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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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The investigation of dynamics of genetic structure for two groupings of round goby from the north and the south part of the Odessa Bay in the Black Sea, utilizing esterases and myogenes loci had been conducted. The number of general and polymorphic loci that encode multiple molecular forms of esterases and hydrophilic muscle proteins had been estimated. These findings allow to consider that analyzed fish localities are groupings with subpopulation rank that related to the same population from coastal zone near Odessa. The analysis of allele and genotype frequencies utilizing polymorphic loci in round goby grouping from the south part of the Odessa Bay indicates the possibility of fish migration from other localities.

Key words: round goby, allele and genotype frequencies, allozymes, the dynamics of genetic structure.

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INTRODUCTION

Genetics methods are widely applied for investigating intraspecific structure of fish in the last decades. The study of population differentiation of animal species is often evaluated based on analysis of individual gene variability or their gene products that has theoretical and practical significances. Historically constituted population of commercial fish is a unit of human economic activity and represents an integral structure. The elimination or significant change of its parts due to trade or another anthropogenic influences may negatively affect the stability of whole fish grouping in a changing environment. Thus, the investigation of dynamics of genetic structure in fish populations allows to understand mechanisms of their group adaptation, develop a set of measures and technological approaches that aimed at rational usage and preserving the biodiversity of intraspecific structure

of ichthyofauna (Брыков, Полякова, 2011; McElroy, Kandl, 2011).

To investigate the genetic structure of populations and other intraspecific fish groupings enzymatic systems had been widely used, because they characterized by simplicity of histochemical detection, reflect inter- and intraspecific variation with high degree of accuracy. A significant amount of data had been collected about population-genetic structure of different commercial fish species utilizing these marker loci in recent decades (Cordes, Perkins, 2005; Okumu, Ciftci, 2003). However, unfortunately, the dynamics of genetic structure for round goby *Neogobius melanostomus* (Pallas) groupings, which are an important components of food chains in coastal ecosystems, had not been discovered properly. The presence of seasonal migrations, which related to relocation of fish between feeding areas, wintering and spawning may significantly change inter- and intraspecific population
structure of this species (Фауна Украины, 1986; Заморов, Радионов, 2014).

Thus, the aim of these studies were clarifying the temporal and spatial dynamics of genetic structure utilizing biochemical marker loci in round goby in the Odessa Bay in the Black Sea.

MATERIALS AND METHODS

The round goby specimens that were caught in the south (locality "Biostanciya" nearby cape Malyj fontan) and the north part (locality "Lisky" nearby cape Pivnichnyj) in the Odessa Bay during 2013-2016 years were used in the study. Each cohort consists of 40 fish specimens (20 male and 20 female). The analysis of genetic structure for groupings had been conducted utilizing loci for hydrophilic β-specific esterases and myogenes.

To identify the spectrum of molecular forms for biochemical markers, individual homogenates of goby's muscle tissue had been used from obtained cohorts. Samples of white skeletal muscle had been selected and prepared in the following way:

1) at the base of the dorsal fin the muscle probe had been obtained that by its size corresponds to 1/3 of total volume of eppendorf tube;
2) each probe was embedded by 50 μl of 20% saccharose;
3) eppendorf tubes were deep-frozen and kept at 4 °C;
4) to obtain protein supernatant eppendorf tubes were defrosted and centrifuged during 20 minutes at 12000 rpm and -2 °C.

7% polyacrylamide gel and Tris-borate-EDTA (TBE) buffer had been utilized for electrophoretic fractionation of hydrophilic esterases and myogenes. Electrophoresis had been conducted in *VE4* vertical plate gel system (size 140×120×1 mm, Russian Federation). The running gel had been kept in electrophoretic tray for 12 hours. After loading samples, pre-run had been conducting for 20-30 minutes until entering bromphenol blue dye in running gel at 80 mA. After that amperage had been increased to 200 mA. Pre-run and actual electrophoresis had been conducted in the cooled buffer at 4 ^{O}C. To identify molecular forms of enzymes and myogenes classical histochemical methods had been applied after electrophoresis procedure (Корочкин, 1977; Уолкер, 2015).

The interpretation of obtained allozyme spectrums had been conducted using classical methods (Пудовкин, 1979; Avise, 2004). The allozyme with smaller electrophoretic mobility marked as *S* (Slow), and with bigger electrophoretic mobility – as *F* (Fast). The Hardy-Weinberg formula had been utilized to calculate frequencies of genes and genotypes in fish cohorts. The index of heterozygosity (H) had been estimated as ratio of heterozygotic individuals to the whole cohort. The polymorphism of locality (P) had been calculated as ratio of polymorphic loci in analysis to all investigated loci (Тоцький, 2008).

The degree of compliance for observed frequencies of genotypes to theoretically expected had been discovered using $\chi 2$ method (Атраментова, Утєвська, 2007). To compare genetic structure of one type of fish grouping that inhabiting different localities, the index of genetic similarity (Ney index) had been used (Avise, 2004).

RESULTS

The analysis of spectrum for molecular forms of tissue hydrophilic esterases in muscles of round goby from the north part of Odessa Bay indicates the presence of 5 main zones of esterolytic activity. Multiple molecular forms for proteins from each zone, most likely, had been encoded by separate autosome locus. These loci had been marked as *Es1 – Es5* (loci for esterase 1 – esterase 5) while numbering had been conducted in respect to a decrease in the anode mobility of proteins during electrophoretic separation. The suggested titles and principle for numerating of loci are generally accepted and utilized in population-genetic studies of fish (Shaklee, 1990).

The results of analysis for multiple molecular forms of tissue esterases shew that round goby groupings from the north and the south part of the Odessa Bay are polymorphic by *Es2* and *Es3* during the whole period of investigation.

Moreover, the investigation of genetic structure for round goby grouping had been conducted for loci that encode hydrophilic muscle proteins – myogenes. This group comprises of wide range of molecules with different electrophoretic mobility that make these markers suitable for investigating genetic structure of fish populations. The analysis of electrophoretic spectrum of myogenes in round gobies revealed a large number of electroforms for these proteins. The genetic control of these proteins remains poorly studied, therefore population studies accept the most suitable and simple genetic scheme for interpretation results from electrophoretic studies that can be defined by formula “one gene – one colored gel zone”. Using aforementioned approach, the maximum assessment can be completed for loci that encode myogenes in investigated fish. By utilizing this approach the maximum number of genes in analyzed spectrums of round goby’s myogenes had been estimated and was equal 14. The numerating for myogenes had been conducted in the same way as for esterases by decreasing in the anode mobility of proteins during electrophoretic method (Shaklee,1990; Avise, 2004). The polymorphism had been detected in loci that encode myogenes 3 and 7.

The analysis of electropherograms shew that for fish from 2013 the frequency of *S* alleles in esterase 2 locus had been significantly higher in comparison to another variant of allele. This index corresponded to 0,66 and 0,81 in the south and the north part of Odessa Bay, respectively (Fig. 1). The decrease in frequency of gene variant that encoded less movable molecular form of esterolytic enzyme had been observed in 2014. The analysis of genetic structure of round goby grouping in the south part if the bay during the whole period of time had shown that investigating frequency decreased to the minimum value of 0,47 in 2015 and after that it was not changing drastically till the autumn of 2016 (Fig. 1). It should be noted that allele’s frequencies for genome *Es2* were significantly different in groupings from various parts of the Odessa Bay.

By studying the frequencies of *S*-allele in locus that encoded myogene 3 we found that this allele had been detected much more frequent in both groupings than another allele variant of hydrophilic muscle protein (Fig. 2). The highest occurrence of *S*-allele had been detected in 2013 in the south part of the Odessa Bay. In 2015 in the south part, as well as in 2013 and 2014 in the north part of the bay the frequencies of this gene were significantly lower.

In addition, the analysis of observed frequencies of genotypes in round goby groupings and their corresponding expected frequencies utilizing polymorphic loci had been conducted

using Hardy-Weinberg formula. Although this formula had been derived for ideal populations, it is one of the main analytical instruments for population processes. This formula allows to connect frequencies of alleles and genotypes in interspecies groupings using simple quadratic relationships. If genotype frequencies that identified in natural or laboratory groups correspond to frequencies that calculated by Hardy-Weinberg formula, it can be postulated that analyzed groups or populations are in equilibrium and close to ideal. This means that factors of population dynamics (natural selection, genetic drift, migrations, gene flow and mutational process) are not influence on this locality or their influence is minimum and do not introduce significant changes in the genetic structure of analyzed grouping (Тоцький, 2008).

The analysis of genotypes by polymorphic loci that encode molecular forms of biochemical markers in the south part of the Odessa Bay revealed that in 2013 and 2015 observed frequencies of genotypes were significantly different from theoretically expected (Table 1). Significant deviations in frequencies of genotypes by polymorphic loci had not be indicated during another periods of time.

In the north part of the Odessa Bay the frequencies of genotypes by polymorphic loci of biochemical markers did not differ from theoretically expected in all identified genotypes according to Hardy-Weinberg law (Table 2).

The quantitative assessment for genetic variability of round goby groupings by investigated loci of biochemical markers had been conducted using two classic and the most common indicators: polymorphism (P) and heterozygosity (H). The data analysis revealed that the level of polymorphism in groupings from both parts of the Odessa Bay was equal to 0,21. The obtained result is corresponding to average level of polymorphism indicator for fish that may vary between 0,11 and 0,30 (Алтухов, Салменкова, 1972). The average value for heterozygosity during four years of investigations in grouping from the south part of Odessa Bay was equal to 0.063. In the other part of the bay in 2013-2014 this value was higher and corresponded to 0,071. The obtained value corresponds to low level of genetic variability

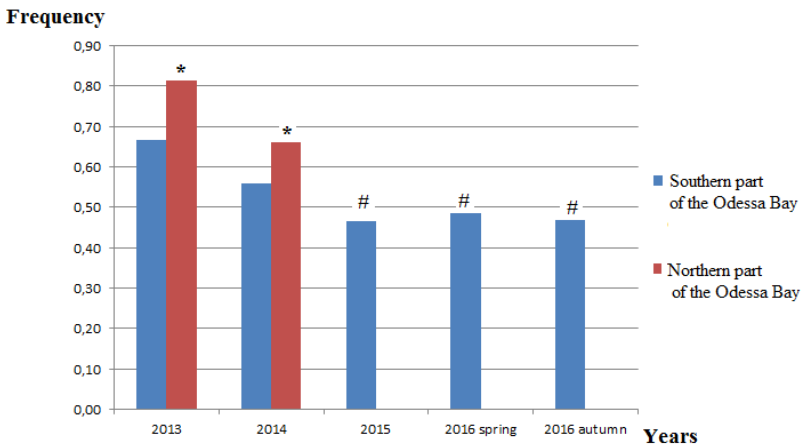

Fig. 1. Frequencies of S-alleles by polymorphic locus Es2 in round goby groupings from different parts of the Odessa Bay.

* – significant deviation of allele frequencies in round goby groupings from the south and the north part of the Odessa Bay (P = 0,05);

– significant deviation of allele frequencies in round goby groupings in the south part of the Odessa Bay in 2015-2016 in comparison to 2013-2014 (P = 0,05).

Table 1. The distribution of genotypes by polymorphic loci of biochemical markers in round goby groupings in the south part of the Odessa Bay

Genotype	Locus	Frequency (observed)	Frequency (expected)	χ^2
1	2	3	4	5
2013				
S / S	Esterase 2	0,53	0,44	**5,06**
S / F		0,28	0,44	
F / F		0,19	0,11	
S / S	Myogene 3	0,91	0,91	0,07
S / F		0,09	0,08	
F / F		0,00	0,01	
2014				
S / S	Esterase 2	0,35	0,31	0,53
S / F		0,42	0,49	
F / F		0,23	0,20	
S / S	Myogene 3	0,86	0,84	1,79
S / F		0,11	0,15	
F / F		0,03	0,01	
2015				
S / S	Esterase 2	0,31	0,22	**4,11**
S / F		0,31	0,50	
F / F		0,38	0,29	
S / S	Myogene 3	0,66	0,64	0,28
S / F		0,29	0,32	
F / F		0,05	0,04	
2016 (Spring)				
S / S	Esterase 2	0,28	0,23	1,12
S / F		0,41	0,50	
F / F		0,31	0,27	
2016 (Autumn)				
S / S	Esterase 2	0,26	0,22	0,77
S / F		0,42	0,50	
F / F		0,32	0,28	

Note: * – null hypothesis about equality of frequencies in cohorts had been rejected at $\chi^2 \geq 3{,}84$ (P = 0,05).

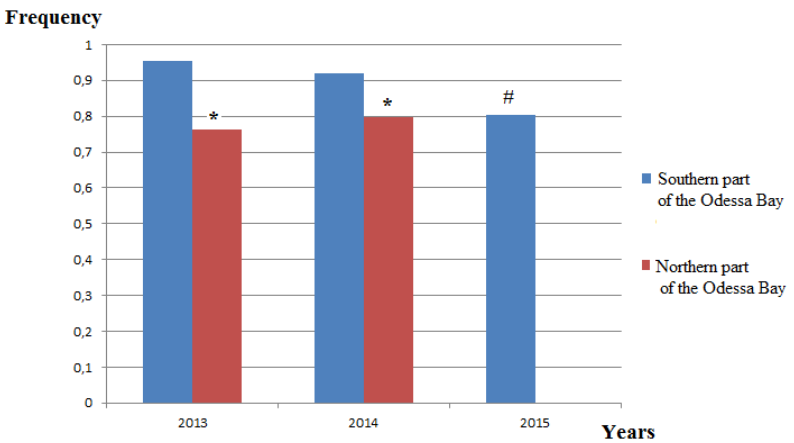

Fig. 2. Frequencies of S-alleles by polymorphic locus of myogene 3 in round goby groupings from different parts of the Odessa Bay

* – significant deviation of allele frequencies in round goby groupings from the south and the north part of the Odessa Bay in 2013 and 2014 (P = 0,05);

– significant deviation of allele frequencies in round goby groupings from the south part of the Odessa Bay in 2013-2014 in comparison to 2015 (P = 0,05).

Table 2. The distribution of genotypes by polymorphic loci of biochemical markers in round goby groupings in the north part of the Odessa Bay

Genotype	Locus	Frequency (observed)	Frequency (expected)	χ^2
1	2	3	4	5
2013				
S / S	Esterase 2	0,71	0,66	2,40
S / F		0,21	0,30	
F / F		0,08	0,04	
S / S	Myogene 3	0,60	0,58	0,42
S / F		0,33	0,36	
F / F		0,08	0,06	
2014				
S / S	Esterase 2	0,43	0,44	0,04
S / F		0,46	0,45	
F / F		0,11	0,12	
S / S	Myogene 3	0,62	0,64	0,28
S / F		0,35	0,32	
F / F		0,03	0,04	

Note: * – null hypothesis about equality of frequencies in cohorts had been rejected at $\chi^2 \geq 3,84$ (P = 0,05).

and this indicator may vary for fish from 0,057 to 0,110 (Алтухов, Салменкова, 1972). The value for coefficient of genetic similarity (Ney index) for comparison round goby groupings from both parts of the Odessa Bay was equal to 0,998.

DISCUSSION

The level of genetic similarity (0,998) between two round goby groupings from different parts of the Odessa Bay confirms that these two localities are part of the same population that inhabit coastal zone near Odessa (Яблоков, 1988; McElroy, Kandl 2011). The spatial dynamics of allele frequencies for certain polymorphic genes of biochemical markers (*Es2* locus and myogene 3) shows that this population has sophisticated internal structure and characterized by significant genetic heterogeneity. During two tears of investigation (2013 and 2014) allele frequencies significantly differ between fish cohorts that inhabit the north and the south part of the bay. Thus, it can be concluded that investigated round goby population is separated into groupings with subpopulation rank The temporal dynamics of genetic structure of fish localities had been investigated as a result of that study. It should be noted that from 2013 to 2015 for round goby grouping from the south part of the Odessa Bay constant change in gene frequencies had been observed for esterase 2 locus, while at the same time in 2015-2016 this value had been relatively constant. This fact testifies about partial change of genetic structure in grouping that had been confirmed by comparative analysis of observed and theoretically expected frequencies using Hardy-Weinberg formula for genotypes by *Es2* locus. In 2013 and 2015 these frequencies were significantly different, probably because due to fact that genetic structure of round goby groupings in the south part of the bay were not in equilibrium and factors of dynamics may affect genetic structure of population.

In our opinion the most important factor in this case that may partially change frequencies of allele variants in round goby groupings was migration during the period of time, when fish came back to previous localities from wintering (Фауна Украины, 1986).

CONCLUSIONS

1. The analysis of genetic structure of round goby groupings in different parts of the Odessa Bay in 2013-2016 revealed existence of 14 loci for myogenes and 5 loci for hydrophilic esterases, of which 4 loci characterized by presence of polymorphisms: *Es2*, *Es3,* myogenes 3 and 7.

2. The coefficient of genetic similarity between round goby groupings from the south and the north part of the Odessa bay indicates that they are components of the same population.

3. The genetic structure of round goby population in the Odessa Bay can be differentiated into two groupings with subpopulation rank.

4. The allele and genotype frequencies by polymorphic loci in round goby grouping from the south part of the bay significantly change during three years (2013-2015) that indicates the probability of migration of fish from another localities.

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