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COMPARATIVE STUDY OF ELECTROPHORETIC CHARACTERISTICS OF SERUM ALBUMIN OF ROUND GOBY *NEOGOBIUS MELANOSTOMUS* FROM BLACK SEA AND AZOV SEA

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ABSTRACT
Concentration and electrophoretic properties of blood serum albumin of the round goby *Neogobius melanostomus* caught in Black Sea and Azov Sea were studied in relation to age, sex, maturation stage and season. Electrophoretic mobility of serum albumin in Black Sea fish increased with age, while in Azov Sea goby it varied less. No differences were observed in albumin characteristics between female and male. Albumin mobility increased during maturation process in male and female from both seas. No significant seasonal changes were shown in albumin mobility in Azov Sea goby while in Black Sea fish it was higher in spring than in summer. Our findings indicate that fish physiological status, age, season and geographical locations play an important role in albumin properties. The alterations of albumin electrophoretic mobility could be explained its transport function and the genetic and ecological differences between the fish from Black Sea and Azov Sea.

KEY WORDS: marine fish, serum proteins, age, sex, maturation stage, season

INTRODUCTION

Round goby *Neogobius melanostomus* (Pallas) is highly distributed teleost fish. It is adapted to different fluctuation of salinity, temperature and oxygen concentration in water. It inhabits different geographical locations including marine, saline and fresh waters. Thus the mechanisms of the round goby adaptations to environmental conditions could be associated with the specificity of its metabolic status. Serum proteins are the complex of multifunctional system which may reflect the metabolic level of the organism and its adaptations to environmental fluctuations (Mc Donald & Milligan, 1992; Zowail et al., 1994).

Serum albumin is the major protein of the blood which plays an important role in transport of wide range of physiological and exogenous ligands, and regulation of the colloid osmotic pressure of the blood (Csogor, 1972; De Smet et al., 1998). At the other hand fish albumin has phylogenetic significance. In some elasmobranches it was not shown, among teleost fishes several species demonstrate lack of albumin. In few fish species specific properties of albumin were observed and they were identified as so-called albumin-like proteins (Hasnain et al., 2004).

Besides that, fish physiological status and environmental factors impact albumin and may change its characteristics. Serum proteins composition and albumin characteristics depend on fish ecological status (Luk'yanenko & Habarov, 2005), the maturity process of gonads and maturation stage of fish (Ishioka & Fushimi, 1975), seasonal variations (Schlotfeldt, 1975), pathological processes and parasites invasion (Moeyner, 1993), sex (Zowail et al., 1994), chemical toxicants (Richmonds, 1990) and long-term pollution of habitats (Moussa et al., 1994; Sayed et al., 2011).

Hence the aim of the present study was to compare the electrophoretic properties of serum albumin of *N. melanostomus* from Black Sea and Azov Sea in relation to fish age, sex, maturity process and seasonal variations.

MATERIALS AND METHODS

Animals

Fish (Fig. 1) were collected in spring - autumn period of 2003 year in Sevastopol Bay (n = 84) (Black Sea, Ukraine) and in the Arabat Bay (n = 173) (Azov Sea, Ukraine) (Fig. 2 A, B).

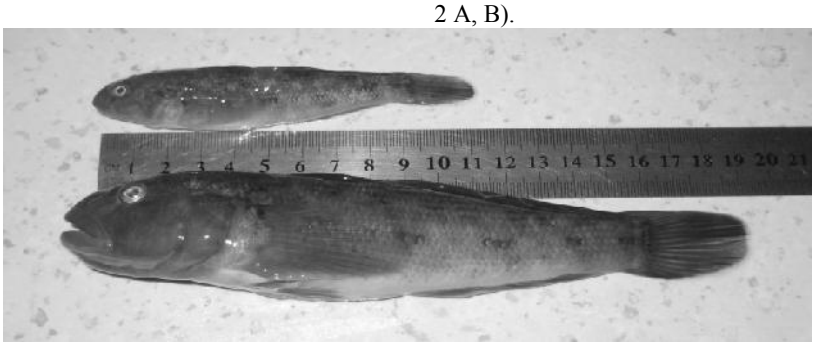

FIGURE 1. Round goby *Neogobius melanostomus* (Pallas)

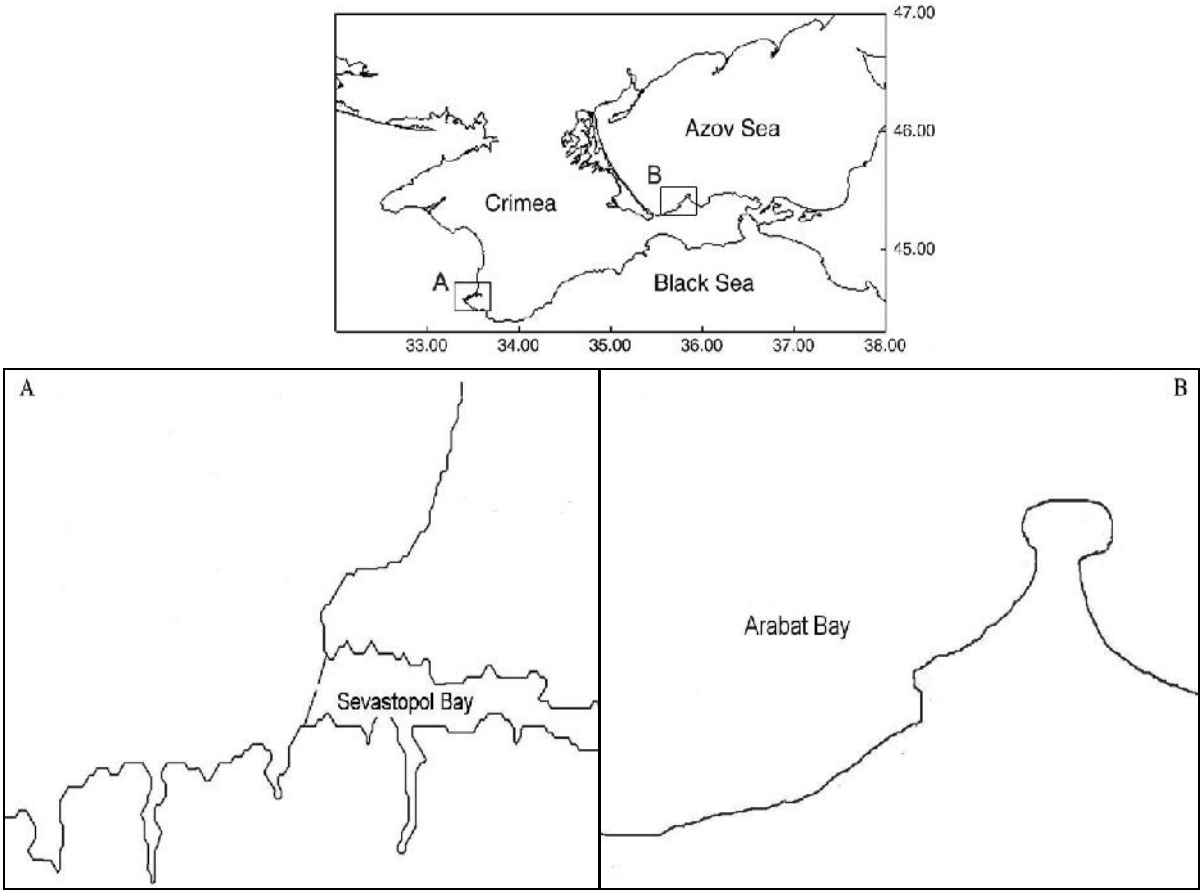

FIGURE 2. Sampling sites of fish specimens. A - Sevastopol Bay (Black Sea, 44° 36′ N - 33° 32′ E); B – Arabat Bay (Azov Sea, 45°31′N - 35°47′ E)

The animals were transported to the laboratory in the containers with marine water and constant aeration. Fish age was recorded by analyzing otolits. Fish sex and stage of maturation were determined by identification of gonads after dissection: II, II – III –non-mature gonads; III, III – IV – mature gonads; IV, IV – V, V – spawning time; VI – II – post-spawning period.

Blood collection and serum preparation

After animal anesthetization blood was taken from individual fish from the caudal arteria. Blood was centrifuged at room temperature and serum (supernatant) was separated and stored at -20^{0}C for biochemical analysis and electrophoretic separation.

Total protein and albumin concentration determination

Total protein concentration in serum was measured according the method of Lowry et. al. (1951). Albumin concentration was assayed spectrophotometricaly at 540 nm by Bromokresol Green binding used the standard kit (Ukraine).

Polyacrylamide disc gel electrophoresis

Serum proteins were analyzed by polyacrylamide disc gel electrophoresis (Davis et al., 1964). The gels contained 7.5% polyacrylamid, electrode buffer was pH 8.5. The electrophoresis was preformed at 2.0 mA per tube during 30 min and increased to 3 – 4 mA at temperature + 4 – 6 ^{0}C. Bromphenol Blue was used as a marker of sample mobility, which was added directly in cathode buffer. After electrophoresis the gels were stained with Sudan Black in 7 % acetic acid. Then the gels were washed in 7% acetic acid water solution and stored in it. Albumin identified according its electrophoretic mobility as compared with human serum albumin mobility. Coefficient of electrophoretic mobility (C_{ef}) was calculated as a ratio of the distance of protein band from the start to the distance of the marker (Bromphenol Blue). The mean value of $\mathbf{C}_{ef}$ was determined for every of comparable fish groups.

Statistical analysis

All values from serum proteins and albumin concentrations and electrophoretic analysis were presented as means ± SEM for each group of fish (Lakin, 1990). Student's t-test was used to assess the differences between measurements of serum proteins and albumin concentrations.

RESULTS

Total protein and serum albumin concentrations of N. melanostomus from Black Sea and Azov Sea

Total serum proteins and albumin concentrations are presented in Figure 3.

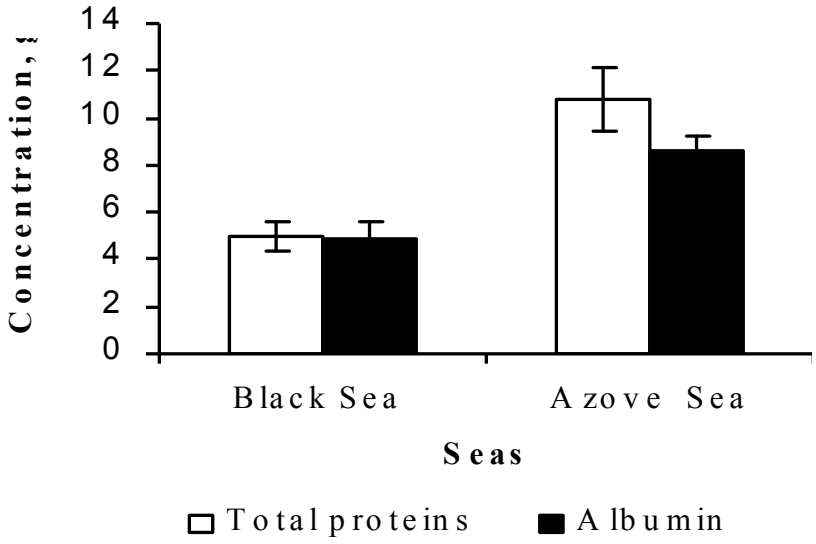

FIGURE 3. Total serum proteins and albumin concentrations (g l^{-1}, mean ± SEM) in fish from Black Sea (n = 13) and Azov Sea (n = 13)

The total serum protein and albumin concentrations were significantly ($p<0.01$) higher in fish from Azov Sea as compared with Black Sea ones. At the same time albumin level in fish from Black Sea was estimated as 97.8 % of the total serum proteins concentration while in Azov goby it was estimated as 79.9 %.

Electrophoresis of serum proteins of N. melanostomus from Black Sea and Azov Sea

Typical patterns of serum samples of the fish from two seas are presented in Figure 4. Serum of *N. melanostomus* contains 19 and 24 bands in fish from Black Sea and Azov Sea respectively. According electrophoretic mobility we identified the major band of C_{ef} 0.8 – 0.7 as albumin. C_{ef} of serum albumin of Black Sea fish was higher as compared with Azov Sea goby (0.75 – 0.8 against 0.70 – 0.73). At the same time the electrophoretiuc mobility of serum albumin depended on some biotic and abiotic factors.

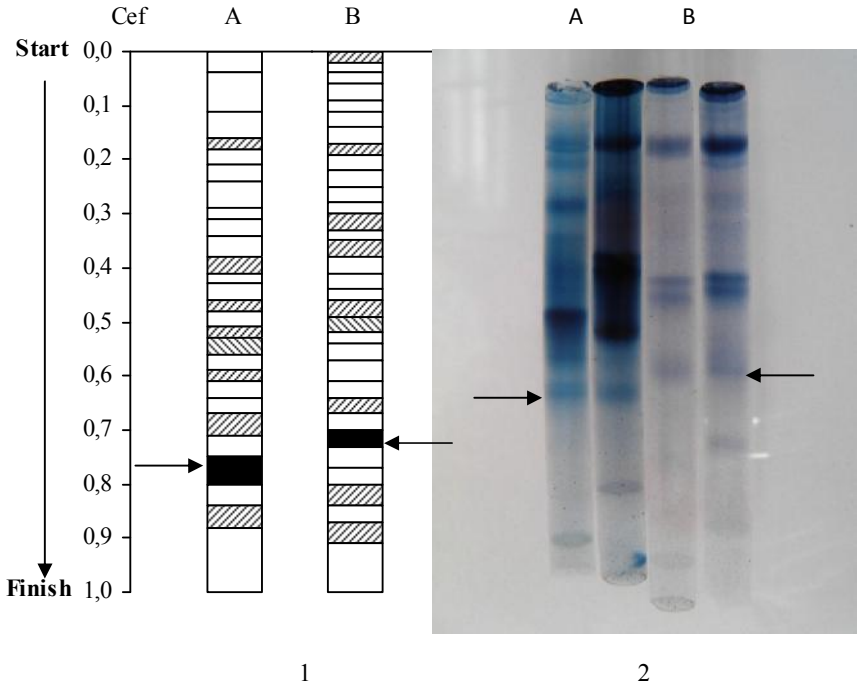

FIGURE 4. Graphical schemes (1) and patterns (2) of the serum of *N. melanostomus* from Black Sea (A) and Azov Sea (B). (→) - Alb – albumin band; C_{ef} – coefficient of electrophoretic mobility; finish – marker distance (Bromphenol Blue), estimated as 1.

Age-related electrophoretic characteristics of fish serum albumin

Electrophoretic mobility of fish serum albumin fluctuated and demonstrated age-related differences in fish (Table1).

Table 1. Age-related changes of serum albumin C_{ef} of fish from Black Sea and Azov Sea

Fish age, years	Serum albumin C_{ef} (min - max)	
	Black Sea	Azov Sea
1 – 1+	0.74 – 0.78 (n = 8)	0.68 – 0.70 (n = 78)
2 – 2+	0.75 – 0.79 (n = 36)	0.69 – 0.73 (n = 68)
3 – 3+	0.72 – 0.77 (n = 22)	0.70 – 0.73 (n = 15)
4 – 4+	0.76 – 0.78 (n = 14)	-

C_{ef} of serum albumin in Azov Sea goby varied less than in Black Sea fish. C_{ef} of albumin in 3-years age Black Sea fish was less than in other age groups. In Azov Sea goby C_{ef} of albumin tended to increase with age.

Sex-related electrophoretic characteristics of fish serum albumin

Electrophoretic mobility of serum albumin in male and female are presented in Table 2.

TABLE 2. Sex-related changes of serum albumin C_{ef} of fish from Black and Azov Sea

Sex	Serum albumin C_{ef} (min - max)	
	Black Sea	Azov Sea
male	0.76 – 0.79 (n = 66)	0.69 – 0.73 (n = 48)
female	0.74 – 0.79 (n = 18)	0.70 – 0.73 (n = 118)

No differences were observed in C_{ef} values of albumin in female and male. In Black Sea goby serum albumin mobility was higher as compared with Azov Sea species both in male and female.

Maturation stages-related electrophoretic characteristics of fish serum albumin

Serum albumin C_{ef} varied more widely and depended on maturation stages of fish (Table 3).

TABLE 3. Maturation stage-related changes of serum albumin C_{ef} of fish from Black Sea and Azov Sea

Maturation stages	Serum albumin C_{ef} (min - max)	
	Black Sea	Azov Sea
	male	
II, II - III	0.72 – 0.76 *(n = 7)*	-
III, III - IV	0.73 – 0.76 *(n = 6)*	0.67 – 0.72 *(n = 5)*
IV, IV – V, V	0.76 – 0.80 *(n = 48)*	0.70 – 0.74 *(n = 24)*
VI - II	-	0.66 – 0.69 *(n = 13)*
	female	
II, II - III	-	0.65 – 0.70 *(n = 2)*
III, III - IV	0.74 – 0.77 *(n = 8)*	0.66 – 0.70 *(n = 6)*
IV, IV – V, V	0.75 – 0.80 *(n = 7)*	0.69 – 0.72 *(n = 104)*
VI - II	-	0.70 – 0.74 *(n = 5)*

In Black Sea male albumin C_{ef} increased during mature process from 0.72 – 0.76 to 0.76 – 0.80. In female the fluctuations were less, but in stages IV, IV – V, V (spawning) albumin band was widely than in stages III, III – IV (gonads mature). Similar trend of albumin C_{ef} was indicated in Azov Sea males, in which C_{ef} of albumin band was higher in fish in stages IV, IV-V, V (spawning) as compared with the males on stages III, III - IV (gonads mature), but in stage VI-II albumin mobility was decreased. In female the similar trend was observed, but albumin C_{ef} in fish on stages II, II - III and III, III - IV was the similar.

Seasonal-related electrophoretic characteristics of fish serum albumin

Seasonal trends of serum albumin C_{ef} in fish from two seas are presented in Table 4.

Table 4. Seasonal-related changes of serum albumin C_{ef} of fish from Black Sea and Azov Sea

Seasons	Serum albumin C_{ef} (min – max)	
	Black Sea	Azov Sea
spring	0.75 – 0.80 *(n = 72)*	0.69 – 0.73 *(n = 22)*
summer	0.71 – 0.76 *(n = 12)*	0.70 – 0.73 *(n = 119)*
autumn	-	0.69 – 0.73 *(n = 22)*

No significant seasonal changes were shown in albumin C_{ef} of Azov Sea goby. Opposite, albumin C_{ef} of Black Sea fish was higher in spring as compared to summer values.

DISCUSSION

Serum proteins play an important role in transport of different substances, defense of the organism against pathological agents, osmotic regulation, and some other functions. Among serum proteins albumin characterized by a high negative charge (isoelectric point, pI = 5.67) and relatively low molecular mass of 66 000. Its main function is the regulation of colloidal osmotic pressure of the blood and transport of some exogenous components (drugs) and endogenous chemicals (fatty acids, hormones, bilirubin) (Csogor, 1975; De Smet et al., 1998; Baker, 2002).

Albumin-like proteins were found in different teleot fish and lamprey, but in elasmobranchs it was absent in some species (Metcalf & Gemmell, 2005). The information of albumin presence in teleosts was also contradicted. Concentration of albumin-like proteins in fish plasma of teleosts can vary from 10 % to 50 % while in terrestrial vertebrates albumin accounts for more than 50 % of the total serum proteins concentration (Mc Donald & Milligan, 1992). Salmonid species contain albumin-like proteins and demonstrated high heterogeneity and dependence on fish ecological status (Luk'anenko & Habarov, 2005). Albumin-like fractions were identified in some other teleost fish species by electrophoretic method (Shagufta & Gayasuddin, 2011).

In our study albumin concentration in blood serum of Azov Sea and Black Sea goby demonstrated high concentration of this protein which was ranged between 74.9 % in Azov Sea fish and 97.8 % in Black Sea fish. Our results agree with the data obtained for bonnet head shark in which albumin was presented 4 g l^{-1} (Harms et al., 2002). At the same time we used the clinical method designed for human samples and it could not be adopted for fish serum. Thus we could propose that the value obtained was more higher which also agrees with the opinion of the other researchers (Metcalf & Gemmell, 2005).

Electrophoretic methods of serum proteins analysis are more preferable. As expected albumin in human plasma emerges as a major spot with molecular mass 66 000 and pI 5.7 and electrophoretic mobility between 0.7 - 0.8. Similar bands we found in Black Sea and Azov Sea goby serum proteins electrophoregrams. Opposite, localization of serum albumin in round goby from Black Sea and Azov Sea was differed each from other: electrophoretic mobility of Azov Sea fish albumin was significantly less than those in Black Sea ones. The difference of albumin mobility in both goby samples may be result of gene mutation or specificity of transport function and binding properties (Michelis et al., 2010). In addition, the differences of food spectra of fish from Black Sea and Azov Sea could be influenced on lipids and fatty acids composition binding with albumin which modified its physical and chemical properties, including electrophoretic mobility (Metcalf & Gemmell, 2005).

Our findings have been demonstrated also some age-related changes of albumin electrophoretic mobility in Black Sea fish, while in Azov Sea animals they were less. It was attributed with the age-related changes of metabolism, including lipid and bilirubin concentration fluctuations in serum during ontogenesis (Hasnain et al., 2004). Besides that the modified forms of albumin may appear during aging and their electrophoretic mobility could be differed in old fish as compared with young.

Albumin binds steroid hormones, including sex hormones (Baker, 2002), but no differences of albumin electrophoretic mobility in male and female were shown. At the same time the differences in albumin mobility of male and female in different maturation stages were revealed by electrophoresis. At spawning time (stage IV - V) both in male and female the electrophoretic mobility of albumin was higher than in fish on stage II - III. It could be proposed that high hormones concentration during the period of fish gonad mature and reproduction changed its physical and chemical properties including electrophoretic mobility (Sayed et al., 2011).

Fish physiological status strong correlated with seasonal fluctuations depending on water temperature, oxygen concentration, food availability, and etc. Our findings demonstrated the differences of albumin electrophoretic mobility in Black Sea fish caught in spring and summer, but no seasonal-related changes were shown in Azov Sea fish. It could be suggested that complex of seasonal factors in Black Sea varies widely than in Azov Sea which influences on fish metabolism especially transport function of serum proteins. In addition, in autumn and summer time pollution level in sampled Black Sea area increased which was attributed with high level of water contamination. Fish consume xenobiotics with food and water which bind with albumin and change its electrophoretic mobility. It agrees with the data of some investigators who documented the changes of electrophoretic spectra of serum proteins in fish from chemical polluted areas (Osman et al., 2010) and microbial parasite infected conditions (Zacharia et al., 2003; Danis et al., 2000).

Hence electrophoretic properties of serum albumin in gobies from both seas demonstrated the similar trends

related to physiological, abiotic and anthropogenic factors, but in Black Sea fish they were greater than in Azov Sea animals.

CONCLUSION

Our findings indicate that fish physiological status, age, season and habitats play an important role in serum protein properties, especially albumin. The alterations of albumin electrophoretic mobility connected for the first time with its transport function and the differences between the round goby from Black Sea and Azov Sea which could be result of genetic mutation and the specificity of environmental factors.

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