as significant levels of anthropogenic load due to the urbanization of the fishing area adjacent to the channel.

The analysis of water quality and fish homeostasis in rivers of Rivne Oblast was conducted during the low summer flows in 2013-2015 in representative control sites. Samples of the following most popular regional river fish species were obtained from the control catches in representative control sites located in the rivers of Rivne Oblast, in particular: common bleak (*Alburnus alburnus* (Linnaeus, 1758)); common rudd (*Scardinius erythrophthalmus* (Linnaeus, 1758)); common roach (*Rutilus rutilus* (Linnaeus, 1758)); silver Prussian carp or Gibel carp (*Carassius auratus gibelio* (Linnaeus, 1758)); common bream (*Abramis brama* (Linnaeus, 1758)); European perch (*Perca fluviatilis* (Linnaeus, 1758)). The sample size for each species was from 19 to 31 fish. The assessment of morphological fish homeostasis was performed by levels of fluctuating asymmetry (FA) of meristic bilateral symptoms. The authors assessed cytogenetic homeostasis by erythrocyte micronucleus test with peripheral blood.

The most relevant finding of the present study is that the research and analysis of the impact of river water quality parameters in Rivne Oblast on the homeostasis characteristics of fish fauna representatives was conducted for the first time. The results of the research lead to the following conclusions:

1. Different representatives of fish fauna have different sensitivity to environmental conditions. In particular, such fish species as common roach and common bleak have demonstrated fish morphological violations (within the rate of IV points of the body developmental instability); in the present study, we have recorded the developmental instability of European perch and common rudd at the level of mainly III points, common bream indicated II points, and Prussian carp showed I point respectively. The

cytogenetic violations were recorded for such species as roach, bleak and perch; excess levels of spontaneous mutagenesis were ranging from 1.1 to 1.7 times.

2. Hence, the morphological homeostasis violations were recorded in four control sites, the cytogenetic homeostasis violations – in seven sites. This confirmed unfavourable environmental conditions of the water environment in the corresponding areas of the studied hydro-ecosystems at different ontogeny stages of the analysed fish species.
3. The multivariate regression analysis of the impact of water quality on fish homeostasis confirmed that different species have a complex and multifactorial process of morphological and cytogenetic homeostasis' formation. Nevertheless, the obtained regression dependence evidenced decisive influence of such factors as oxygen regime of water environment (COD, BOD<sub>5</sub>, O<sub>2</sub>), pollutants (Cu<sup>2+</sup>, Zn<sup>2+</sup>, Mn<sup>2+</sup>) and biogenic substances (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, PO<sub>4</sub><sup>-</sup>) on the formation of fish homeostasis.

Finally, as an outcome of the research we obtained prognostic forms of the relationship between water quality indicators and fish homeostasis that may become a basis of environmental assessment method in which fish characteristics are practically used to assess hydro-ecosystems' health.

## **POSSIBILITY OF *ACUTODESMUS DIMORPHUS* (TURPIN) TSARENKO USING AS A FEED ORGANISM IN *DAPHNIA* CULTIVATION**

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An artificial breeding of hydrobionts is the main way to obtain the natural feed for fishes on their different developmental stages. Freshwater crustacean *Daphnia magna* is a widespread object in aquaculture and its juveniles are usually consumed by fish larvae. The



mature *Daphnia* individuals are the most valuable for young-of-the-year (YOY) fishes and their older groups. A main problem in zooplankton cultivation is a necessity of constant feed supplying as well as an election of appropriate feeding substrate. Due to the small sizes and sufficiently high content of amino acids, proteins, polyunsaturated fatty acids and carotenoids a biomass of *Acutodesmus dimorphus* serves as an alternative valuable feed in *Cladocera* breeding. Using of co-cultivation technology in the system of *Daphnia* and the chosen microalgae would allow the avoiding of problem of constant feed supplying for zooplankton during its breeding. We were trying to create a scheme of co-cultivation where *Daphnia* and feeding microalgae would be coexist at equal parts in the same cultivation system. It's necessary to take into account a lot of factors as well as a content of an optimal cultivation medium. A chosen medium should fulfill all the needs of microalgae and *Cladocera* individuals. The possibility to cultivate both investigated species on the waste water from recirculating aquaculture system (RAS) was shown. It has been found that a usage of such water while crustaceans cultivation allows not only to delay growth deceleration but also to rise the quantity and biomass of zooplankton. For microalgae this medium serves as a source of all important elements and helps to get an actively growing productive culture.

The sequence of inputting both cultivation objects into a system is an important factor in co-cultivation technology. The productive indicators of zooplankton culture will be determined by amount and availability of feeding substrate for crustaceans. It was approved three schemes of co-cultivation that depended by to the date of zooplankton inputting into cultivation system. It was observed quite rapid adaptation of *Daphnia* organisms from each group and their active feeding with available microalgae after system had been inhabited. Keeping of the balance between the amount of grown microalgae biomass and the consumption rate of *Daphnia* while eating them out was our goal on this stage. The delay of crustacean's culture inputting into the cultivation system leads to intensive growth of *A. dimorphus*. Under these conditions the number of *Daphnia* organisms was less than in comparison to the simultaneous inhabiting. It was noticed that the later inputting of *Daphnia*'s culture into the cultivation system is characterized by its slower breeding. It is obviously that the intensive

development of microalgae slows the growth of zooplankton. The qualitative changes in culture medium and the massive accumulation of microalgae biomass serve as explanation. The largest number of *D. magna* individuals was obtained during simultaneous inputting of zoo- and phytoplankton into cultivation system. It's definitely a result of permanent accessibility of feeding substrate for *Daphnia* organisms during co-cultivation with microalgae.

Hence, the scheme of simultaneous inputting of phyto- and zooplankton into the culture medium was found the most effective. The largest number of *Daphnia* individuals was received, and the optimal growth rate of phyto- and zooplankton during the whole cultivating period was managed under the above described conditions.

## **THE OXIDATIVE PROCESSES UNDER TEMPERATURE CHANGES IN JUVENILE FISH COMMON ROACH (*RUTILUS RUTILUS* L.)**

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To determine the limits of climatic factors impact on typical hydrobiont organism the oxidative process in muscle of roach (*Rutilus rutilus*) was studied. Roach *Rutilus rutilus* is one of the dominant fish species in Latvian freshwater reservoirs. Roach is successful generalist fish in Central European fresh water habitats (Schiemer & Wieser, 1992) and therefore a well studied species. This allows to meet the high information requirements of individual based models (Hölker & Breckling, 2001). The aim of this study was to investigate the effect of temperature on oxidative process stress in one of local more frequent fish Common roach, *Rutilus rutilus* (Linnaeus, 1758). All aerobic organisms must „fight” with reactive oxygen species (ROS). ROS are chemically reactive molecules containing oxygen, which forms in normal metabolism of oxygen and have important roles in

cell signaling (Vinagre *et al.*, 2012). ROS levels can dramatically increase due to environmental stress (e.g. ultra-violet radiation, or heat exposure). The process when the production and accumulation of ROS is higher than organism's ability to deal with reactive oxygen species is called oxidative stress. This can damage lipids, proteins and DNA. Lipid peroxidation disrupts biological membranes and subcellular organelles, crosslinks proteins or enzymes and produces alkyl free radicals which display medium reactive activity towards inducing mutations (Chen *et al.*, 2016).

Juvenile *R. Rutilus* were obtained from local hatchery and kept in recirculating water systems consisting of 3 tanks, 20 fish per tank. Temperature was altered at a rate of 1 °C every 2 h until the experimental temperatures of 20 °C, 25 °C, 30 °C were attained. At  $t=0$  days,  $t=7$  days,  $t=14$  days five fish from each tank were sampled, frozen and stored at -20 °C to await further analysis. To determinate lipid peroxidation level was used the process major secondary oxidation product malondialdehyde (MDA) concentration. MDA concentration was detected spectrophotometrically in reaction with thiobarbituric acid; absorbance was read at 532 and 600 nm. To determinate catalase activity was applied spectrophotometric method consisting of measuring the absorbance at 240 nm (Aebi, 1983) in a time interval of 1 min 30 s every 15s. The reaction can be followed by a decrease in absorbance as the peroxide is turned into oxygen and water. The obtained results about oxidative processes under temperature changes in juvenile fish Common roach will be discussed.

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## **SPECIFIC FEATURES OF FISH FARMING EXPLOITATION OF THE INTEGRATED SYSTEM “POND – RAS – BASIN – POND”**

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Current economic realities stimulate the search for new technological schemes and organizational solutions for the optimization of fish farming in water bodies of various types. In this connection, there has been developed and put into operation an integrated system “pond – recirculating aquaculture system – basin – pond” based on the water areas of “Rybatskyi khutor” LLC (Kherson region, Ukraine). The system allowed changing the strategy of the fish-breeding enterprise, creating new jobs, providing rational exploitation of available water resources, expanding the range of fish production, and introducing additional types of services.

“Rybatskyi khutor” LLC exploits two ravine-type ponds of long-term regulation (upper and lower), with an area of 4 and 16 hectares, respectively. The main source of water supply for the ponds is spring water, which conditions the corresponding quality of water and fish production.

The upper pond is used for recreational purposes and amateur fishing, which required equipping appropriate recreational areas and bridges for fishing. The species composition and density of cultivated fish are formed answering the needs of amateur fishing (golden carp, carp, pike, perch, European catfish) and biomelioration (Chinese carp, silver carp, hybrid carps). The lower pond is mainly used for pasture aquaculture (commercial fish production from 540 to 780 kg / ha), and partly to provide amateur and sport fishing services.

A hotel, fish restaurant *Rybatskyi khutor* and a fish-breeding shop are equipped and functioning at the production site located between the upper and lower ponds and adjacent to the highways Kherson - Dnepr and Kherson - Mariupol. The fish-breeding shop includes a basin facility with three 400 m<sup>2</sup> reinforced concrete pools with an average depth of 1 m and running water supply from the upper pond providing water exchange every 5 days. The infrastructure of the fish-breeding shop is supplemented with a recirculating aquaculture system including 4 plastic tanks with a volume of 5.5 m<sup>3</sup> each and 4 tanks of 2 m<sup>3</sup> each.

The recirculating aquaculture system is used to rear and grow out the stocking material of rainbow trout (*Parasalmo mykiss* Walb.), sterlet (*Acipenser ruthenus* L.), European catfish (*Silurus glanis* L.), pike

perch (*Sander lucioperca* L.). In 2016, the complex was involved in the cultivation of *Clarias gariepinus* juveniles, resulting in the production of the stocking material with an average weight of 105.2 g (survival rate of 78%, feed conversion ratio of 1.5), which was used for further commercial cultivation.

The basin facility is used for commercial cultivation of rainbow trout and starlet; in 2016 it reared air-breathing catfish that reached a weight of 940 g (survival rate of 82%, feed conversion ratio of 3.0) within 120 days (from May 20 to September 20) under natural thermal conditions and feeding with frozen fish of low value (sprat, atherine, small goby).

It should be noted that the company does not incur significant water treatment costs, since the water, used in the recirculating aquaculture system and basins, saturated with fish metabolism products and feed residues, is discharged into the lower pond, thereby stimulating the development of food supply, which is important for pasture aquaculture.

## **PROSPECTS OF ORGANIC AGRICULTURE AS “NICHE” DOMESTIC PRODUCTS**

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As part of the implementation of the Association Agreement between Ukraine and the EU and the reforms realization, Ukrainian domestic products competitiveness is rising, while technical barriers in trade are gradually decreasing and Ukrainian products are entering the markets of the European Union and other developed countries. Agricultural products are one of the main export goods. It should be noted that European consumers who care for their health and environment protection prefer organic products.

Organic product output in Ukraine has grown twice over the past five years and according to the experts' estimates it is going to increase

several times more. The outlook seems quite promising: in Europe which is currently considered the main market for Ukrainian products, the volume of organic products consumption is rising annually by 10% due to the growing interest of customers, raising consumer awareness of the organic products benefits and the emergence of a significant number of the organic sector active representatives. For example, in 2016 Ukraine exported 165 000 tons of organic products, which is 2.5 times more than in 2015 (€40 000 000 against €21 000 000, respectively).

Today a new notion of “niche products” has appeared, these are the products which can fill the “niche” on foreign markets. According to experts, organic aquaculture products can rightfully be considered just such products. Organic aquaculture has just started its development in the world, its share amounting to only 0.1% of the total volume, that is, 100 000 tons annually. The EU consumer organic market has been growing steadily, while this niche remains unfilled. At present up to 100 organic fish breeding farms that operate in Europe produce about 10% of the total product volume. For example, in Sweden the amount of organic aquaculture products constitute 25-30%. Experts predict the threefold growth of organic fish farmers by 2020.

The EU organic market is strictly controlled and regulated by EC Regulations № 834/2007 and № 889/2008 which stipulate the requirements of feeding, maintenance facilities for aquatic organisms, cultivation conditions that are to meet the environmental needs of each species ensuring minimal impact on the environment, conformity assessment and marking. Thus, a domestic manufacturer of organic fish who wants to occupy this “niche” has to understand that meeting these requirements is mandatory to him.

The provisions for the production of organic aquaculture products in Ukraine are stated in the Ukrainian law “On the production and turnover of organic agricultural products and raw materials” and “The detailed rules for organic production (raw materials) in aquaculture”. To coordinate Ukrainian and European legislation, the draft law “On general principles and requirements of organic production, organic products turnover and labelling” has been prepared. Ukraine does not have its organic standards but the Ukrainian organic market has been successfully developing on the basis of the equivalent standard which

is designed for non-EU countries and adapted to the European law. In what way can a domestic producer of organic fish products confirm the product compliance with the standard? In accordance with the EU Regulation № 1235/20081 of 08.12.2008 (last updated EU Regulation № 2015/9312 of 17.06.2015) the official list approved by the European Commission includes 16 international accredited certification bodies for organic production that are carrying out their operations in Ukraine and are recognized by the European Commission. In addition, Ukraine has potential water resources for the development of organic aquaculture.

## **SPERM CRYOPRESERVATION AS A TOOL FOR CONSERVATION AND SELECTION IN AQUACULTURE**

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Sperm cryopreservation is an assisted reproductive technique that allows the storage and conservation of preserved cells at ultralow temperatures for an infinite period of time. Sperm cryopreservation was first developed for the dairy cattle industry in the 1950-ies and quickly became a multi-million dollar business. Although the first experiments on fish sperm were reported in 1953, the technology of cryopreservation is still not part of everyday hatchery management, except for some isolated cases.

Fish sperm cryopreservation is a relatively simple and cheap technology. It requires the use of isotonic extenders that keep spermatozoa in a quiescent state and reversibly inhibit their motility. Sperm is diluted in the extender typically at ratios ranging from 1:1 to 1:9. Sperm cryopreservation also requires the use of internal, penetrating cryoprotectants that protect cells from the damaging effects of freezing and prevent the formation of ice crystals. These most commonly include methanol, dimethyl-sulfoxide or glycerol, although several others have been tested. Sperm is typically frozen either in the form of pellets on a block of dry ice or loaded into 0.25-

or 0.5-ml straws in the vapor of liquid nitrogen. Following freezing, samples are normally stored in liquid nitrogen in canister storage dewars. After thawing, fertilization is conducted as with fresh sperm, although some fertilizing capacity is lost during freezing and thawing. Sperm-egg ratios between  $6 \times 10^5$  to  $5 \times 10^3$  were reported to give satisfactory fertilization, depending on the species.

Sperm cryopreservation has extensively been studied in several taxa of fishes, including sturgeons, salmonids, cyprinids, catfishes and percids and is closest to a routine application in the farming of Atlantic salmon. Its advantages are obvious: it allows reduction of male broodstock size and off-season spawning, facilitates hybridization of species whose spawning seasons do not overlap and assists in the conservation of valuable genetic resources. In fish, however, it also has severe limitations: males seldom have an individual value, techniques originally developed for bull sperm cannot be adapted without alterations to fish and contrary to mammalian species, the cryopreservation of fish eggs and embryos is still not possible. Thus, the widespread use of cryopreservation in aquaculture is not expected. It will be applied to species where its use will demonstrate a significant advantage compared to conventional spawning, otherwise it will only be used in niche areas related to conservation.

In order to overcome the problems related to egg and embryo cryopreservation, research currently focuses on other cell types. These include primordial germ cells or spermatogonia and oogonia which can be frozen and then transplanted into suitable recipients. The results of these studies are promising, however, they are still far from application.

## **NUTRIENT COMPOSITION AND HYDROLYTIC ACTIVITY OF ARTEMIA NAUPLII SATURATED WITH $\omega$ -3 FATTY ACIDS**

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Using of bioencapsulation technologies for live feeds enrichment with target products allows to supply the fish larvae with essential substances, probiotics, etc. However, a question whether the enrichment of feed organisms with some substances causes the deterioration of their nutrient composition still remains open. Furthermore, it is well known that live feed serves as a source not only of nutrients, but also of hydrolytic enzymes, which fish larvae extremely need while switching to exogenous feeding. According to pointed out above, the purpose of the study was to evaluate the nutrient composition and hydrolytic activity in *Artemia* nauplii saturated with essential fatty acids.

The brine shrimp nauplii obtained after cysts incubation (*Artemia* sp., «Ocean Nutrition», Belgium) were enriched with polyunsaturated fatty acids (PUFA) using Easy DHA Selco («INVE Aquaculture», Belgium). The share of PUFA's in the supplement is about 35% of total fatty acids, and the ratio of docosahexaenoic / eicosapentaenoic acids is approximately 2.5. The procedure for bioencapsulation with fatty acids was carried out according to 4 schemes: Easy DHA Selco was applied once at the beginning of enrichment at doses of 0.6 g/l (scheme 1), 0.9 g/l (scheme 2) and 1.2 g/l (scheme 3); in scheme 4, an emulsion at a dose of 0.6 g/l was added twice in equal parts at the beginning and after 12 hours of cultivation. The control group was consisted of nauplii, that did not receive Easy DHA Selco, but were cultured under the same conditions as in the experimental groups. The biochemical parameters were determined at 6, 12, 18 and 24 hours of the experiment.

The share of total proteins and lipids of freshly hatched nauplii in terms of dry weight was 57% and 17%, respectively. An all day starvation of studied organisms in the control group was accompanied by a decrease in the protein content to 41% and total lipids to 13%. The lipid content of *Artemia* from all experimental groups during 24 h enrichment was higher than in the control group, that may indicate about accumulation of lipid origin substances, containing in tested supplement, in fodder organisms. The protein content at the end of the experiment remained at the level of 57% only in nauplii from the 4th

group, in nauplii from other groups the protein content during the experiment decreased to values reaching control ones.

Incubation of brine shrimps nauplii with Easy DHA Selco led to an increase in the share of docosahexaenoic acid from 0.1% in freshly hatched nauplii to 1.3%, 2.1%, 6.5% and 1.4% of the total fatty acids in nauplii of 1-4 experimental groups, respectively. The total share of PUFA's was within 37-40%.

An important feature is absence of protease activity inhibition in feed organisms during their cultivation with using of Easy DHA Selco supplement. As it was already known, the most active proteases in *Artemia* brine shrimp are alkaline. Due to obtained results of investigation, the highest increase in proteolytic activity at alkaline pH was observed in nauplii from the first experimental group. In nauplii from other groups proteolytic activity did not differ from that one of control organisms. A significant increase in lipolytic and amylolytic activities in comparison with control data was observed in brine shrimps, received supplement according to schemes 1, 2 and 3.

## **IMPACT OF $\omega$ -3 PUFA BIOENCAPSULATION TECHNOLOGY ON THE GROWTH AND SURVIVAL RATE OF ARTEMIA NAUPLII**

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*Artemia* nauplii, frequently used in industrial aquaculture as a starter feed, are often deficient in polyunsaturated fatty acids, especially eicosapentaenoic acid. The solution of this problem is realized by using of bioencapsulation technology. However, the issue of such technologies' influence on the survival rate of nauplii themselves, as well as on the increase of feed organisms in the size, remains open, that might negatively affect on their availability for fish larvae. Taking

into account the all above, the purpose of the work was to evaluate the effect of the bioencapsulation regime of PUFA supplement on the survival rate and the increase in size of brine shrimp's nauplii.

Incubation of *Artemia* sp. cysts («Ocean Nutrition», Belgium) was carried out in the Weiss apparatus with a volume of 8 liters within 24 hours under the constant illumination, aeration and water temperature of 28°C. The bioencapsulation of nauplii with polyunsaturated fatty acids (PUFA) was conducted using Easy DHA Selco supplement («INVE Aquaculture», Belgium). The percentage of PUFA in the supplement is about 35% of total fatty acids, and the ratio of docosahexaenoic/eicosapentaenoic acid (DHA/EPA) is approximately 2.5. Before the enrichment procedure, a stable emulsion of Easy DHA Selco was preliminarily prepared with a small amount of water, followed by inputting of obtained emulsion into the Weiss apparatus with *Artemia*. Four schemes of bioencapsulation procedure have been tested: Easy DHA Selco was applied once at the beginning of enrichment at doses of 0.6 g / l (scheme 1), 0.9 g/l (scheme 2) and 1.2 g/l (scheme 3); in scheme 4, an emulsion at a dose of 0.6 g/l was added twice in equal parts at the beginning and after 12 hours of cultivation. The control group was consisted of nauplii, that did not receive Easy DHA Selco, but were cultured under the same conditions as in the experimental groups. The mortality rate of *Artemia* nauplii was determined at 6, 12, 18 and 24 hours of the experiment. For this purpose, the number of dead and living individuals in 1 ml of the incubation medium was calculated using a binocular microscope. Mortality was expressed in % as a ratio of dead individuals to the total number of all selected nauplii. The length of *Artemia* nauplii was defined in randomly selected 100 individuals.

It has been shown that during one-stage enrichment of *Artemia* nauplii with a supplement in doses of 0.6 g/l and 1.2 g/l the mortality index remains stable for 24 hours and equal to 9.3% and 13.5% at the end of bioencapsulation procedure, respectively. It should be noted that the mortality of *Artemia* nauplii in the control group at 24 hours after hatching was 22%. During the enrichment procedure by the second and fourth scheme, mortality at the end of saturation period was 26.8% and 25.0%, respectively. The mortality of *Artemia* in the first 12 hours of enrichment with essential fatty acids in all experimental groups was

higher than in the control group that is obviously connected with adaptation of cultured organisms to an emulsion presence in the culture medium.

The average length of *Artemia* immediately after cysts hatching was  $553 \pm 32 \mu\text{m}$ . Over a 24 hour period, the length of nauplii increased to  $631 \pm 22 \mu\text{m}$  and  $650 \pm 16 \mu\text{m}$  in the control and first experimental groups, respectively.

Thus, there were no significant differences in nauplii sizes during one-day saturation with PUFAs, although their length was 14-18% greater than that of the newly hatched individuals.

## **COLLABORATION ON ATLANTIC STURGEON RESTORATION IN THE NEMUNAS RIVER BASIN**

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Atlantic sturgeon *Acipenser o. oxyrinchus* population in the Baltic Sea basin probably settled in the Neolithic period, around 7500-6500 BC. Nowadays, the natural occurrence of this species extends to the Atlantic coast of Canada, and spawns on the St. John and St. Andrews rivers. (Stankovič et.al. 2007, Kolman 2008).

The sturgeon was mainly found in the southern part of the Baltic Sea basin, and spawned in all the large rivers from the Oder to the Neva, including Nemunas (Berg 1948, Kesminas 2010). Traditional

spawning places were found in the mainstream of the river and in its tributaries: Neris and Šventoji (Kesminas 2010).

At the beginning of the 20th century, the annual fishing of sturgeon in the Baltic Sea was over 220 tonnes, of which about 23 tonnes were caught in the Nemunas and Curonian Lagoon (Kolman et al. 2008, Pilinkovskij et al. 2014). Environmental changes in the rivers of the southern part of Baltic basin and intensive fishing caused that in the second half of the 20th century the Baltic sturgeon was considered a lost species.

In the mid-2000s the Baltic Atlantic sturgeon population was start of restoration. The starting material for this work was a roe from the St. John (Kolman et al. 2005). Restoration works were initially carried out in the Odra and Vistula River basins, and in 2010 the Nemunas river basin restoration started: Neris and Šventoji (Kolman et al. 2011). Since 2011, stockings of the Nemunas have been regular: annually, thousands of nursed fish are introduced into the Nemunas tributaries with 5-7g average weight (autumn) and 100-400g (spring stocking). The spring fry was equipped with "Floytag" markers with individual numbers that allowed identification of fish caught by fishermen accidentally. A small number of larger individuals were equipped with telemetry micro transmitters. The telemetry receivers along the migration paths allowed to trace the nature of their movement (Pilinkovskij et.al 2014). The time of migrating from the moment of discharge to the river to the lagoon pool was usually 15-82 days (Gushchin et al., 2014).

For the period of restoration work carried out, 108,201 pieces of sturgeons were introduced into the basin of Nemunas, of which over 95% were fry with an average weight of 5 to 7 g and about 5% with a weight of 100-400g. From the river flooded into the Curonian Lagoon. Some of them migrate to the Russian part of the Lagoon. The sturgeon caught in the nets were monitored. For the period of research, in 2012-2016 on the side of the Russian part of the lagoon, 145 pieces of sturgeon were caught. More than 90% of the caught individuals were alive and, after recording their individual number, were released to the lagoon. The good condition, as well as the weight gain of the sturgeon caught, showed that there were good conditions in the Curonian Lagoon. Out of the lagoon they go out into the open sea and take a

long hike. One of the released individuals was caught near the Alans Islands (Pilinkovskij et al., 2014).

In order to enter the Nemunas River, the sturgeons could continue to migrate to the Baltic Sea and create a stable population there, in order to preserve the ecological status quo of both the river and the lagoon. Therefore, it is necessary to extend monitoring especially in the area of planned construction of NPS water intake.

## **A TECHNOLOGICAL SOLUTION FOR THE REMOVAL OF PHOSPHORUS COMPOUNDS FROM THE CIRCULATING WATER OF RECIRCULATING AQUACULTURE SYSTEMS**

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When fish are grown in recirculating aquaculture systems (RAS), effective purification of the circulating water from pollutants including fish metabolism products and indigested feed remains is especially important. The restoration of water quality to the parameters when it was first fed into the fish-breeding tanks allows us to reuse the water and realize the main advantages of RAS. Technologies and schemes used for circulating water purification are undergoing certain changes related to the scientific and technological progress, a deeper study of the influence of individual abiotic factors on the rate of fish growth, and because of stricter environmental legislation on the discharge of insufficiently treated sewage. The examples of such changes can be seen in the improvement of the

design of filters for mechanical cleaning and of structures for the compaction of sediments formed, as well as in the substantiation of the expediency of including denitrifying agents in the biological treatment unit.

More attention has been paid recently to the processes of removing phosphorus compounds from the water of RAS. The need to clean the circulating water of this contaminant is due to its relatively high levels in fish metabolism products, as well as in its ability to accumulate in water in a soluble form. Priority is given to applying biological methods for the removal of dissolved phosphorus compounds because of the danger of poisoning fish when using chemical reagents for sedimentation, and formation of a significant amount of waste that must be disposed of. The processes of extraction and transformation of phosphorus by phosphorus-accumulating organisms depend on many factors. Modern biotechnologies for the removal of phosphorus compounds from wastewater are characterized by a large number of circulatory flows, significant energy inputs, difficulties in cultivating phosphorus-accumulating organisms in bioreactors.

An alternative method for the removal of phosphorus compounds engages floating aquatic plants in water purification processes, with the possibility of further using those plants as food for fish. A special advantage of such biotechnology lies in the fact that the expediency of using higher aquatic plants in water purification is due to their potential to assimilate nitrogen compounds. Our experimental studies have shown that representatives of duckweed *Wolffia arrhiza* and *Lemna minor* are the most promising for cultivation in RAS. Thus, the use of the phytoreactor with plants will allow us not only to remove the compounds of biogenic elements from the recirculating water but also to transform them into the phytomass available to fish.

## **EXPERIENCE OF CULTIVATION OF AUSTRALIAN CRAYFISH (CHERAX QUADRICARINATUS) IN THE CONDITIONS OF THE SOUTH OF UKRAINE**

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In our country, the purposeful cultivation of traditional crayfish is rather problematically. Biological features of the species, high energy prices and equipment predetermine significant investments that make such production unprofitable. But, *Cherax quadricarinatus* has a number of physical and biological advantages over other classes of crustaceans. The main producing countries that are engaged in its commodity cultivation are China, Indonesia, Israel, Spain, USA. Today this industry is successful and has a strong foundation that reflects its profitability and potential. In Ukraine, this crayfish has become widely known among aquarists due to its bright coloration, rapid growth and easy breeding in aquarium conditions. The technology of cultivation on the territory of Ukraine has not yet been developed, the main problem is the provision of an optimal water temperature of 23-30 ° C, which is impossible in the climatic conditions of Ukraine. Therefore, the cultivation of *Cherax quadricarinatus* requires the development of a specific technology, which provides for two stages: cultivation in an artificial environment of closed water supply systems and cultivation to the stock in ponds, which is best suited for fishing ponds.

In 2015, on the basis of the problem research laboratory of the Department of Aquatic Bioresources and Aquaculture of the Kherson State Agricultural University, there was imported a batch of *Cherax quadricarinatus* of different generations with a mass of 0.15 to 1.3 g. The cultivation in aquarium conditions was carried out with a water temperature of 24-26 °C. The feeding was carried out with granulated feeds of its own formulation. The results of the cultivation of Australian crayfish in the autumn-spring period have indicated a systematic increase in both linear and mass indices. Thus, over a period of 250 days, crayfish have reached an average weight of 41 g and a length of 12.5 cm. The growth data indicated the highest growth rate in the younger age groups (from 50 to 130 days). The fluctuation for the relative increase ranged from 14.6 to 37.0%, and for the mass from 16.1 to 46.9%. Fecundity of females fluctuated in insignificant borders depending on age and accordingly on the size of females: from 100 to 170 eggs under the mother's ridge. After the arrival of optimal air temperatures and the optimum temperature of water for cultivation, crayfish were planted in a pond with an area of 0.48 hectares. Four groups (variants) of crayfish have been introduced: an average weight



of 0.56 g, 25 and 40 g; the fourth group was adult females with caviar. Unfortunately, for some reasons, the introduction of crayfish was possible only in early July, and the cultivation period was only three months. At the same time, the rather high results were obtained: first group of crayfish within three months has reached a weight of 34 g, the second - 75 g, the third - 110 g (some specimens reached 187 g, which is almost four times their original mass of 40 g). The growth rates of crayfish that were grown in aquarium conditions with the use of artificial feeds, compared with the specimens that were grown in pond conditions due to natural forages, were twice as large. In the burrows there were pairs of females with males, females with caviar at the different stages of development. This phenomenon has indicated a high adaptive capacity of this species and its ability to protect and reproduce in the conditions of the research pond. Thus, we have had many reasons to assert that ponds of the south of Ukraine with their large natural forage base can be used for extensive cultivation of *Cherax quadricarinatus*. At the same time, it is necessary to ensure adequate conditions for their maintenance in winter, using the closed water systems or other systems that will provide this kind with warm water.

## **USE OF RECIRCULATING AQUACULTURE SYSTEM IN SALMON AND SEA TROUT RESTOCKING IN LITHUANIA**

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Currently, the natural production of salmon smolts in the rivers of Lithuania increased. Salmon and sea trout recovery programs began in 1998. In these programs artificial propagation of salmon and sea trout were included, along with such measures as the protection of spawning grounds, fish passage construction, control of pollution and poaching, etc. As a part of the program, Žeimena salmon hatchery was constructed in 1999, based on a recirculating aquaculture system. Since the beginning of its activity, it produces more than 200 thousand

of juvenile sea trout and more than 150 thousand young salmon of different age stages which are released into Lithuanian rivers by the Fisheries Service each year. Stocking efficiency is estimated annually by the Nature Research Centre and Fisheries Service based on salmonid monitoring by electro-fishing and trap net surveys. According to the results, salmon populations were recovering quite slowly in the initial stages of the restocking program. However, noticeable increase in abundance was observed from 2008. The use of RAS facilitated the cycle of artificial production of salmonids for enhancement of wild populations and reintroduction of stocks into potential rivers. Fry and smolts of both species are reared in shorter times compared to the conventional flow-through systems.

The presentation details some of the aspects of RAS pros and cons in restocking. Data on status of salmon and sea trout stock parameters in the natural water bodies are given. We also present developments in genetic studies of salmon and sea trout populations in Lithuania and their involvements in the stock management. Lower genetic diversity of salmon populations was detected by mtDNA and SNP methods. Sea trout populations displayed higher levels of variability. The results suggested that some precautions should be taken towards the management of small local sea trout populations.

## **ASSESSMENT OF VITAL CAPACITY AND ECOLOGICAL FUNCTIONS OF ALIEN HYDROBIONTS OF WATERS IN-STEPPE UKRAINE**

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According to experts of FAO and IUCN, number of invasive hydrobionts with high adaptive capacity is increasing annually, which are influenced by direct or indirect human actions and fall into an extrinsic new conditions of existence and cause enormous damage to natural ecosystems and fisheries. The rapid spread of new species is

caused by their adaptive capacity to stress factors on the biochemical, cellular, species and population levels.

The research results of leading scientists concern the study of biology, ecology, distribution of alien species in the reservoirs of integral use (Marlis et al., 2015), as well as socio-economic impacts of biological invasions (Lotz, Allen, 2013). European scientists have researched vectors of migration and spread of invasive species (Frances et al., 2016), but studies of adaptive capacity of species were almost never carried out, indicating the urgency of such works.

Thus, the purpose of our research is to determine the ecological characteristics of invasive aquatic organisms in waters used for fishery in steppe zone of Ukraine, which give the species some advantages during entering and interfering in local ecosystems and study changes of food chains that occur under the influence of life of invasive species and crustaceans. Research has received state support within the Contest of scientific papers, scientific and technical (experimental) developments of young scientists who work (study) in higher educational establishment and research institutions belonging to the Ministry of Education and Science of Ukraine (state registration № 0116U008040).

We conducted a comprehensive survey of key aquatic ecosystems of steppe zone of Ukraine to search and evaluate the environmental impact of invasive aquatic species that gave an opportunity to get current information about the status of their populations, range of power and their role in the ecosystem. A laboratory model experiments were carried out to study the mechanisms of adaptation of alien species (pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) and the marbled crayfish *Procambarus fallax* (Hagen, 1870) f. *virginalis* (Decapoda) as an example) using biochemical markers. The effect of different concentrations of heavy metals (Cu – 10 MAC, Zn – 10 MPC, CD – 2 MAC) (based on their content in water of Zaporozhye (Dnipro) reservoir which is the main recipient reservoir for this species) on the physiological state, linear-weight indicators, fertility, vitality and histostructure of body tissues and organs was determined. It was determined the behavioral aspects of joint maintenance of crayfish of genus *Astacus* and marbled crayfish *P. fallax* (Hagen, 1870) f. *virginalis*, their territorial and food behavior.

Biochemical and histological studies of blood, kidney, liver of pumpkinseed *L. gibbosus* (Linnaeus, 1758) and Prussian carp *Carassius gibelio* (Bloch, 1782) were carried out (representatives of rapid biological invasions with widespread area of habitat). It was determined the reaction of red blood of research species on the impact of anthropogenic factors and patterns of age-related changes in red blood cells were determined. Aspects of the behavior of these species of fish in the model experiments were researched.

As a result of research recommendations was developed on measures use of which will help to predict the spread of hazardous invasive hydrobionts and predict their impact on native ichthyocomplex and the environment. The research results can be the basis for improving existing methods of finding and assessment of adverse environmental impacts of invasive species under conditions of steppe zone of Ukraine.

## **ESTIMATION OF AQUACULTURE PRODUCTION OF UKRAINE**

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Every year in Ukraine reduced fish catch, in 2015 was caught just 88.55 thousand tones. (Excluding the temporarily occupied territory Autonomous Republic of Crimea and the city of Sevastopol), Which is 59.5% lower compared to 2010, and compared with the previous, 2014 - 3%.

With inland waters in 2015 was caught 38.5 thousand tones, which represent 43.5% of the total catch. With freshwater bodies were caught 37.3 thousand tones. The bulk of the catch constituted freshwater ponds and reservoirs, which together caught nearly 32.8 thousand tons, which correspond 87.8%.

Carp fishes were caught 34.2 thousand tons, of which for the bulk accounted on bighead carps 11.5 thousand tones, or 33.7%, carps 9.8 thousand tones - 28.6% and crucians 5 3 thousand. tones - 15.5%.

Other carps occupied in the structure of the catch just 22.2%.

The prices of carps species fishes in 2015 compared to 2014 increased on average by 43%.

With most predatory freshwater fish have been caught pikeperch 733 tons.

The catch pikes, catfishes and perches was almost at the same level and amounted to 270-297 tones.

The comparing the average cost of 1 ton of fish in 2014, it should be noted that it has grown on all types of fish, an average of 25.3%. The highest rise in prices was the on pikeperch and perch by 41.5 and 36.3% respectively. Group of salmon was mainly represented by one representative trout, catch it last year amounted to 405 tons. The sturgeons in 2015 was growing and caught only 36 tons. The largest share of sturgeon and paddlefish sturgeon occupied, their catch was 25 tones, or 69.4% of the total catch of sturgeon.

With the herrings fishes prevailed sprat, which virtually amounted to the bulk of the catch of 11.8 tones, or 98.6%.

The average price for salmon and sturgeon fish is traditionally the highest. Thus, the average cost of 1 ton of trout amounted to 91.1 thousand UAH and increased from the previous year by 94.8%. Thus, the average cost of 1 ton of trout amounted to 91.1 thousand UAH and increased from the previous year by 94.8%.

The average price of sturgeon in 2015, compared to 2014 increased by 2.7 times.

The most high cost paddlefish 3.1% and sturgeon in the 2.3 times the cost of sturgeon increased slightly - by 34.8% and has not changed the value better.

Marine fish species in total catch fishes was 31%. Of these, the goby fish of the occupies 15.6 thousand tons, or 68.2%

The average cost of marine fish was within 1,3-35,5 thousand UAH. The most expensive were flounder, mullet and rays fish whose price was within 23,1-35,5 thousand UAH. / tons.

On the aquatics invertebrates in catch accounted for just 14.6 thousand tons, or 16.5%. The bulk of aquatic invertebrates were crustaceans and molluscs 13.1 and 0.9 thousand tons.

Fisheries Ukraine production only one third of citizens with fish and seafood, other 2/3 comes from abroad, mainly frozen.

The main supplier of frozen fish into Ukraine is Norway. The other importing countries is Estonia, Lithuania, Iceland, Spain, the United States and other countries.

The imports of chilled fish products decreased from 23.3 thousand. tones. In 2013 to 9 thousand. tons in 2015, that 2.5 times. The leader in the supply is Norway with a share of 88.3% in imports.

At the considering the situation on the market of fishery products importers were forced to the review the share assortment and increas the of cheaper varieties of fish such as hake, pollock, capelin and others, and reduce - expensive, salmon, tuna, trout and others.

Last year, imported fish took about 75% of the total consumption of fish products. The own raw base every year more decrease, and production figures do not inspire optimism.

But however, Ukraine has a great potential for inland waters towards the development of fisheries and fish farming. In the Ukraine 400 thousand hectares of estuaries, lakes and ponds and about 700 thousand hectares of reservoirs, fish productivity which can be enhanced by intensification. The increase in fishes breeding 1.5-2 times - up to 60-65 thousand tons - is real possibility of domestic aquaculture.

## **FRACTIONATION OF SUSPENDED PARTICLES AS A WAY TO STUDY THE DISTRIBUTION OF HEAVY METALS BETWEEN THEIR CHEMICAL FORMS IN WATER**

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Assessment of the level of heavy metals is necessary for determining whether a certain water source is ready for use in aquaculture, and for calculating the water treatment system parameters. It should also be monitored during the whole fish breeding process.

Heavy metals constitute one of the key groups of natural water pollutants. It is necessary to monitor the changes of their concentrations in water basins; information about chemical forms of the heavy metals is also helpful to assess the hydrochemical state of the water. Those forms may be considered corresponding to the fractions of suspended particles with the following sizes:  $> 1.2 \mu\text{m}$  is mostly a fraction of inorganic compounds of metals;  $0.2$  to  $1.2 \mu\text{m}$  is mostly an organic fraction mainly consisting of biomass of microbes;  $< 0.2 \mu\text{m}$  is predominantly a fraction of metal ions.

A simple laboratory method of analyzing the distribution of heavy metals between such fractions has been designed during this study. The water samples were consequently filtered with membrane filters having  $1.2$  and  $0.2 \mu\text{m}$  pores. The filters with sediments on them are digested in a microwave oven with a solution chemically close to aqua regia; concentrations of heavy metals were measured with ICP-MS, similar methods of analysis are also acceptable. The results of the method testing have clearly shown differences between the distributions of heavy metals in variously polluted water from different samples. It is possible to make approximate conclusions about chemical forms of the metals in water using such data.

The method is promising and may be used in aquaculture for water quality estimation as well as in monitoring of fish breeding in open and closed systems or artificial and natural water bodies.

# **PHYTOREMEDIATION AS PERSPECTIVE ENVIRONMENTAL TECHNOLOGY FOR CONTROL OF HUMAN IMPACT ON AQUATIC ECOSYSTEMS**

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The contamination of aquatic ecosystems by toxic metals is a serious environmental and medical problem that badly needs an effective and affordable technological solution. Heavy metals (TM) are on the list of the most dangerous chemical pollutants. Excessive inflow of TM into aquatic ecosystems by anthropogenic means often leads to irreversible changes and disturbances of vital functions of living organisms and poses a serious threat to human health. Plumbum, cadmium, arsenic and hydrargyrum are the main non-biodegradable TM, which are especially dangerous for the human body.

Thus, instead of treating TM poisoning, a prophylactic approach should be used as an effective alternative for the removal of xenobiotics from the aquatic environment. Extended scientific research on the introduction of environmentally friendly and cost-effective technologies, led to a relatively new approach to the rehabilitation of inland freshwater reservoirs and wastewater treatment.

Phytoremediation is a relatively new environmental technology that uses plants to remove, transform or stabilize various contaminants in water and sediment. The great popularity of this technology is associated with efficiency, low cost, a wide range of absorbed pollutants, environmental friendliness and the ability to apply directly in the contamination area, which helps reduce costs and reduce contact with people and the environment. The positive effect of phytoremediation depends on the determination of suitable types of macrophytes that can accumulate TM. To date, there are practically no scientifically substantiated criteria for selecting such hyperaccumulators from the point of view of their potential ability to purify water bodies, which gives grounds for a more detailed study of the issue of phytoremediation technology of reclamation measures in



wastewater and inundated inland water bodies with a certain area and pollution degree of TM.

## **FIRST RESULTS OF WWF PROJECT «LIFE FOR DANUBE STURGEONS» IN UKRAINE**

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Not so long ago in the 20th century sturgeons was common fishes in Low Danube and North-Western Black Sea. But now according to the IUCN, Family *Acipenseridae* is more critically endangered then any other group of species worldwide. Out of six Danube sturgeon species, five (*Huso huso*, *Acipenser gueldenstaedti*, *A. sturio*, *A. stellatus*, *A. ruthenus*) are classified as “critically endangered”, with one extinct in the Danube (*A. nudiventris*). Also the European Red List of Freshwater Fishes states that the conservation status of Europe’s eight sturgeon species is particularly worrying. All Danube sturgeon species are listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The most direct threats to Danube sturgeons are overfishing before fishing ban, poaching after fishing ban, fragmentation of Danube by dams and loss of spawning sites, channelization of the river, gravel extraction and navigation, illegal wildlife trade. Native population trends for all sturgeon species in Ukraine are also decreasing because the most sturgeons which released into the Danube were from hatcheries and carried tags and require urgent conservation measures.

WWF project «LIFE FOR DANUBE STURGEONS» funded by the EU Life+ programme starts working with fishermen, enforcement agencies, decision makers and aquaculture industry in four countries (Romania, Bulgaria, Serbia and Ukraine). Aim of the project is the protection of globally threatened wild sturgeons from illegal fishing and trade in a part of the EU with particular importance for their survival. Project started in Ukraine at the 1<sup>st</sup> of October 2016, and target area is Vilkovo, Leski and Primorskoe in the Danube delta. The project includes three main objectives: work with Law Enforcement Agency, fishing communities and markets. The following results are expected: law enforcement is implemented more effectively through enhanced capacity and practical knowledge of authorities and higher acceptance – and consequently compliance – by stakeholders to achieve a long-term reduction of illegal sturgeon fishing and trade in the lower Danube region; targeted fishing communities in Vilkovo, Leski and Primorskoe positively take on ownership for sturgeon conservation and are willing and able to use alternative income sources to compensate profits from sturgeon fishing and to give stocks a break to recover; the availability of legal and illegal sturgeon products on the market is better known and under stronger surveillance by authorities, and respective retailers are aware of legislation and enabled to prevent illegal products from reaching the market.

By March 2017 project team has identified key stakeholders in targeted fishing communities and established good relationship with them. The project team produced and distributed information materials (sturgeon guides, sturgeon factsheets, sturgeon posters) for active fishermen, future fishermen/young people in fishing communities, local authorities, Fishing agency. Project expert conducted three Sturgeon specific practical trainings within the training courses for new inspectors of Fish Patrol in Kiev, Kherson, Kremenchuk. We start to set up a system of voluntary “Sturgeon Watchers” to protect wild sturgeons together with enforcement agencies as a model case in Ukraine. In strong relationship with Kherson State Agriculture University created the small groups of fishery students will surveil water and river banks and announce potential poaching. Additionally, volunteering students from the field of fishery or aquaculture will develop skills for their careers as well as awareness for the problem and will be familiar and sensibilized in their

future work. This approach will be tested in the Ukrainian Danube delta but shall be applied in other project countries (esp. Romania) in the near future and also presented to other range states for potential replication.

## **EXPERIENCE OF USING GASTROPODS FOR THE TRANSFORMATION OF ORGANIC POLLUTANTS OF RECIRCULATING AQUACULTURE SYSTEMS**

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In the process of growing fish in recirculating aquaculture systems (RAS), there are formed various fine-dispersed water pollutants characterized by poor sedimentation properties. This group of pollutants is represented mainly by components of organic origin, which have the capacity for rapid oxidation and transition to soluble forms. These properties of suspended pollutants should be taken into account when organizing a water purification system in RAS.

To solve this problem, we conducted experiments on the transformation of undissolved organic pollutants in RAS with the help of gastropods *Physa fontinalis* and *Planorbarius corneus* that provided a positive effect due to the mineralization and increase in size of undissolved contaminants.

In nature, mollusks graze on seaweed and periphyton, and swallow detritus. Therefore, organic contaminants of RAS, the solid part of which is mainly composed of fish feces, small particles of feeds and mucus, are a good nutrient substrate for mollusks. Given the ability of

gastropods to scrape the fouling and plaque off solid objects, any inert carrier (for example, filamentary fibers of Viya type) should be placed in the bioreactor, and a biofilm absorbing fine-dispersed and dissolved organic compounds will develop on its surface. The experiments showed that gastropod mollusks *Physa fontinalis* and *Planorbarius corneus* adapt well to the conditions of the bioreactor with synthetic filamentous fibers, and are able to intensively eat up their surface, and assimilate most of the organic matter. The feces excreted by mollusks and characterized by high density, resistance to decomposition in water and a high level of mineralization settle in the bioreactor collector as sediments.

In addition, there have been conducted experiments on the use of these species of gastropod mollusks for the utilization of organic contaminants in a thin layer sedimentation tank that has inclined shelves. The findings show that the mucus with excellent sorption properties secreted by mollusks enhances the effect of subsequent purification. The organic pollutants accumulated on the inclined shelves are utilized by mollusks; the excreted feces slip down, which makes it possible to form a stabilized silty mixture at the bottom of the tank, and it is easily removed during sedimentation or filtration, as mollusk feces are strong enough to settle quickly and not disintegrate in water.

The experiments performed have the following positive results: the sedimentation effect is improved by 15-20%; the amounts of waste formed are reduced by 50-60%; total mineralization of the sediments is significantly increased (ash content increased from 10-12 to 25-30%).

## **RECREATIONAL FISHING IN UKRAINE AND ITS ECONOMIC AND ENVIRONMENTAL VALUES IN REGIONAL SUSTAINABLE DEVELOPMENT**

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Today, amateur and sport fishing may be seen as recreational, which refers to the use of water biological resources not only for the purpose of extraction (catch) of fish but also recreation, fishing (green) tourism, conservation and restoration of objects of fishing and the environment.

Each year, the total number of amateur fishermen in Ukraine is growing, but only a relatively small part of the fishermen are members of various public organizations and private fishing clubs. According to some in our country there are about 7 million unorganized amateur fishermen. According to various sources, amateur catches in inland waters Ukraine compared with industry range from 70 to 400 percent. Although these large fluctuations are possible due to different conditions in different regions of the country, but at the same time they also show quite estimates the scale recreational fishing.

Organization of recreational fisheries in most countries is one of the most profitable industries of economic complex that brings 40-50 percent of net profit. For example, the total revenue from this direction of the economy in North America ranging 80-100 billion dollars.

However, in our view amateur and sport fishing should not be seen as one of the components of the fishing industry of the country. Much more it belongs to one of the varieties of tourism on the one hand, very close in nature to the modern environmental or "green" tourism, on the other hand takes into account the centuries-old traditions of indigenous peoples and rapidly growing every year the demand of our urbanized society in recreation outdoors.

So, recreational fishing today is an important factor of social and economic development, which can not be ignored. On the one hand, recreational fishing allows developing those reserves of water resources which are not fully covered or not fishing including low-value fish species, which increases the efficiency of use of fishery reservoirs. On the other hand, recreational fishing - recreation means millions of citizens Ukraine, this is its great social importance.

The basis of eco-tourism on five basic principles, which fully meet the essential requirements of the recreational fishery, and defining the main directions of further economic and social development of the industry:

1. The object of intellectual and emotional satisfaction should act natural objects.
2. The management and operation of all types and categories of natural resources.
3. Do not harm the environment and minimum impact on the environment.
4. Preservation of social, cultural and religious traditions of the local environment and the region.
5. The above principles of eco-tourism should have a positive impact on economic development as separate objects, regional tourism in general.

Today, tourism industry in Ukraine, taking into account the performance of the recreational fishery is only fifth in importance as a component of filling budget and its share in the coming years should increase significantly with all existing objective preconditions: favorable geopolitical location in the heart of Europe, favorable climate, high levels of security in different tourism resources, the availability of significant recreational and health potential, developed network of protected areas with a rich flow swarm and fauna.

According to estimates of experts fund potential environmental, health resort and recreational area of 12.1 million hectares. Ie almost 20% of Ukraine, which generally meets international indices rational exploitation and conservation of natural and recreational resources. Capacity landscapes Ukraine, including natural and artificial water bodies for recreational fishing organizations, including acceptable environmental standards, more than 40 mln. people.

## **ACCLIMATIZATION AND PROSPECTS OF CULTIVATION SHRIMP *MACROBRACHIUM NIPPONENSE* IN THE WATERS OF UKRAINE**

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Eastern freshwater shrimp *Macrobrachium nipponense* (de Haan, 1849) is widely distributed in the Indo-Eastern Pacific region. It

occurs in freshwater and brackish waters of Japan, China, Korea, Taiwan and Vietnam. Due to its amazing ecological plasticity, in the 60s Japanese shrimp was widely used for acclimation in cooling reservoirs of power stations in Russia, Belarus and Moldova. In 1986, *M. nipponense* was introduced from the reservoir-cooler of the Berezovskaya water-power plant (Belarus) to the Kuchurgan reservoir (Moldova). By the end of the previous century, eastern freshwater shrimp had already been acclimated in the Kuchurgan reservoir and formed a stable population here, as well as penetrated the Turunchuk and Dniester rivers, spreading throughout the waters from Tiraspol to the Dniester estuary.

*M. nipponense* is an important fishery and aquaculture object in China, Korea, Japan and other countries of South-East Asia. The volume of Japanese freshwater shrimp production in China is growing and now is estimated at 100 000 tons (about 50% of the total production of shrimp in aquaculture). Efficient reproduction, rapid growth and high market value make Japanese shrimp a promising object of cultivation in ponds and cages, intensive and semi-intensive methods in mono and polyculture. The widespread distribution of *M. nipponense* in the Dniester river basin and its amazing tolerance to adverse environmental conditions, combined with high fecundity and growth rate, unpretentiousness in nutrition, high cost and excellent gastronomic qualities, make *M. nipponense* a promising and valuable object for aquaculture in Ukraine.

For the successful introduction of *M. nipponense* into the aquaculture of the south of Ukraine, it is necessary to develop modern methods for the formation and maintenance of a herd of producers, artificial reproduction, larval growth and commercial cultivation of shrimp in mono- and polyculture.

The conducted researches are directed on optimization of methods of feeding shrimps at all stages of ontogenesis. It has been established that the selection of adequate food rations, their composition and magnitude have a significant effect on the rate of growth and survival of shrimps in the growing process. It is shown that the proper selection of a diet provides a high percentage of producers maturing and can significantly reduce cannibalism. This is the crucial aspect of the biotechnology of industrial shrimp cultivation. Based on the results of

the studies, a set of measures was proposed, including optimization of feeding conditions, the use of shelters and the creation of recirculation systems of directional flow of water with certain intensity in the rearing basins. The proposed technology reduces the level of cannibalism and increases the yield of marketable products.

## **A NEW STRATEGY OF A PASTURE MARICULTURE IN THE LIMANS OF THE NORTH-WESTERN BLACK SEA REGION**

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Limans and lagoons of the North-Western Black Sea are the highly productive ecosystems that have a great fishery and recreational importance. These water bodies are subject to a significant anthropogenic impact, which is typical for the entire Black Sea and, especially, for its north-western part. The peculiarities of the natural conditions (shallow water, desalination, high temperature, abundance of nutrients, etc.) combined with the presence of freshwater, brackish and sea forms in the flora and fauna determine a great biological diversity and a high productivity of these water bodies. For a long time, the brackish-water limans of the north-western Black Sea region serve as the feeding grounds for marine game fish and are traditionally used for a pasture mariculture.

For centuries the sea fish pasture cultivation was practiced in the limans and lagoons of the Mediterranean, Azov and Black Seas. Aboriginal species of mullet and some other fish were the objects of cultivation. Cultivation was carried out at an annual turnover, its technology provided stocking the limans with *Liza auratus* fry born in the sea. In spring through special channels they went into the quickly warmed up and abundant with fodder limans, and in autumn in the same channels the two-year-old commodity fish, striving to go to the sea was caught.

In the sixties of the last century, the technology of mullet pasture growing during a two-year cycle, which included using except *Liza*



*auratus* and *Mugil cephalus* and *L. saliens* stored in the special winter huts that were built on the Shabolat Liman was developed.

Under the influence of a number of anthropogenic factors at the end of the last century, the ecological state and the production capabilities of the most seaside water bodies underwent significant changes, and a depressive state of a mullet population in the sea did not allow to provide the mullet-growing farms with the necessary amount of the fish planting material.

The possibility of reviving a pasture mariculture suggested an introduction of fish breeding techniques for the artificial breeding of marine fish, but due to the deterioration of the economic situation in Ukraine the nurseries have not ever built, and the developed biotechnologies have not find their wide industrial application. The situation was worsened by the closure of a mullet-growing farm on the Shabolat Liman with its unique wintering complex.

Under the prevailing conditions the traditional technologies of a pasture mariculture proved to be ineffective. A need to develop a new concept of the fish-breeding use of the brackish-water seaside limans arised and, on this basis, a need to develop a modern technology of a pasture mariculture arised too.

The main principle of this technology is to form the living self-reproducing marine fish populations (*Liza hematocheilus*, *Platichthys luskus*, *Neogobius melanostoutus*, *Zosterisessor ophiocephalus*) in the limans to ensure the stocking of the liman mainly due to juveniles from the natural spawning, and to introduce the new objects such as *Mesogobius barhocephalus*; *Dicentrarchus labrax*; *Morone saxatilis*; *Oncorhynchus mykiss* and others into a polyculture.

The completed complex study allowed to develop a modern strategy of a pasture mariculture, to identify the most promising growing objects, taking into account the modern ecological state of the brackish-watered seaside limans. On this basis, a mathematical model that allows to manage the fish-breeding process, to estimate the necessary volumes of stocking and commercial fish products cultivation using the Shabolat liman as an example has been developed.

## EXPERIENCE IN GROWING OF PIKE-PERCH (*SANDER LUCIOPERCA* L.) LARVAE ON LIVE FOOD TO VIABLE STAGES

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Currently, pike-perch (*Sander lucioperca* (L.)) is a popular fish in the European market and its cost in some countries (France, Germany) reaches up to 21 Euros per 1 kg. Currently, two methods are used for the initial feeding of larval pike-perch: 1. In this method, both commercial feed and *Artemia nauplii* are fed to the larval pike-perch during the first 14 days. During this period, brine shrimps are provided with ~200-300 nauplii/fish/day, given in minimum three portions a day. After 14 days, the larvae are fed on commercial feed exclusively. The granule size in the first 10-14 days of rearing is 0.1-0.3 mm, while in the third and fourth weeks it is 0.2-0.4 and 0.3-0.5 mm respectively. The feed has a high protein content (55-62%) and a lipid content of 10-16%. Feed is delivered ad libitum 24 h/day with an automatic feeder; 2. In this method, larval pike-perch is fed on brine shrimp exclusively for the first 14 days at a rate of ~500 nauplii/fish/day, delivered every 1-1.5 h for a minimum of 16 hours per day. After 2 weeks, the larvae are trained to accept commercial feed; the period required to do this is usually 3 days. After 3-4 weeks of rearing (BW >50 mg), the fish is sorted (FAO, 2017). However, as practice has shown, shrimps of *Artemia salina* are large food objects for sander-

larvae, when transferred to external nutrition. The mortality of larvae, when transferred to external feeding and for lack of food, can reach 90%. One of the reasons for this phenomenon may lie in the absence of smaller feed for the pike-perch larvae of this age. Our research objective was to enhance survival and viability of the pike-perch larvae transferred to external feeding, using the elements of “Blue Bioeconomy” during the feeding. The works were carried out at the fish hatchery of the Latvian fish farm “K3K” LLC, where the works on the artificial reproduction of pike-perch were performed. The incubation process of fertilized eggs before the larvae hatching was carried out in Weiss apparatus and the keeping of larvae before their switching to external feeding and then growing was done in trays measuring 4.5x1x0.5 m. For feeds, freshwater rotifers of *Brachionus* genus were proposed, which constitute complete food for sander larvae in natural conditions of natural reservoirs. By size characteristics, these objects are half the size of brine shrimp entomostracans. Rotifers were grown in tanks with a volume of more than 1 m<sup>3</sup>. As a food for rotifers is used (*Chlorella vulgaris*) and (*Scenedesmus acutus*). Subsequent growing of larvae to an average mass of 250 mg was done on live cladoceran without use of expensive artificial feeds. During the works performed, it was found that the survival rate of sander larvae grown on live feeds was 50-65%, which is 1.4 times higher than on the dry starting feed. Thus, the economic expediency of the “Blue Bioeconomy” technology, using small living objects in feeding of pike-perch fingerlings, has been shown that makes it possible to reduce losses of larvae in the early stages of development.

## **EXPERT ESTIMATION OF PRODUCTION CHARACTERISTICS OF ECOSYSTEMS OF SMALL LAKES WITH THE HELP OF REGRESSION MODELS**

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Such parameters of lake ecosystems as total phosphorus concentration and phytoplankton production are the most important for characterizing the reservoir's productive potential. Usually, obtaining this information requires regular field observations and laboratory analysis, which is not always possible. However, it is possible to build expert systems based on the analysis of existing information on lake ecosystems to determine these parameters.

The aim of this paper is to construct regression models for reliable prediction of such important parameters of small lake ecosystems as the concentration of total phosphorus and phytoplankton production (primary production).

In this work we relied on information from the limnological database of small lakes that we built. It was based on published research data, conducted by the staff of the Limnological of the Russian Academy of Sciences (Saint-Petersburg) in 1975-1992.

This database includes information on 21 small lakes (surface area ranged from 0.04 to 14.2 km<sup>2</sup>) located on the Karelian Isthmus (the Leningrad region, Russian Federation) and in the eastern part of the Republic of Latvia (Latgale).

The database consists of 63 records (rows) and contains 20 parameters (columns). All lakes included in it were investigated during at least one growing season (ice-free period); morphometric, hydrological, hydrochemical and hydrobiological characteristics (parameters) are average estimates for the specified season of the year.

As a method for the implementation of expert estimation (prediction), a linear multi-regression model (ridge regression) and a neural network regression model were chosen. As predictors, only those parameters that were easily determined were selected. Therefore, these models are more focused on the practical application of the results obtained than on clarifying the nature of the phenomenon. Therefore, we were not interested in the specific kind of dependencies between variables. The main attention was paid to the search for solutions on the basis of which reliable forecasts could be built.

The result of this work is the construction of multi-regression models for predicting the average values of total phosphorus and primary

production, the relative standard error of which does not exceed 15%. Predictions made by neural network regression models turned out to be more accurate, their relative standard error does not exceed 10%. The constructed neural network models are stored as C ++ codes.

The models constructed by us can be recommended for practical use by specialists of limnologists and ichthyologists to assess the potential production of small lakes.

## **THE COMPACT RECIRCULATING AQUACULTURE SYSTEMS (RAS) FOR FISH FARMING**

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Prydniprovska region has a favorable base for the development of aquaculture. The solution of the problem of further development of the fisheries is possible only through an integrated approach in the development of modern fishery technology in Dnieper reservoir. Implementation of advanced European technologies to the manufacturing process of growing fish in our country will help change the situation in the fishing industry and ensure its stable development. Improvement of fishery technologies in Dnieper reservoir will increase the commercial output of fish products and restore the natural ichthyocomplex of the reservoir.

Today a promising direction is use of modern technology of growing fish in recirculating aquaculture system (RAS). Since these technological aspects provide the possibility of maximum control over cultivation parameters, they allow receiving quality biological production of fish.

We have developed a scheme and justified the use of RAS at growing young fish to increase resistance of carp and quality of biological products. It was found that RAS with working volume of 500 liters each, allow growing 1 thousand yearlings of carp species with a mass of 50 g. For the first time, it was developed and proposed

biotechnology of growing fingerling of carp using RAS and feeding carp yearlings with mixture of natural feed to adapt them to water conditions, followed by stocking reservoirs.

RAS has a tank 1 for culturing fish, mechanical and biological filters, pump, reflector, column for ozonation, ozonizer, aeration column, supply line of treated water, line for water discharge, an additional source of energy, solar panels. Expanded clay is used as a biological filter.

Our experimental work on RAS in the future will allow holding the species of fish that are characterized by relatively high requirements for cultivation. That is, the RAS can be used in the future for obtaining viable young native fish species, populations of which are suppressed such as zander, sterlet, pike.

It should be noted that growing fish in developed RAS can be combined with the cultivation of useful crops (aquaponics). The use of plants in integrated RAS allows full use of fishery area and creates additional financial income from crop sales.

The compact RAS installation for growing fish larvae was designed in order to obtain a viable youth of valuable fish species and its adaptation for further stocking ponds to restore native fish fauna. Implementation of the proposed scheme to the manufacturing process of fish-breeding farms will increase the rate of fingerling's survival after stocking reservoirs, improve the economic and biological indicators of fish.

## **QUICK-TEST METHOD FOR EVALUATING PHYSIOLOGICAL STATE OF STURGEONS UNDER THE AQUACULTURE CONDITIONS**

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Current intensification of fishery requires a new look at the issue of fish state diagnostics at all levels of breeding.

Strict requirements are imposed to the applied methods of evaluating fish physiological state in terms of their information content, injury-free and easy application and possibility to obtain results within a short period of time. The method of wedge-shape and marginal dehydration meets all these parameters; it allows for performing multi-parameter monitoring of biological objects' state and controlling deviation that happens during the process of their adaptation to the changing environment.

By applying the marginal dehydration method, we studied structural and optical properties of blood serum of the sturgeons – 10 individuals of two-year-old sterlet *Acipenser ruthenus* and 20 individuals of one-year-old and two-year-old Russian sturgeon *Acipenser gueldenstaedtii*. In order to perform the marginal dehydration, the blood serum was applied to the slide and covered with a coverslip; then it was dried at the room temperature. The morphology of the generated structures was studied with a standard light microscope under different magnification. Such morphological types of crystals as flaky, dendritic and transitional were registered in the analytic cells.

The analysis of the obtained results showed that the solid phase of the sterlets' blood serum was characterized by the presence of flaky structures (in all the samples). The transitional forms of crystals were observed less frequently (60% of cases). The dendritic structures were registered in 30% of cases. The one-year-old Russian sturgeons are also characterized by frequent flaky structures (90%). However, the number of registered dendritic and transitional crystal forms significantly increased (90% and 100% respectively) in comparison with the number of these forms in the two-year-old sterlets. The permanent morphological elements of the solid phase of the two-year-old Russian sturgeons' blood serum were dendritic and transitional structures (100% of cases). Flaky structures were registered in 2 out of 10 individuals under examination.

According to the literature data, the presence of dendritic and transitional crystal structures indicates normal physiological state of fish homeostasis and web-like and flaky forms indicate pathological processes. The conducted research showed that both structures indicating normal state (dendritic and transitional) and those

indicating pathological changes (flaky) are present in the analytic cells of the examined fish blood serum.

The blood serum morphology of the majority of the two-year-old Russian sturgeons indicated physiological state of their homeostasis. The one-year-old Russian sturgeons and two-year-old sterlets had markers of destructive changes, which may be related to significant accumulation of the metabolic products in their organisms due to the wintering. The flaky structures in the one-year-old Russian sturgeons may indicate a low level of adaptation mechanisms of young sturgeons being under stress.

Therefore, the obtained results show that the marginal dehydration method is informative enough for evaluating fish physiological state and its adaptation abilities. The findings indicate the possibility of applying this method in order to identify lowering resistance of the fish to the breeding conditions, to prevent this process and to take measures for improving the conditions in due time.

## **PROSPECTS OF USING THE RECIRCULATING AQUACULTURE SYSTEMS (RAS) IN THE AQUACULTURE OF EGYPT**

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The cultivation of fish in recirculating aquaculture systems (RAS) in many countries is actively practiced in recent years. They are used mainly for the cultivation of valuable fish species, such as eel, tilapia, sturgeons and others, since the production of marketable products of low-value fish is not very profitable. The costs for the acquisition, installation and operation of RAS are high, but this system has a number of advantages compared to traditional technology of growing with the direct water supply. The advantages of RAS include the possibility of providing the optimal hydrological conditions for fish rearing, ensuring high survival and growth rates of fish, applying high



stocking densities, increasing fish productivity and reducing the consumption of fresh water by almost 10 times compared to direct water supply. The last circumstance plays a decisive role in the further development of Egypt's industrial aquaculture.

The current level of aquaculture development in Egypt can be assessed as ineffective, since extensive cultivation methods are mainly used. Of the total volume of fish products, 60% are fish grown in natural water reservoirs without feeding at the low landing densities, 39% - fish cultivated in semi-intensive methods with partial feeding and only 1% fish production is produced under intensification conditions. Intensive methods of cultivation of water organisms in the country are applied extremely limited and make up no more than 10 pilot farm projects. Now the intensification of technological processes is carried out mainly due to the increase in the stocking densities and by using compound feeds in conditions of direct water supply.

Unfortunately, in Egypt the highly efficient RAS in industrial aquaculture are almost nonexistent. Scientific research in this direction in Egypt are not carried out. It should be noted that in the country there are practically no sources of fresh water, and available sources have water of low quality. In this regard there is an urgent need for innovative developments in the application of RAS in Egypt's aquaculture, which will allow the production of fish products to modern high levels and reduce dependence on unsuitable natural factors.

Achieving this goal will yield the following results:

- increasing the volume of aquaculture production in the country;
- a significant expansion of scales of intensive fish farming in Egypt (cultivation of marine and freshwater aquatic organisms);
- increasing the number of profitable hatcheries through using of modern high-developed technologies;
- transfer of semi-intensive tilapia farms into the category of intensive fish farming and increase their economic indicators;
- increasing the efficiency of technological processes in the early stages of ontogenesis (incubation, hatching, growing larvae) and

improving fish-breeding parameters: survival, growth rate and general resistance of fish;

- improving the quality of commercial fish products and meeting the requirements of environmental safety;

- improving the technologies on Egyptian fish farms to meet international standards (HACCP and GMP system standards).

## **SPECIFIC FEATURES OF BREEDING STERLETS TO MATURE STATE IN THE RECIRCULATING AQUACULTURE SYSTEM**

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**Introduction.** In the commercial sturgeon breeding, intensive methods of fish breeding, in particular with the use of the recirculating aquaculture system (RAS), have been widely spread as they have such significant advantages as full control over technological processes, independence from natural conditions, minimal water flow, environmental compliance, increasing volume of commercial output per unit of area, production in any season of the year and reduction of the time (by almost a half) for maturation and other processes in comparison with the once-through water supply system. In 2014, a sturgeon fishing company LLP “Caspian Royal Fish”, located in Atyrau, Kazakhstan, put in operation a RAS with Dutch equipment. The system receives water from the Ural River according to the standard procedure – mechanic and biological filters for disinfection (ultraviolet and ozonation) and oxygenation. The schemes of breeding sturgeons (in particular, sterlets) were worked out in order to harvest caviar.

**Study materials and methods.** The research was conducted in 2014-2017 and concentrated on studying sterlets that were bred in 37m<sup>3</sup> RAS ponds at temperatures of 21-22<sup>0</sup>C; their sex was identified through the ultrasound-scanning with MyLab Five Esaote that is

equipped with a linear sensor with a working area of 40mm and working frequency of 5-10 mHz. The sterlets were fed with Aller Aqua feeds - Aller Metabolica 4.5 mm, Aller Bronze 3 mm, Aller Bronze 4,5mm, Aller Sturgeon Rep EX 6 mm. The polarization index of fish eggs was examined by taking biopsy samples of gonads.

**Study results.** In 2014, the sturgeon farm received sterlet stock and in 2017 the first caviar was harvested from these 2.4-year-old sterlets. During the process of sterlet breeding, when the sterlets reached the mass of 500 g, the ultrasound-scanning was carried out in order to identify their sex. The identified males were grown up to the commercial weight of 800 g and sent for sale after preliminary 2-week wintering (pre-sale preparation) at a temperature of 7°C. The selected females were also grown up to the weight of 800 g and then another ultrasound-scanning was carried out in order to identify their maturity stage. The females with the 2nd and 3rd stages of gonads maturing were sent to further feeding with Aller Sturgeon Rep EX 6 mm. The mature fish with the 4th maturity stage was sent to the wintering unit where the water temperature was gradually lowered without any rapid change.

Artificial wintering of the females was carried out with once-through water supply at temperatures of 6-7 °C during 2-2.5 months. During this period, the fish were washed clean and matured up to the necessary stage of raw eggs for obtaining a high-quality caviar. When the wintering was over, the temperature was gradually raised from 7 to 14-16°C, thus becoming suitable for spawning, and the fish was kept 3 days under these conditions. The caviar was obtained according to S.B. Podushka's intravital method. Five different ways of the hormone injection were worked out while searching for the best options of gonadotropic stimulation of fish gonads maturation. The best results were obtained with 2-times injection of carp hypophysis hormone and using of Surfagon during the first injection and Raunatin during the second one in order to enhance the effect. After the first injection, the water temperature was gradually raised by 0.5-1°C.

**Conclusions.** The conducted research showed that the sterlets which were grown in the RAS ponds matured after 2.4 years for the first time and the caviar output was 10% of the body weight. We can expect that the period of these females' next maturation will be reduced to 0.5-1

year and the caviar output could increase. The obtained results indicate that sterlets grown in the recirculating aquaculture system mature faster than in the wild (5-6 years) or under the conditions of once-through water supply (3-4 years).

## **CURRENT STATE OF COMMERCIAL FISH FAUNA OF THE URAL RIVER WITHIN THE BOUNDARIES OF THE WEST KAZAKHSTAN REGION**

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**Introduction.** The Ural River is one of the main fishery reservoirs in Kazakhstan. The central and northern parts of its lower course that are 761 km long are located in the West Kazakhstan region. The waters play an important role for reproduction of fishing resources of the Ural-Caspian basin as it concentrates the main spawning grounds of sturgeons (more than 1000 ha), as well as about 5 thousand bottomland spawning areas of phytophilous fish. The spawning of both river and semi-anadromous fish takes place in the bottomland overflow. As the area is quite far from the sea, only the strongest fish comes there for spawning. Therefore, the natural reproduction of semi-anadromous and anadromous fish in this area is very important for improving the population qualitative composition in the region.

**Study materials and methods.** In order to study the fish fauna, materials were collected during the spring, summer and autumn periods of 2008-2016, and the study resources were populations of 9 fish species: common carp, pike-perch, asp, freshwater bream, european catfish, silver bream, blue bream, sabre carp and Volga pike-perch that are of commercial value.

**Study results.** The Ural commercial fishing resources have been decreasing from year to year: by 2016, the biomass of commercial fish decreased 5 times in comparison with 2008. The resources that decreased the most are of common carp (7.6 times), freshwater bream (7.2), blue bream (14), silver bream (3.8), sabre carp (2.1), asp (3.2),

pike-perch (4.1), Volga pike-perch (2.3) and european catfish (3,0 times).

The analysis of water content influence on forming fish resources shows that in low-water years the watering of bottomland spawning areas worsens, spawning areas become smaller resulting in less efficiency of natural reproduction. In comparison with 2007 that was optimal in terms of watering, the next low-water years led to the decrease in young fish yield capacity by 46% in 2008, 70% in 2009, 48% in 2010, 9% in 2011, 39.5% in 2012, 35.3% in 2013, 12.6% in 2014 and by 42.9% in 2015. As the natural reproduction and yield capacity of young fish went down in 2008-2013, in 2016 the fishing resources of these species decreased due to their smaller share in the populations aged 3, 4, 5, 6, 7 and 8 years. The research conducted in 2015-2016 showed that natural reproduction of sturgeons was significantly impaired – full absence of spawning was identified in all (50) natural spawning areas of the Ural River in the West Kazakhstan region.

Wide-spread amateur fishermen and illegal fishery also have a negative impact on spawning. A densely populated basin of the Ural River is a home for dozens of thousands of amateur fishermen and some of them are poachers. Due to the long-standing Ural traditions of fishing, these people are quite high-skilled. Moreover, the number of amateur fishermen is not registered and, of course, illegal catch is not taken into account.

**Conclusions.** In order to improve the conditions of spawning semi-anadromous and river fish, it is necessary to hold regular activities on amelioration of spawning areas, to deepen connecting channels and migration paths annually and to raise the efficiency of artificial reproduction of sturgeons and common carps. For providing efficient conservation of fish resources of the Ural River and adjacent areas of the Caspian sea, it is proposed to develop and introduce a high-technology system of full visual control over the water areas with unmanned aerial vehicles (UAV) of midsize and small class in the places of feeding, migration and spawning of valuable fish; to develop and introduce a mechanism for analyzing obtained visual data and for prompt and efficient response on the registered cases of illegal catches, destruction of spawning areas, contamination, etc.

**THE DYNAMICS OF GENETIC STRUCTURE OF ROUND  
GOBY *NEOGOBIUS MELANOSTOMUS* (PALLAS)  
GROUPINGS IN THE ODESSA BAY OF THE BLACK SEA  
UTILIZING BIOCHEMICAL MARKER LOCI**

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To investigate a genetic structure of intraspecific fish groupings, enzyme systems are commonly used as markers due to their simplicity in histochemical detection and accuracy in reflecting a level of species variability. A significant amount of data have been collected about population genetic structure for many species of commercial fish in terms of biochemical marker loci during recent decades. Unfortunately, population genetic structure of round goby *Neogobius melanostomus* (Pallas) groupings, which is a crucial component of food chain in aquatic coastal ecosystems and a subject for commercial fishing, is still poorly studied. Considering this, the goal of this study is conducting investigation about spatial and temporal dynamics for genetic structure of round goby *Neogobius melanostomus* (Pallas) groupings in the Odessa Bay of the Black Sea utilizing biochemical marker loci.

Round goby individuals were caught in the southern (localities "Biostation") and northern (localities "Leski") parts of the Odessa Bay during 2011-2016 years and were chosen as a subject for that study. The spectrum of molecular forms for biochemical markers (esterases and miogenes) have been analyzed by electrophoretic separation in 7% polyacrylamide gel for individual homogenates derived from round goby muscle tissue (20 males and 20 females). The identification of molecular forms for biochemical markers was performed by classical histological methods.

The electrophoretic spectrum for esterases in muscle tissue show the presence of polymorphisms for 2<sup>nd</sup> and 3<sup>rd</sup> loci. The analysis of round goby molecular forms for miogenes revealed a huge number of electromorphs. Utilizing the most appropriate and simple genetic scheme to interpret these results “one gene – one dyed gel zone”, the maximum number of loci have been obtained that corresponding to coding sequences of miogenes across investigated individuals. According to results, 14 genes have been determined for round goby groupings. The polymorphic loci have been detected for 3<sup>rd</sup> and 7<sup>th</sup> loci.

The analysis of genetic structure for round goby *Neogobius melanostomus* (Pallas) groupings in the Odessa Bay revealed statistically significant differences between allele frequencies for two polymorphic loci – esterase 2 and miogene 3 in 2013-2014 years between individuals that were living in the northern and the southern part of the Odessa Bay. This fact proves that round goby groupings in the Odessa Bay are partially isolated. The dynamics of genetic structure for round gobies from localities "Biostation" pointed on significant changes in terms of allele frequencies of polymorphic loci for esterases and myogenes in 2014 and 2015 years. There is no statistically significant changes in frequencies of polymorphic genes for esterases and miogenes in round goby groupings that have been detected in another years.

**CONSERVATION HERPETOCULTURE OF *BOMBINA BOMBINA* IN RECIRCULATING AQUACULTURE SYSTEM (RAS) IN LATVIA: LARVAE KEEPING TECHNOLOGY**

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Aquaculture is a target cultivation of hydrobionts in a regulated environment (Plotnikov et al., 2017). One of such purposes can be the nature protection, including the cultivated species and its natural populations: as a reduction in the use of natural populations by humans, and by the release into nature of bred and cultivated individuals to enhance or restore the natural populations. Now, these methods are widely used in the conservation of herpetofauna.

Hydrobiont Fire-bellied toad *Bombina bombina* is a rare and protected species of amphibians in Europe. In Latvia, there is the northern border of the European range of *B.bombina* (Kuzmin et al. 2008). For a long time, only two populations of *B.bombina* (Siliņš, Lamsters 1934), with a total number of up to 20 singing males, were known in Latvia. In the Red Data Book of Latvia (2003) *B.bombina* is listed in the category "1" - "very rare species". Currently, there are known seven species populations in Latvia, the largest of which is the Demene population. Despite this, the number of *B.bombina* and its area in Latvia is extremely low: all localities found are at a distance of not more than 20 km from the borders with Lithuania and Belarus.

The main natural limiting factors for *B.bombina* in Latvia are the overgrowing of water habitats and the cold climate that slows the development of eggs and larvae, increases the risks of wintering in shelters on land, slows down the reproduction and growth rates of *B.bombina*. Limiting factors of anthropogenic origin include melioration, destruction *Castor fiber* dams, transformation of habitats and migration routes; predation and competition from invasive species introduced by human: exotic turtles species (Pupins 2007, Pupins, Pupina 2011, Pupina, Pupins 2016), *Nyctereutes procyonoides*, *Carassius carassius*, *Perccottus glenii* (Pupina et al., 2015), invasion of parasites *Batrachochytrium dendrobatidis* (found in Rigas Zoo in 2017) and *B.salamandrivorans*. Most of these limiting factors are dangerous for *B.bombina* at all stages of their development (eggs, larvae, juveniles, adults).



In 2006, *B.bombina* Conservation Plan in Latvia was developed and approved (Pupins, Pupina 2006). One of its main activities is the breeding and keeping of autochthonous *B.bombina* in herpetoculture and releasing in wild.

We incubated eggs and kept *B.bombina* larvae in the aqua-herpetoculture (cultivation of herpetofauna hydrobionts) in Recirculating Aquaculture System (RAS in the next text) in our Latvian Rare Amphibian and Reptile Centre from 2010 (Pupina, Pupins 2014). The conditions of the keeping of *B.bombina* larvae in present time in our RAS are:

Pool: from food plastic of white colour, with built-in crane at the bottom. The total volume of the water part of one basin is 100 x 120 x 30 cm. The height of the above-water part, open from the front for service is 40 cm. Water level: 25 cm. Illumination: UVB lamp (ReptiGlo 5), incandescent lamp 40 W at a height of 20 cm. Water temperature: 23-25 ° C. Daylight hours: 14 hours. The total volume of water used in one basin is 360 l. Filter: homemade plastic biofilter with volume 20 l, filler - plastic fishing line and foam mats. The filtration rate is 100 l / h. Change of water with washing of filters is made once a month. The pool is equipped with an air compressor. In the pool, there are plastic plants. The density of planting *B.bombina* - 300 larvae in one pool. Feed: fish food "Vipan". Feeding: twice a day. Before the metamorphosis, 4 rafts of foam and cork are placed in the pool.

In a result 4 069 *B.bombina* juveniles, kept according to this technology, were released in wild in 27 new created or improved ponds in micro-reserves Katriniski and Strauti in 2012-2014 (Project LIFE-HerpetoLatvia), as well 1 000 in 2 ponds in Ainavas (South-East Latvia) (Pupina, Pupins 2016). In 2015 and 2016, we there observed strong breeding populations, that confirms the effectiveness of using the described RAS aquaculture technology for preserving *B.bombina* in Latvia. The technology will be used in 2018 creating RAS for *B.bombina* exhibition basin in Latgales Zoo according the Latvian Environmental Protection Fund co-financed Project "LV100-Eo-LV200".

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## **CONSERVATION HERPETOCULTURE OF *EMYS ORBICULARIS* IN RECIRCULATING AQUACULTURE SYSTEM (RAS) IN LATVIA: KEEPING TECHNOLOGY IN FIRST YEAR AFTER HATCHLING**

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The hydrobiont European pond turtle *Emys orbicularis* is a rare and protected species of reptiles in Europe. The most northern border of the European range of *E.orbicularis* (Berdnikovs 1999) passes along Latvia. The first published findings of *E.orbicularis* in Latvia date back to 1820 (Siliņš, Lamsters 1934). In the Red Data Book of Latvia (2003) *E.orbicularis* is listed as "0" category - "extinct species". Currently, the *E.orbicularis* population in Latvia is extremely small (Meeske et al., 2006, Pupins, Pupina 2008), the total number of *E.orbicularis* individuals in Latvia is estimated to be no more than a half of *E.orbicularis* in the more southern Lithuania (Red Data Book of Lithuania 2007) , that is, no more than 250 - 300 adult individuals.

The main limiting factor for *E.orbicularis* in Latvia is the cold climate that does not allow the successful development of deferred eggs, increases the risks of wintering under ice, slows the reproduction and growth tempo of *E.orbicularis*, thereby significantly prolonging their juvenile vulnerability to predators. The limiting factors of anthropogenic origin include the transformation of habitats and migration routes; predation, competition and introduction of parasites from invasive species introduced by human: exotic turtles species (Pupins 2007, Pupins, Pupina 2011, Pupina, Pupins 2016), *Neovison vison*, *Nyctereutes procyonoides*, *Ondatra zibeticus*, *Carassius*

*carassius*, *Perccottus glenii* (Pupina et al. 2015). All these limiting factors are most dangerous for *E.orbicularis* in the early stages of their development (eggs, hatchlings, young juveniles) and their danger decreases for semi-adult and adult *E.orbicularis*.

Therefore, in 2007 *E.orbicularis* Conservation Plan in Latvia was officially approved by Ministry of Environment (Pupins, Pupina 2007). One of its main activities is the breeding and keeping of autochthonous *E.orbicularis* in herpetoculture and releasing of semi-adult and young adult *E.orbicularis* in wild. Such release of grown and already big *E.orbicularis* allows minimizing many of the above-described risks.

We kept *E.orbicularis* in aqua-herpetoculture (cultivation of herpetofauna hydrobionts) in Recirculating Aquaculture System (RAS in next text) and in our Latvian Rare Amphibian and Reptile Centre since 1984 (Pupins, Pupina 2014a,b, Pupins et al. 2016). During this time, we tested different conditions of *E.orbicularis* keeping and found that the most critical is the breeding of *E.orbicularis* during the first two years of life. Conditions of the first-year keeping of *E.orbicularis* aqua-herpetoculture in the present time in our RAS are:

Pool: from the food plastic of white colour, with a built-in crane at the bottom. The total volume of the water part of one basin is 100 x 120 x 40 cm. The height of the above-water part, opened for keeper service from the front, is ~40 cm. Water level: during the two first months - 5 cm, two subsequent months - 10 cm, then 25 cm. Illumination: LED lamp 5 W, UVB lamp (ReptiGlo 10), 2 incandescent lamps of 40 W at a height of 20 cm are located above the place for sun-basking from a solid plasticized mesh. Water temperature: 25-27°C. Daylight: 16 hours. The volume of water used in one basin is 300 l, the volume of biofilter is 50 l. The swimming pool is equipped with an air compressor. RAS: 1) homemade plastic biofilter, filler - plastic fishing line and foam mats, and 2) consequently mounted canister filter with built-in UV sterilizer, filler - foam mats. The filtration rate is ~180 l/h. Filtered water comes on an algae scrubber S=900 cm<sup>2</sup>. Change of water with washing of filters is made once a month. In the pool, there is an island for sun-basking, many plastic plants. The density of planting juvenile of *E.orbicularis* is 20 turtles per pool.

Food: main - moist canned food "KitKat" (chicken), dried Gammaridae, once a week additionally *Shelfordella tartara*, *Zophobas morio*. Feeding: once a day in the morning, until satiety. In the pool, there are always many pieces of a calcareous stone for turtles and sepia cuttlebone.

Intensive cultivation of *E.orbicularis* under the conditions described allows them to grow much faster in the first year than in the natural conditions. When hatching *E.orbicularis* average, the length of their carapace is CL = 26.13 mm, average body weight BW = 5.23 g.; After 8 months of such cultivation, CL = 55.41 mm (an increase of 2.12 times,  $p < 0.05$ ), BW = 37.13 g (increase in 7.1 times,  $p < 0.05$ ).

The first 42 *E.orbicularis* grown by this technology became already young adults and were released in the wild nature in the Nature park Silene in 2014 (Project LIFE-HerpetoLatvia). They successfully adapted, wintered and were observed here in 2015 and 2016, that confirms the effectiveness of using the described technology of aquaculture RAS for the conservation of *E.orbicularis* in Latvia.

The next releasing in wild of 100 adult *E.orbicularis* is planned in 2018 - 2028 according the Latvian Environmental Protection Fund co-financed Project "LV100-Eo-LV200".

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## **RECIRCULATING AQUACULTURE SYSTEMS (RAS) IN SMALL ZOO EXHIBITION: APPROACH OF NATURE-FRIENDLY LATGALES ZOO, DAUGAVPILS, LATVIA**

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Aquaculture (hereinafter referred to as AC) is a target cultivation of hydrobionts in a regulated environment (Plotnikov et al., 2017). The goal of the AC is to satisfy the individual needs of a person by providing certain products. According to the purpose, requirements and product, all ACs can be divided into the following 4 types:

1. Material-productive (MAC). Purpose: to satisfy the human need for substances (food, raw materials for industry, etc.). Product: biomass of hydrobionts. Example: the most aquaculture farms.
2. Function-productive (FAC). Purpose: to satisfy the human need for the processes (removing of "weedy" fish in the pond, the desired functioning of ecosystems and populations, etc.). Product: functional service of hydrobionts. Example: aquaculture for the restoration of populations in the nature conservation projects.
3. Information-productive (IAC). Purpose: to satisfy the human need for knowledge (genetics of hydrobionts, their behaviour, physiology, etc.). Product: information on hydrobionts. Example: laboratory aquaculture of *Danio rerio*.
4. Emotion-productive (EAC). Purpose: to satisfy a person's need for emotions (aggression, joy, fear, pleasure, etc.). Product: emotions caused by hydrobionts. Example: aquaculture of a home aquarium.

One and the same species of hydrobionts can be cultivated in any type of aquaculture, while, despite the fact that the basic biological, social and psychological needs of the hydrobiont remain the same, the technologies for their cultivation differ significantly. The exhibition aquaculture of Zoos refers to 3 and 4 types.

Latgales Zoo is a very small Nature Friendly Zoo and has its own conceptual principles of organization, according to which its RAS solve non-standard tasks. These are, for example:

1. The keeping of several species of hydrobionts in one basin with one RAS (for example, in the "Tropical Lake" basin there

- are 2 species of crocodiles, 3 species of turtles, 10 species of fishes).
2. The need for a nature-like masking of all technical elements of RAS from visitors.
  3. Ensuring the necessary water turnover with the undesirability of creating a perceptible current in the basin (for example, for water turtles).
  4. Provision of water exchange of all zones of the basin with the undesirability of creating a tangible current in the basin.
  5. Increased efficiency of RAS with small room dimensions.
  6. Ergonomics of work with RAS for employees, etc.

The successful solution of these tasks allowed creating a system RAS in Nature-Friendly Latgales Zoo and successful keeping of hydrobionts for 25 years (110 species and more than 500 individuals in 2017), that attracts up to 45 000 visitors per year. The technology will be used in 2018 creating RAS for hydrobionts of 100 species 16 exhibition basins in Latgales Zoo according the Latvian Environmental Protection Fund co-financed Project “LV100-Eo-LV200”.

## **VETERINARY MEDICATION ANTHELMINTIC OF ACTION "DIPLOCIDUM" FOR FIGHT AGAINST DIPLOSTOMUM OF FISHS**

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## **ВЕТЕРИНАРНЫЙ ПРЕПАРАТ АНТИГЕЛЬМИНТНОГО ДЕЙСТВИЯ «ДИПЛОЦИД» ДЛЯ БОРЬБЫ С ДИПЛОСТОМОЗОМ РЫБ**

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Ветеринарный препарат антигельминтного действия «Диплоцид» был разработан сотрудниками лаборатории

болезней рыб РУП «Институт рыбного хозяйства» Национальной Академии наук Беларуси в 2014 году. Препарат предназначен для лечения, а так же профилактики диплостомоза рыб. Полностью отвечает требованиям, предъявляемым к препаратам антигельминтного действия. В экспериментах и на практике показал себя как высокоэффективное средство против церкарий и метацеркарий трематод р. *Diplostomum*. Является не токсичным для разновозрастных и разно видовых групп рыб, быстро метаболизируется в печени и выводится из организма. После применения «Диплоцида», товарную продукцию можно реализовывать через 20 суток. Имеет несколько вариантов применения, которые без проблем реализуются в производственных условиях и не требуют каких – либо затрат. Стоимость 1 кг препарата составляет около 40 \$, что на порядок ниже импортных аналогов.

Для лечебных мероприятий препарат применяется двумя методами:

#### 1. Метод группового скармливания.

Доза вводимого препарата в комбикорм зависит от вида рыб. Для рыб сем. *Карповые* - 4,0 кг препарата, рыб сем. *Осетровые* и *Лососевые* - 13,0 кг препарата на 1 тонну комбикорма. Лечебный комбикорм задают рыбам из расчета: 5% от массы тела для рыб сем. *Карповые*; 1,5% от массы тела для рыб сем. *Осетровые* и *Лососевые*. Большую рыбу прокармливают двукратно, с интервалом 20 дней.

#### 2. Метод лечебных ванн.

Препарат разводится из расчета 20 мг препарата на 1 л воды (20 г/м<sup>3</sup>), экспозиция – 60 мин.

Препарат так же может применяться в качестве профилактического средства. Для этого раствор, с концентрацией 20 мкг препарата на 1 л (20 мг на м<sup>3</sup>) вносят по поверхности воды в прудах. Раствор вносится на мелководных участках, где отмечаются массовые скопления моллюсков.

При использовании лечебных ванн или внесении по поверхности воды, следует учитывать то, что препарат является

слаборастворимым в воде. Поэтому для приготовления ванн, сначала нужно приготовить маточный раствор – залить необходимое количество препарата теплой водой (40-50 °С) и тщательно растереть, до образования молочно-белой жидкости. Получившийся раствор, в зависимости от способа и метода применения необходимо разбавить водой до необходимой концентрации.

## **REARING OF LARVAE OF COMMON CARP CYPRINUS CARPIO ON DIFFERENT DIETS IN CLOSED RECIRCULATORY SYSTEM ON DIFFERENT FEEDING REGIMES**

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The aims of the present paper is to analyze the carp larvae initial feeding habits via a pilot experiment on different diets and to evaluate the efficiency of the changeover from live food to artificial. The experiment was conducted in the recirculation system in two steps. At the first stage, the changeover to the artificial food was carried out for 12 days, on each day with a separate subgroup. At the second stage, two starters were compared; each starter was fed to a separate control group from the 12th to the 26th day. In addition, put on weight was assessed.

The research showed that the most efficient diet for the larvae in closed recirculation system is as following: to feed the carp larvae for 18 days on artemia salina vitalized larvae and on artificial Aller Futura MP EX 0,2 mm from the seventh day. The described feeding schedule has a higher coefficient of adaptivity for the larvae than the other commonly used schedules. The experiment on the control group lasted for four weeks and viability was 92 per cent. This proves that the carp larvae is possible to rear in the artificial habitat with great success if the population density is about 14 fish per liter.



Nevertheless, despite the fact that on the 12th day of the experiment the carp larvae in the closed system were twice shorter than the control group in the pond, it was not considered as a serious obstacle to the further growth. In the autumn, the larvae in the closed system weighed on average 195.5 grams, while in the pond only 22.2 grams.

The experiment on feeding the carp larvae from the beginning on prestarter failed. The experiment on feeding the larvae on the prestarter Perla Larva Proactive 6.0 from the third day gave encouraging growing results at first, but failed on the sixth day with the cease to feed on artemia; the mortality was 32 per cent.

Comparing the artificial starters, only Aller Futura MP EX gave tangible result; the carp larvae viability on Veronesi VITA starter was only 36.6 per cent.

The novelty of the research lies in the described feeding schedule. Thus, the larvae significant growth in the artificial habitat proved that it is feasible to rear carp larvae in closed recirculation systems and produce advanced larvae for fish farms.

## **ROLE OF EPIGENETICS IN AQUACULTEURE**

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Epigenetics in contrast to genetics study the stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence. Epigenetic marks such as DNA methylation, histone modifications and chromatin conformational changes can be triggered by environmental stimuli and can persist throughout life or across multiple generations. Methylation of DNA is a process in which a methyl group (-CH<sub>3</sub>) is added to the carbon 5 position of a cytosine residue, mostly within a CpG dinucleotide, although the mechanism has also been reported outside of the CpG context. Through conformational changes to DNA and histone structures, the level of methylation at the promoter region is usually inversely correlated with

the transcriptional activity of a gene. The «resetting» of epigenetic status occurs *in each generation by* extensive demethylation and subsequent *de novo* DNA methylation during gametogenesis and early development, mostly symmetric CpG sequences.

The effect of environmental factors on epigenetic inheritance is equally important to the aquaculture. The environmentally-induced DNA methylation causes phenotypic heterogeneity. There are a number of nutritional stimuli that are known to affect the differentiation of fish cells during development and possibly do so by changing the epigenetic markings in particular cells. For example, it is known that a diet can be influence on body color, a lack of vitamins in sea bass has can be disrupt the temporal sequence and coordination of growth factor gene expression which affects the differentiation of osteoblasts so that some are converted into adipocytes resulting in deformities. The conversion of osteoblasts into adipocytes can also occur when dietary highly unsaturated fatty acid levels are too high during the early stages of the development of marine fish. Salmon pre-adipocytes seem to possess the ability to differentiate into cells of the immune lineage depending on environmental conditions. However, epigenetic cues affecting cellular differentiation during the development of fish have not yet been investigated. The regulatory effects of DNA methylation could be divide: specific (a particular gene activity is directly influenced by methylation of its regulatory regions), and general (methylation causes changes in the chromatin structure).



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