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APPLICATION OF ESTERASE POLYMORPHISM TO SPECIFY POPULATION GENETIC STRUCTURE OF *ENGRAULIS ENCRASICOLUS* (PISCES: ENGRAULIDAE) IN THE BLACK AND AZOV SEAS

Genetic structure of anchovy populations was studied using genetic-biochemical markers. Anchovy samples collected between 1980 and 2006 from different Black Sea regions (off the Bulgarian, Turkish, Ukraine, and Georgian coasts) as well as from the Sea of Azov were analyzed. Three methods of electrophoresis were applied: starch gel electrophoresis, vertical polyacrylamide electrophoresis, and isoelectric focusing on thin polyacrylamide ampholine gel plates. On the base of long-term monitoring of allelic frequencies of the polymorphic non-specified esterases loci (*EST-1** and *EST-2**), wintering migration of Azov anchovy may have alternative routs along the western Black Sea coasts. Typical Azov anchovy winter together with the Black Sea anchovy form the mixed populations along the Ukrainian, Georgian, and Turkish Black Sea coasts. Azov anchovy was also registered along Bulgarian coast during the second part of May and July. The results allow supposing the north-western Black Sea as complementary spawning areas of Azov anchovy.

Key words: The Azov and Black Sea anchovy, genetic markers, electrophoresis, isoelectric focusing (IEF), migration routes

The Azov anchovy (*Engraulis encrasicolus maeoticus*) and Black Sea anchovy (*Engraulis encrasicolus ponticus*) are considered as two subspecies (races) [1]. Further using genetic biochemical markers allowed finding out significant differences between them. Dobrovolov [10] identified Black Sea and Azov races of anchovy on the base of general muscle proteins and lactate dehydrogenase analyses. Kalnina and Kalnin [15] studied European anchovy biochemical polymorphism and found out significant differences between two races in the allele frequencies. Kalnin and Kalnina [16] examined introgressive hybridization of two anchovy races in the Black Sea and the Sea of Azov analyzing genetic frequencies of isocitratedehydro-genase and esterase by electrophoresis in PAAG. Dobrovolov [9, 10, 12], Ivanova and Dobrovolov [14] studied the phylogenetic relationship between *E. encrasicolus* from the Atlantic Ocean, the Black Sea, and Sea of Azov using allozyme analyses. Long-term monitoring of genetic biochemical markers proved existence of two anchovy populations (visiting) in Bulgarian waters [11]. Erdoğan *et al.* [13] studied genetic variations of *E. encrasicolus* from the Black, Marmara and Aegean Seas using allozyme and morphologic analyses. Based on polymorphism of general muscle proteins, Bat *et al*. [4] investigated population structure of anchovy from some regions of the Black Sea and established two sub-populations (western and eastern). Zuev et al. [25] studied fish otoliths shapes and indicated two statistically dissimilar forms of anchovy wintering at the south-western coastal waters off Crimea.

Anchovy from the Sea of Azov and the Black Sea undertake long migration within the both basins. Azov anchovy usually spend spring, summer and early autumn in the Sea of Azov, where spawn and then accumulate lipid reserve. After temperature decline, it starts wintering migration to the Black Sea. The main migration routes of the Azov anchovy to the place of winter depends on wind direction and pass either along Caucasus or Crimean coasts. In April – May Azov anchovy go back to the Sea of Azov [20].

The Black Sea anchovy is distributed over the whole Black Sea during spawning and feeding period [6]. In October – November, under the temperature decline, Black Sea anchovy migrates to the wintering grounds approaching warmer Turkish or Georgian coasts and forms dense wintering concentrations [20]. In spring, Black Sea anchovy move back to the northern Black Sea. The Azov and Black Sea anchovy migration route often overlapping and both anchovy also may winter in mix populations together.

The aim of the study is to clarify population structure of anchovy in the different regions of the Black Sea and the Sea of Azov and to specify their migration routes using unspecified muscle esterase polymorphism as genetic-biochemical markers.

Material and Methods. Anchovies were caught by the fishing vessels or by trap-nets in different Black Sea regions along Bulgarian (Kavarna, Varna, Byala, Sozopol), Turkish (Samsun and Sinop), Ukrainian (Sevastopol), and Georgian (Poti) coasts and in the Sea of Azov (Jurkino and Kerch strait) between 1980 and 2006. The fish were frozen immediately after catching and delivered to the laboratory in portable refrigerators or in dry ice for further muscle esterase analysis. In total, 2091 samples from 21 catches were analyzed.

The system for decoding nonspecific muscle esterase of anchovy, different from the schema presented by Kalnin *et al*. [17], was proposed. Three methods of electrophoresis were applied in this study: starch gel electrophoresis, vertical polyacrylamide electrophoresis, and isoelectric focusing on thin polyacrylamide ampholine gel plates.

Proteins from dorsal muscle were separated by horizontal starch gel electrophoresis according to Smithies [23] with Dobrovolov's modifications [8]. The visualization of nonspecific esterases was made using Fast Red TR. Buffer systems were elaborated by Clayton and Gee [5] and Dobrovolov [9]. Vertical polyacrylamide electrophoresis was carried out after Truveler and Nefedov [24]. Isoelectric focusing (IEF) on thin polyacrylamide Ampholone gel with pH gradients between 3.5–10.0 with the equipment of LKB (Stockholm, Sweden) was used. The proteins were stained with Commassie Brilliant Blue R-250.

Gene frequencies of the polymorphic loci were calculated using the Hardy-Weinberg equilibrium. Calculation of indices of genetic similarity and genetic distance were performed after Nei [19].

We used here the nomenclature of loci and alleles following the recommendation of Shaklee et al. [21]. The ratios between Azov and Black Sea anchovy populations assuming their mechanical mixing were calculated using estimating Altukhov's formula [2].

Results and discussion. Ten zones with esterase activity in anchovy muscles were visualized on the starch gel electrophoregrams (Fig. 1 and 2).

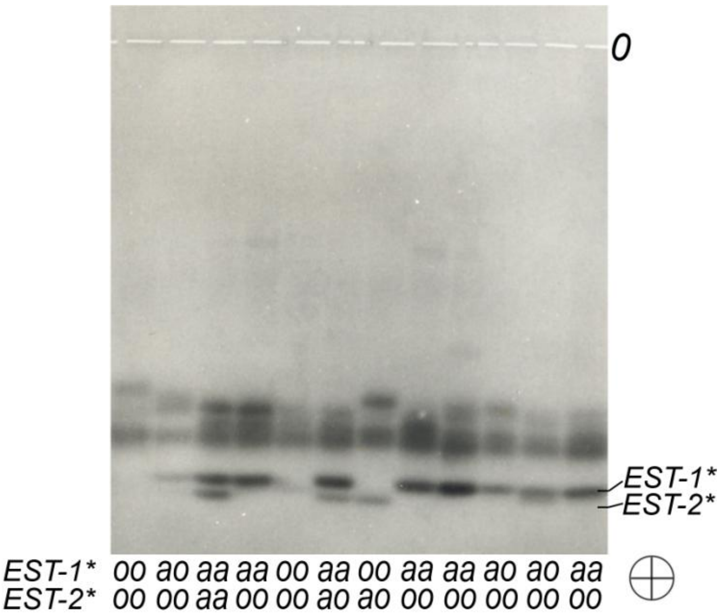

Fig. 1 Electrophoregrams on starch gel of nonspecified muscle esterase of anchovy from the Black Sea. Polymorphism with null alleles in *EST-1** and *EST-2**: AA и AO and OO phenotypes, *O* – origin.
Рис. 1 Электрофореграммы неспецифичных эстераз мышц хамсы из Чёрного моря на крахмальном геле. Полиморфизм с нуль-аллелями в *EST-1** и *EST-2**: *aa, ao* и *oo* – фенотипы, *O* – старт.

There were two polymorphic zones in the anodal part which are coded from two loci (*EST-1** and *EST-2**). Polymorphism of both esterase loci in anchovy from different Azov and Black Sea areas were observed, and gene frequencies were calculated (Table 1).

Table 1 Gene frequencies of non-specified muscle esterases loci (*EST1** and *EST2**) in anchovy from different regions of the Black Sea and the Sea of Azov (N – number of the samples; B – Black Sea anchovy, A – Azov anchovy, and B/A – mechanical mixing populations
Табл. 1 Частоты генов неспецифичных эстераз мышц локусов (*EST1** и *EST2**) у хамсы из различных районов Чёрного и Азовского морей (N – количество проб, B – черноморская хамса, A – азовская хамса, B/A – механически смешанные популяции

Sampling location	The Black Sea																	The Sea of Azov			
	31.01.1980, Varna	07.07.1980, Varna	31.05.1982, Kavarna	29.06.1982, Sozopol	07.12.1982, Varna	19.01.1988, Byala	11.1988, Sozopol	18.05.1994, Varna	15.07.1997,Varna	09.11.2005, Sinop	11.11.2005, Sinop	10.12.2005, Sinop	17.11.2006, Sinop	21.11.2006, Sinop	16.01.2006, Samsun	06.04.2006, Sevastopol	17.11.1988, Poti	01.05.1981, Kerch	16.06.1989, Jurkino	30.04.1990, Jurkino	11.06.1994, Kerch
*EST-1*a*	0.598	0.619	0.696	0.604	0.600	0.779	0.850	0.757	0.766	0.703	0.580	0.640	0.703	0.790	0.568	0.720	0.704	0.790	0.783	0.799	0.757
*EST-1*o*	0.402	0.381	0.304	0.396	0.400	0.221	0.150	0.243	0.224	0.297	0.420	0.360	0.297	0.210	0.432	0.280	0.296	0.210	0.217	0.201	0.243
*EST-2*a*	0.143	0.126	0.114	0.143	0.126	0.061	0.026	0.093	0.100	0.117	0.120	–	0.119	0.053	0.169	0.109	0.116	0.084	0.080	0.082	0.035
*EST-2*o*	0.857	0.874	0.886	0.857	0.874	0.939	0.974	0.907	0.900	0.883	0.880	–	0.881	0.947	0.831	0.891	0.884	0.916	0.920	0.918	0.965
N	68	160	102	102	85	68	135	34	80	68	102	85	102	68	102	102	229	136	85	74	102
Populations:	B	B	B/A	B	B	A	A	A	A	B/A	B	B	B/A	A	B	B/A	B/A	A	A	A	A

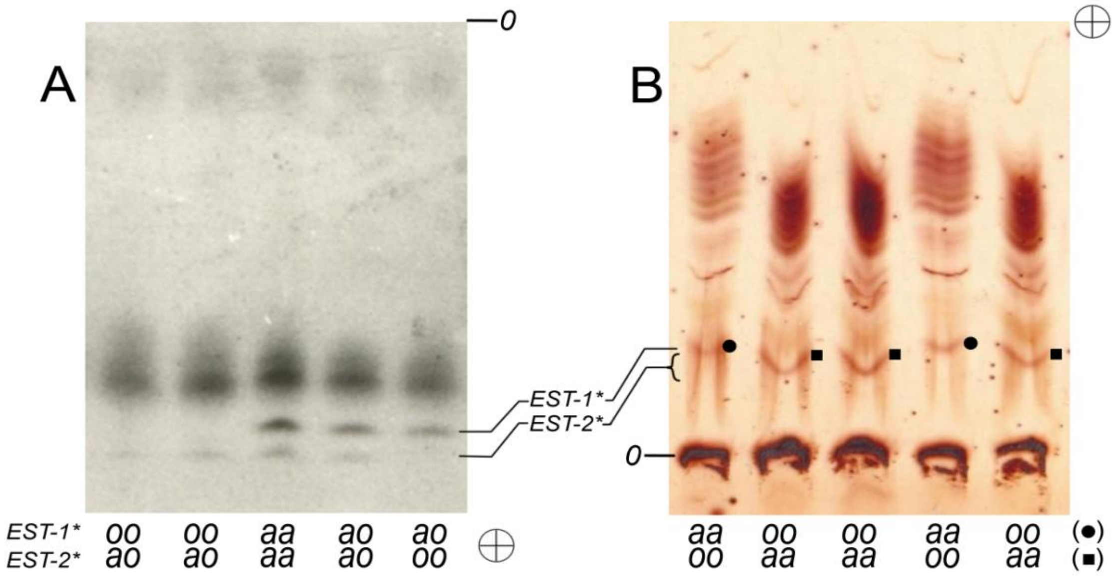

Fig. 2 Electrophoregrams of non-specified muscle esterase of anchovy from the Black Sea on vertical polyacrylamide electrophoresis (A), and isoelectric focusing (B). Polymorphism with null alleles in EST-1* and EST-2*: AA и AO and OO phenotypes, *O* – origin.

Рис. 2 Электрофореграммы неспецифичных эстераз мышц хамсы из Чёрного моря: в вертикальном полиакриламидном геле (A) и изоэлектрическом фокусировании (B). Полиморфизм с нуль-аллелями в *EST-1** и *EST-2**: *aa, ao* и *oo* – фенотипы, *O* – старт.

The *EST-1** locus was polymorphic in all samples. Allele frequencies on *EST-1**o which was typical for Black Sea anchovy populations ranged 0.350 to 0.440 while for the same allele in Azov anchovy ranged 0.150 to 0.250. The *EST-2** locus had two allele system of inheriting in the populations analyzed with null allele and polymorphism was found in all samples. In this locus, null allele was most often presented. Alternative allele *EST-2*a* frequencies ranged from 0.120 to 0.169 and 0.020 to 0.100 for the Black Sea and Azov anchovy, accordingly. Intermediate values of the frequencies of *EST-1*o* and *EST-2*a,* were accepted as a mechanical mixed population of two subspecies.

According to allelic frequencies (Table 1), typical Azov anchovy were found in November and January in the Bulgarian Black Sea coast (Varna, Byala, and Sozopol) as well as near the Turkish coast (Sinop) in the end of November (Fig. 3).

Pure Black Sea anchovy population was found also during winter in the same Bulgarian areas and near Sinop and Samsun (Turkey). Mechanical mixed populations of Azov and Black Sea anchovy with intermediate value of the gene frequencies were established off Kavarna (Bulgaria), Sinop (Turkey), and Sevastopol (Ukraine) as well as near Poti (Georgia) during winter months (Table 1, Fig. 3). Using our data of allelic frequencies of esterase loci and Altukhov's formula [2], the proportion of each population in the mixed pool were estimated (Table 2). The results showed that the share of the Azov sea fish in all mixed populations was about 50 %. Spatial overlapping as well as mixed schools of Azov and Black Sea anchovy populations during winter are known long ago [2, 3, 7, 18]. However, according to Chashchin [6], during wintering migration, the fish of Azov population, which mingled with Black Sea anchovy in the almost equal share, moved frequently in eastern direction along Caucasus coast and reach Georgian and Turkish waters.

The presence of Azov anchovy near Bulgarian Black Sea coast, which was shown in our study, allows presuming that Azov anchovy may also take the alternative route to wintering areas in western direction (Fig. 4).

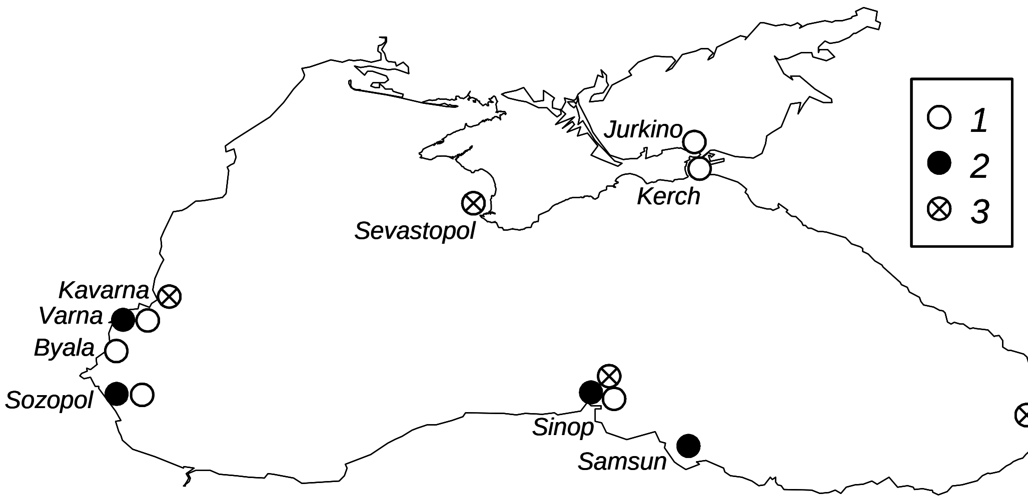

Fig. 3 Detection of Azov anchovy (1), Black Sea anchovy (2), and their mixed populations (3) in different sampling locations in the Black Sea and the Sea of Azov on the basis of allelic frequencies of *EST-1*o* and *EST-2*a* loci
Рис. 3 Обнаружение азовской хамсы (1), черноморской хамсы (2) и их смешанных популяции (3) в различных районах Чёрного и Азовского морей по частотам аллелей локусов *EST-1*o* и *EST-2*a*

Table 2 Shares of Azov anchovy in mechanical mixing populations in the Black Sea regions estimated from gene frequencies of esterases loci (*EST1** and *EST2**) using Altukhov's formula [2]
Табл. 2 Относительные доли азовской хамсы в механически смешанных популяциях в районах Чёрного моря, рассчитанные по частотам локусов эстераз (*EST1** и *EST2**) с применением формулы Алтухова [2]

	Bulgarian coast	Turkish coast		Ukrainian coast	Georgian coast
	31.05.1982, Kavarna	09.11.2005, Sinop	17.11.2006, Sinop	06.04.2006, Sevastopol	17.11.1988, Poti
*EST-1*o*	0.475	0.436	0.436	0.343	0.431
*EST-2*a*	0.623	0.670	0.699	0.555	0.656
Average value	0.549	0.553	0.568	0.449	0.544
N	102	68	102	102	229

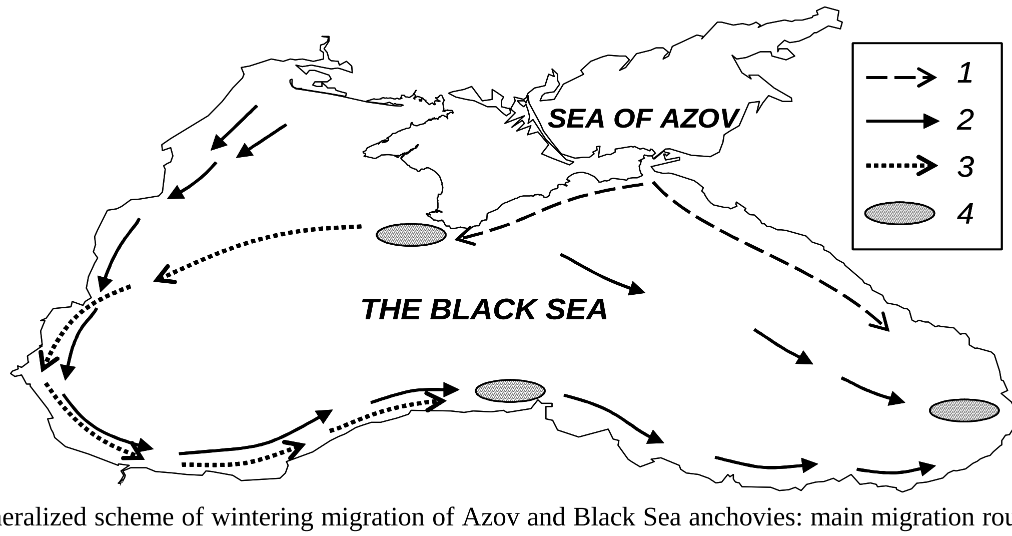

Fig. 4 Generalized scheme of wintering migration of Azov and Black Sea anchovies: main migration routes of Azov anchovy (1), Black Sea anchovy (2) after [6, 22], and alternate migration route of Azov anchovy (3) and mechanical mixed populations on wintering grounds according to our data
Рис. 4 Обобщённая схема зимовальной миграции азовской и черноморской хамсы: основные пути миграций азовской (1) и черноморской (2) хамсы по [6, 22], и альтернативный путь миграции азовской хамсы (3) и механически смешанные популяции на местах зимовки согласно нашим данным

The Azov anchovy spring migration back to the Sea of Azov begins in the mid of April and is over by the end of May [6]. We found out these months typical Azov anchovy in Kerch Straight (Table 1, Fig.3). We also registered typical Azov anchovy along Varna coast and in mixed population with Black Sea anchovy along Kavarna in April and even in July (Fig. 3, Table 1). It is possible to assume that the Azov anchovy, which wintered along the southern part of Crimea Peninsula, may take alternative migrating route: it may direct in spring not to the Sea of Azov, but to the north-western or western Black Sea in the areas with lower salinity. That could be the reason why typical Azov anchovy were registered during spawning period in the western Black Sea (in front of Varna coast).

Thus the data for gene frequencies of polymorphic esterase loci, verify the existence of two anchovy subspecies (races). The following hypothesis was proposed. We supposed that before last opening of Bosphorus, the frequency of *EST-1*a* in the Azov sea anchovy was close to the fixation (close to 1) and respectively the frequency of the alternative allele *EST-1*o* was very low (close to the null). After supposed introgerssive hybridization with Black Sea anchovy the frequencies of the first allele decreased from 1.00 to 0.757–0.799 and respectively the alternative allele frequency increased up to 0.150–0.250. If the monitoring of gene frequencies of the mentioned locus continued and increasing of the frequencies of null allele in Azov anchovy will be detected, that could be feature for estimation of the intensity of interrace hybridization.

Conclusions. Long-term monitoring of allelic frequencies of polymorphic esterases loci were applied to distinguish Azov and Black Sea anchovy. The results allowed us to suppose the winter migration of Azov anchovy along the Bulgarian Black Sea coast. The new data were presented on Azov anchovy wintering along the Turkish Black Sea coast. In the Sinop area, the both anchovy races wintered together. Mechanical mixed populations of two subspecies were proved in the areas off Sevastopol, Kavarna, Sinop, and Poti. Spawning of Azov anchovy in the western part of the Black Sea was presumed.

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1. *Alexandrov A. I.* The anchovy of the Azov-Black Sea region, its origin and taxonomic designation // Trudi Kerch Nauch. Ryb. Stant. – 1927. –**1**. – P. 37 – 93 (in Russian).
2. *Altukhov Y. P.* Population genetics of fish. – Moscow: Pishchevaya Promishlennost, 1974. – 245 p. (in Russian).
3. *Artemov A.G., Chashchin A. K.* Assessment of anchovy biomass with hydroacoustic method // Fish industry. – 1982. – N 12. – P. 45 – 79 (in Russian)
4. *Bat L., Ivanova P., Dobrovolov I.* et al. The population structure of the Black Sea anchovy. AquaCulture & Fisheries. – 2007. – **2,** N 8. – P. 51 – 56.
5. *Clayton J. W., Gee G. H.* Lactate dehydrogenase isozymes in long nose and black nose Dace (*Rhinichthys cataractae* and *R. atratulus*) and their hybrid // J. Fish. Res. Bd. Can. – 1969. – **26**, № 11. – P. 3049 – 3053.
6. *Chashchin A. K.* The Black Sea population of anchovy // Sci. Mar. – 1996. – **60** (Suppl. 2). – P. 219 – 225.
7. *Danilevsky N. N.* The penetration of the Black Sea anchovy into the Sea of Azov and concomitant environmental conditions // Trudy AzcherNIRO. – 1960. – **18. –** P. 118 – 129 (in Russian).
8. *Dobrovolov I. S.* Micro starch gel electrophoresis // Proc. Inst. Oceanogr. Fisheries. – Varna, 1973. – **12. –** C. 157 – 162 (in Bulgarian, with English summary).
9. *Dobrovolov I. S.* Multiple forms of tactate-dehydrogenase in anchovy (*Engaulis encrasicolus* L.) from the Black Sea, the Sea of Azov and the Atlantic ocean // Comptes rendus de l’Academie bulgare des Sciences. – 1976. – **29**, № 6. – P. 877 – 880.
10. *Dobrovolov I. S.* Polymorphism of muscle proteins of anchovy *Engraulis encrasicolus* (L.) from Azov-

Black Sea basin and Atlantic Ocean // J. Ichthyol. – 1978. – **18**, N 3. – P. 534 – 539 (in Russian).

11. *Dobrovolov I. S.* Biochemical and population-genetic investigations of commercial fish species from Bulgarian waters and World Ocean: Dr. Sci. Thesis. – Moscow, 1987. – 533 pp.
12. *Dobrovolov I. S.* Study of the intraspecific divergence of anchovy *Engraulis encrasicolus* L. // Comptes rendus de l'Academie bulgare des Sciences. – 1992. – **45,** N 2. – P. 63 – 65.
13. *Erdoğan Z., Turan C., Koç H. T.* Morphologic and allozyme analyses of European anchovy (*Engraulis encrasicolus* (L. 1758)) in the Black, Marmara and Aegean Seas // Acta Adriatica. – 2009. – **50,** N 1. – P. 77 – 90.
14. *Ivanova P., Dobrovolov I.* Population-genetic structure on European anchovy (*Engraulis encrasicolus*, Linnaeus, 1758) (Osteichthyes: Engraulidae) from Mediterranean Basin and Atlantic Ocean // Acta Adriatica. – 2006. – **47**, N 1. – P. 13 – 22.
15. *Kalnina O. V., Kalnin V. V.* Genetic differentiation and reproductive relationship between the Black Sea race and Azov Sea race of European anchovy. 2. Genetic differences between the Black Sea race and the Azov Sea race and their intrarace heterogeny // Genetics. – 1984. – **20**, N 2. – P. 309 – 313 (in Russian).
16. *Kalnin V. V., Kalnina O. V.* Genetic differentiation and reproductive relationship between the Azov and Black Sea races of anchovy. 3. Introgressive race hybridization and population structure of the Black Sea anchovy // Genetics. – 1985. – **21**, N 8. – P. 1352 – 1360 (in Russian).
17. *Kalnin V. V., Kalnina O. V. Dashova M. B.* Genetic differentiation and reproductive relationship in the Azov and Black Sea races of anchovy. 1. Biochemical polymorphism // Genetics. – 1984. **– 20**, N 2. – P. 303 – 308 (in Russian).
18. *Marty Y.Y.* Migrations of the marine fish. – Moskow: Pishchevaya Promishlennost, 1981. – 248 p. (in Russian).
19. *Nei M.* Genetic distance between populations // Amer. Naturalist. –1972. – **106.** – P**.** 283 – 292.
20. *Pusanov I. I.* The anchovy // Uchenije Zapiski Gorkovskogo Univ. – 1936. – N 5. – P. 3 – 64 (in Russian).
21. *Shaklee J., Allendorf F. W., Morizot D. C., Whitt G. S.* Gene Nomenclature for protein-coding loci in fish // Trans. Amer. Fish. Soc. – 1990. – **119. –** P. 2-15.
22. *Shulman G. E.* Anchovies of the Sea of Azov and the Black Sea regulates of wintering migration (brief review). // Mar. Ecol. J. – 2002. – **1**, N 1. – P. 67 – 77.
23. *Smithies O.* Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults // Biochem. J. – 1955. – **61. –** P. 629 – 641.
24. *Truveler K. A., Nefedov G. N.* Multifunctional apparatus for electrophoresis in the parallel polyacrylamide plates // Biol. Sci. – 1974. – N 9. – P. 137 – 140 (in Russian).
25. *Zuev G. V., Gutsal D. K., Goralevich K. G., Bondarev V. A., Murzin U. L., Novoselova U. V.* Intraspecific morphological, ecological and biological variability of Azov-Black Sea anchovy *Engraulis encrasicolus* (Pisces: Engraulidae) wintering at Crimea coastal waters // Mar. Ecol. J. – 2011. – **11**, N 4. – P. 5 – 18.

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Застосування поліморфізму естераз у дослідженнях популяційно-генетичної структури *Engraulis encrasicolus* (Pisces: Engraulidae) у Чорному та Азовському морях. **П. П. Іванова, І. С. Доброволов, Л. Бат, А. Є. Кідейс, В. М. Нікольський, Т. В. Юньова, А. М. Щепкина, Г. Є. Шульман.** Генетичну структуру популяцій камси (*E. encrasicolus*) аналізували із застосуванням генетико-біохімічних маркерів. Було проаналізовано 1692 зразків камси з різних районів Чорного моря - біля берегів Болгарії, Туреччини, України та Грузії і 399 зразків з Азовського моря, зібраних в період з 1980 по 2006 роки. Три методи електрофорезу були застосовані у цьому дослідженні: електрофорез в крахмальному гелі, вертикальний електрофорез у поліакриламидному гелі, ізоелектричне фокусування на пластинах з поліакриламид амфолиновим гелем..На основі довгострокового моніторингу частот алелей поліморфних локусів (*EST-1* * and *EST-2* *) неспецифічних естераз було показано, що зимувальна міграція азовської камси може здійснюватися уздовж західного узбережжя Чорного моря. Генетичні дані показали, що біля берегів Синопа (Туреччина) поряд з чорноморською зимувала і азовська камса. Змішані популяції цих риб під час зимівлі були виявлені як у берегів України, так і Грузії. Азовська камса, поряд з чорноморської була виявлена біля берегів Болгарії також у другій половині травня і червні. Генетичні маркери дозволяють припустити, що розмноження азовської камси може здійснюватися в північно-західній частині моря.

Ключові слова: азовська та чорноморська камса, генетичні маркери, електрофорез, ізоелектричне фокусування (ІЕФ), міграційні шляхи.

Применение полиморфизма эстераз в исследованиях популяционно-генетической структуры *Engraulis encrasicolus* (Pisces: Engraulidae) в Чёрном и Азовском морях. П. П. Иванова, И. С. Добровoлов, Л. Бат, А. Э. Кидейс, В. Н. Никольский, Т. В. Юнёва, А. М. Щепкина, Г. Е. Шульман. Генетическую структуру популяций хамсы (*E. encrasicolus*) анализировали с применением генетико-биохимических маркеров. Было проанализировано 1692 образца хамсы из различных районов Чёрного моря – у берегов Болгарии, Турции, Украины и Грузии и 399 образца из Азовского моря, собранных с 1980 по 2006 гг. Три метода электрофореза были использованы в этом исследовании: электрофорез в крахмальном геле, вертикальный полиакриламидный электрофорез, изоэлектрическое фокусирование на пластинах с полиакриламид амфолиновом геле. На основе долгосрочного мониторинга частот аллелей полиморфных локусов (*EST-1** and *EST-2**) неспецифических эстераз показано, что зимовальная миграция азовской хамсы может осуществляться вдоль западного побережья Чёрного моря. Генетические данные показали, что у берегов Синопа (Турция) наряду с черноморской зимовала и азовская хамса. Смешанные популяции этих рыб во время зимовки были обнаружены как у берегов Украины, так и Грузии. Азовская хамса, наряду с черноморской, обнаружена у берегов Болгарии также во второй половине мая и июне. Генетические маркеры позволяют предположить, что размножение азовской хамсы может осуществляться в северо-западной части моря.

Ключевые слова: Азовская и черноморская хамса, генетические маркеры, электрофорез, изоэлектрическое фокусирование (ИЭФ), миграционные пути.