

# БИОТЕХНОЛОГИЧЕСКИЕ АСПЕКТЫ АКВАКУЛЬТУРЫ

## BIOCHEMICAL POLYMORPHISM OF BLOOD PLASMA ENZYMES OF DIFFERENT AGE GROUPS OF RAINBOW TROUT (*O. MYKISS*) ON THE EXAMPLE OF A LOCAL CHERNIVTSI STOCK

O. Bielikova, S. Tarasjuk, A. Mruk, A. Mariutsa, O. Zaloilo

*Institute of Fisheries NAAS of Ukraine, Kyiv, Ukraine, belikova.e.y@gmail.com*

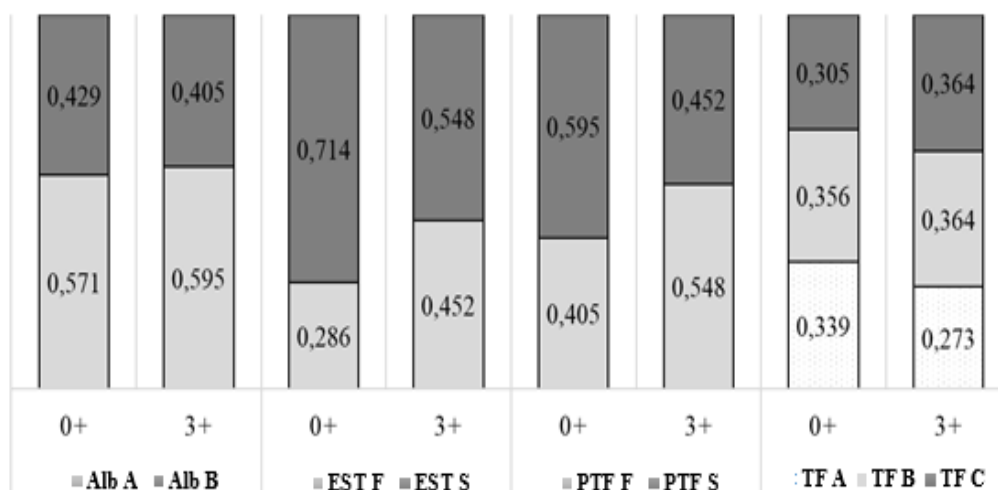
Breeding of the rainbow trout in conditions of industrial aquaculture occupies the main share in the production of salmonids both in Ukraine and in the world [1-4], since this species is not only highly productive, but also has significant commercial value caused by considerable consumer interest [5]. Farms that cultivate such a commercially valuable and expensive fish species as rainbow trout need a detailed analysis of the genetic diversity of their stocks at the molecular level in order to conduct breeding works. The genetic potential of local trout stocks can be assessed based on the analysis of the polymorphism of genetic-biochemical markers (isoenzymes). Such markers allow studying not only the diversity and structure of the population, but also allow analyzing the processes of adaptation of the organism to changes in conditions during fish farming at the genetic level. This is due to the fact that allozyme markers give the concept of changes in proteins with known biochemical functions at the metabolic level [6-8]. Comprehensive systematic studies using genetic-biochemical systems will allow tracking stability characteristics at various stages of the life cycle and variability of fish under the effect of various environmental factors and conditions of keeping fish in aquaculture [9-10].

Therefore, the aim of the work was to study the allelic and genotypic diversity of the local stocks of the rainbow trout using genetic-biochemical markers.

**Materials and methods.** Isoenzyme analysis of the rainbow trout (*O. mykiss*) plasma proteins was performed on the age-3+ (n = 21) and age-0+ (n = 21) fish of a Chernivtsi local stock (Beregomet township). Following genetic-biochemical systems were selected for polymorphism analysis: transferrin (TF), posttransferin (PTF), albumin (ALB) and esterase (EST) (EC 3.1.1.1.). Electrophoretic separation of blood plasma proteins was carried out in 8% polyacrylamide gel (PAGE) with further histochemical staining [11-13]. Statistical analysis of allele and genotype frequency distribution, calculation of heterozygosity values and F-statistics were performed in BIOSIS-1 and GelStat. The G<sub>st</sub> differentiation index for the Tf locus was calculated according to the Nei model (1975) [14-15].

**Results and discussion.** The genetic structure and polymorphism at the esterase, albumin, posttransferin and transferrin loci for different age groups of the rainbow trout of a local stock from the Chernivtsi region were characterized according to the results of studies using genetic and biochemical systems. Two allelic variants (slow and fast migrating) were found in the studied trout groups at the Est, Alb, and Ptf loci (Fig. 1), whose frequencies were equally distributed.

The of Est, Alb, and Ptf allele frequencies ranged from 0.4 to 0.6; except the Est allele frequencies in the age 0+ group, where the slow-migrating Est S allele had a higher frequency (0.714) than the fast Est F allele (0.286). Three alleles: Tf A, Tf B, and Tf C were found at the transferrin locus. It should be noted that the Tf allele frequencies in two studied age groups were in equal proportions. Maintenance of the fast to slow-migrating allele ratio in dynamics indicates that the local stock is in a stable state, therefore, these trout groups can be used as producers for breeding works.



**Fig. 1. – Distribution of allelic frequencies of isoenzyme loci (ALB, EST, PTF, TF) in age 0+ and age 3+ groups of the rainbow trout**

The deviation of the actual genotype frequencies from theoretically expected ones for the studied loci was calculated using the Pearson criterion with a 5% level of error significance (Table 1.) in order to assess the stability of the local stock of the rainbow trout according to Hardy-Weinberg equilibrium.

The actual frequency of heterozygous genotypes by albumin loci in the age 0+ group exceeded the expected value by 17.7%. At the esterase and posttransferin loci in this group, the actual frequency of heterozygous genotypes predominated over their expected frequency (6.8% and 4.2%, respectively).

**Table 1. – Distribution of genotypes frequencies**

Loci	Allele	G <sub>o</sub>	G <sub>e</sub>	$\chi^2$	p-level	Loci	Allele	G <sub>o</sub>	G <sub>e</sub>	$\chi^2$	p-level
Age 0+						Age 3+					
EST	FF	1	1.718	0.583	0.747 p>0.05	EST	FF	4	4.290	0.069	0.966 p>0.05
	FS	10	8.577				FS	11	10.403		
	SS	10	10.706				SS	6	6.306		
Alb	AA	5	6.847	2.737	0.254 p>0.05	Alb	AA	6	7.435	1.702	0.427 p>0.05
	AB	14	10.288				AB	13	10.121		
	BB	2	3.865				BB	2	3.445		
PTF	FF	3	3.445	0.159	0.924 p>0.05	PTF	FF	7	6.306	0.383	0.826 p>0.05
	FS	11	10.121				FS	9	10.403		
	SS	7	7.435				SS	5	4.290		
TF	ABC	17	9.188	11.965	0.102 p>0.05	TF	ABC	14	9.188	7.844	0.347 p>0.05
	AB	3	6.891				AC	1	6.891		
	BC	1	4.922				BB	1	0.984		
							BC	5	3.938		

Note: G<sub>o</sub>- observed genotypes; G<sub>e</sub>- expected genotypes

In both age 0+ and 3+ groups, for the albumin locus, there was also a predominance of the actual frequency of heterozygous genotypes over the expected one by 13.7%. As for the esterase locus, the observed frequency of heterozygous genotypes was 2.8% higher than expected.

It was previously shown [16] that the number of heterogeneous genotypes by the esterase loci in older age groups is greater than in younger ones.

The predominance in favor of heterozygous genotypes, which have a number of advantages over homozygotes as noted by many authors [17-19], may indirectly indicate a selection towards an increase in heterozygosity, and, consequently, outbredness of the population.

The genotype frequencies at the transferrin locus were calculated using the equation for multiallelic systems. In both age groups, the observed frequency of the ABC genotype was dominated over the expected one according to the splitting  $(3 + 1)^n$  by 37% and 23% in the age 0+ and age 3+ groups, respectively.

The  $\chi^2$  values indicate that older age groups had a more balanced state in accordance with the Hardy-Weinberg equation due to an increase in the number of heterozygotes. According to the distribution of genotype frequencies in the studied groups, no statistically significant deviations from the theoretically expected at the selected loci were observed ( $p > 0.05$ ).

The analysis of the level of heterozygosity for biallelic loci (Fig. 2) was carried out using the fixation index, average values of the observed and expected heterozygosity. The average values of the expected heterozygosity  $H_e$  in the groups age 0+ and age 3+ were 0.460 and 0.491, respectively, while the average values of the observed heterozygosity  $H_o$  were 0.556 and 0.524. Since the value of observed heterozygosity is higher than expected, it can be concluded that the inbreeding phenomenon is not characteristic of the studied populations.

The fixation index in both age groups assumed negative values (-0.205 and -0.070 in the groups of age 0+ and age 3+, respectively), which indicated the predominance of heterozygotes in both age groups. It should be noted that heterozygotes were more prevalent in the age 0+ group than in the age 3+ group.

As for the albumin locus, heterozygous genotypes prevailed with the greatest extent over homozygous ones, so  $F_{is}$  ranged from 0.285 to 0.361. As for the Ptf locus, a deficiency of heterozygotes was observed in the age 3+ group ( $F_{is} = 0.133$ ). Based on the values of the fixation index, we can conclude that the equilibrium state of hetero- and homozygotes for the two studied age groups was observed by other studied isoenzyme loci.

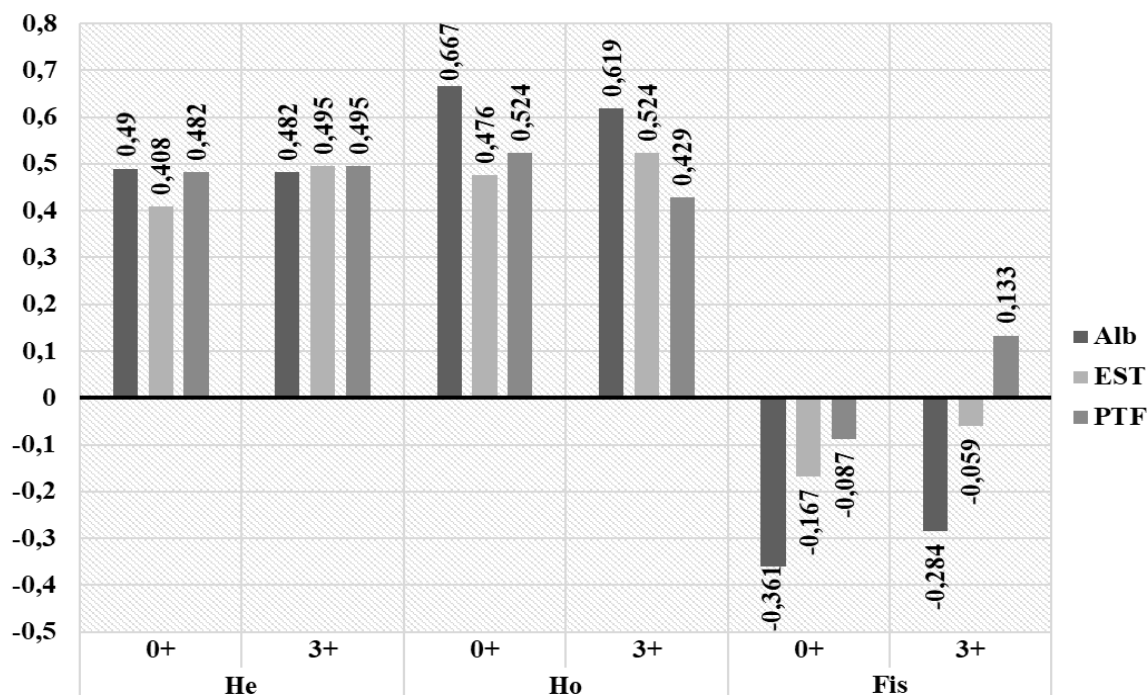


Fig. 2. – Fixation index, observed and expected heterozygosity in the local rainbow trout stock

Wright's F-statistics were calculated to assess the degree of differentiation of the studied groups at three levels of genetic variation (Table 2.). The subpopulation  $F_{st}$  was 0.017 indicating an average level of divergence of age groups in this local stock. The population  $F_{it}$  had a negative value (-0.117), which indicated an excess of heterozygotes in the studied rainbow trout groups.

Table 2. – Assessment of the differentiation degree by Wright's F-statistic

	$F_{is}$	$F_{it}$	$F_{st}$	$H_t$	$H_i$	$H_s$
Alb	-0,323	-0,322	0,001	0,486	0,643	0,486
EST	-0,107	-0,074	0,030	0,466	0,500	0,452
Post TF(PTF)	0,026	0,045	0,020	0,499	0,476	0,489
Mean	-0,135	-0,117	0,017	0,482	0,488	0,470

Since Tf in the rainbow trout is a multiallelic locus, the differentiation index  $G_{st}$  (an analogue of the F statistic) was calculated according to the Nei model (1975). For the transferrin locus,  $G_{st}$  was equal to 0.003 suggesting no variation at this locus between the two age groups and that the studied age groups were affected by stabilizing selection.

**Conclusions.** An analysis of the polymorphism of blood plasma enzymes of different age groups of the rainbow trout of Chernivtsi local stock was performed using genetic and biochemical markers. The indicators of genetic variation were also calculated and a high level of heterozygosity was noted. The average value of the fixation index (-0.14) indicated the absence of inbreeding in the studied local rainbow trout stocks.

As a result of using isoenzymes, the studied populations were found to be in equilibrium and had no statistically significant deviations from the Hardy-Weinberg equation.

Based on F statistics, both groups were characterized by a predominance of heterozygotes in the population and an average level of divergence between the two studied groups.

Performed isoenzyme analysis allows cost-effective and reliable monitoring of dynamic processes in local stocks, which are usually caused by the selection effect and changes in the fish habitat. The practical significance of the results is to confirm the effectiveness of using molecular genetic markers for a comprehensive assessment of the genetic diversity of the local herds of rainbow trout.

The obtained information with its appropriate assessment and use with the classical methods of breeding, makes it possible to assess the genetic variability of the rainbow trout in artificial populations directly by molecular genetic markers, as well as the formation of brood fish groups with the desired economically valuable characteristics through a targeted genetic selection.

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