I.J.A.B.R., VOL. 2(2) 2012: 203-208 ISSN: 2250 - 3579

INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

COMPARATIVE STUDY OF SERUM ALBUMIN LEVELS IN ROUND GOBY *NEOGOBIUS MELANOSTOMUS* FROM BLACK SEA AND AZOV SEA

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ABSTRACT
Concentration of blood serum albumin of the round goby *Neogobius melanostomus* caught in Black Sea and Azov Sea was studied in relation to age, sex, maturation and season. Albumin concentration in fish from Azove Sea was 3-fold higher than in the animals from Black Sea. Albumin level in fish serum depended on fish physiological status, maturation, age, season and geographical location. The variations of albumin concentration in serum could reflect the differences between the fish from Black Sea and Azov Sea and the specificity of their genetic status, ecological and biological characteristics of the habitats.

KEY WORDS: marine fish, serum albumin, age, sex, maturation, season

INTRODUCTION
Serum proteins play an important role in transport of various endogenous and exogenous chemicals, defense of the organism against infections, parasites and xenobiotics, and some other functions. Our studies of serum proteins composition of Black Sea scorpion fish *S. porcus* showed the differences between fish caught in polluted and non-polluted locations (Rudneva *et al*., 2005) and thus serum proteins may use as biomarkers of negative impact on the organism. Among serum proteins albumin is the major component which characterized by a high negative charge (isoelectric point, pI = 5.67) and relatively low molecular mass of 66000. Its main function is the regulation of colloidal osmotic pressure of the blood and transport of some exogenous chemicals (drugs) and endogenous metabolites (fatty acids, hormones, bilirubin) (De Smet *et al*., 1998; Baker, 2002).

Fish albumin has phylogenetic significance, because in some elasmobranches it was not found, and among teleosts several species have lack of albumin. In few fish species specific properties of albumin were shown and they were identified as so-called albumin-like proteins (Hasnain *et al*., 2004). Albumin-like proteins were found in different bony fish and lamprey, while 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). Our previous studies have been shown that fish physiological status, age, season and habitats influenced on serum protein properties, especially albumin. The alterations of albumin electrophoretic mobility in round goby caused its transport function and the differences between the animals from Black Sea and Azov Sea were observed (Rudneva & Kovyrshina, 2011).

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

MATERIALS AND METHODS
Animals
Animals were collected in winter - autumn period of 2009 - 2011 years in Sevastopol Bay (n = 48) (Black Sea, Ukraine) and in the Arabat Bay (n = 52) (Azov Sea, Ukraine) as we described previously (Rudneva & Kovyrshina, 2011).

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

Blood collection and serum preparation
After anesthetization of the animals 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.

Albumin concentration determination
Albumin concentration was assayed spectrophotometrically at 630 nm used the standard kit (Felicit - Diagnosis, Ukraine). Albumin binds to Bromokresol green and forms the green color complex. The color of this complex is directly related to albumin concentration in blood serum. For albumin level determination 2.0 ml of the standard Bromokresol green solution was mixed with 0.02 ml of the sample (blood serum). The albumin concentration was detected used calibration curve and calculated according the following equation

$$\mathbf{C = (E_o / E_k) * 50}$$

where C – albumin concentration in the sample, g l^{-1}; 50 – albumin concentration in the standard solution, g l^{-1}; E_o– extinction of the sample, optical units; E_k – extinction of the standard solution, optical units.

Statistical analysis

All values from serum albumin concentrations were presented as means ± SEM for each group of fish (Lakin, 1990). Student's *t*-test was used to assess differences between measurements of albumin concentrations. The correlations between fish age and albumin concentration in serum were calculated used the program CURFVIT.

RESULTS

Serum albumin concentration in fish from Black Sea and Azov Sea is presented in Figure 1.

Albumin concentration in the serum of Azove goby is approximately 3-fold higher as compared with the values of fish from Black Sea. The differences are significant ($p \leq 0.001$).

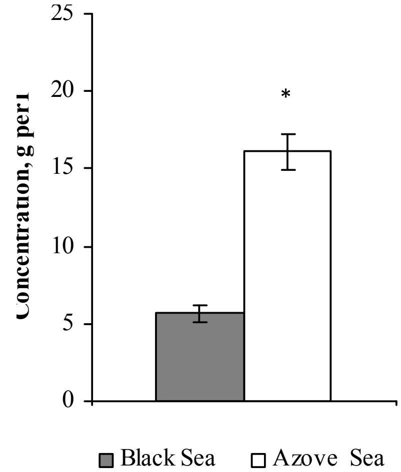

FIGURE 1. Albumin concentration (g l^{-1}, mean $\pm$ SEM) in fish from Black Sea and Azov Sea. Asterisk (*) indicates significant differences between fish used Student *t*-test ($p \leq 0.001$).

Age-related variations of albumin levels in serum were shown in Azove Sea goby while in Black Sea animals the fluctuations were insignificant (Fig. 2). In fish from Azove Sea albumin concentration dropped progressively during aging and it was significantly less in 4 years of age fish than in younger groups. At the age of 2 and 3 years albumin concentration in serum of Azove Sea goby was significantly higher ($p<0.01$) than in Black Sea goby. At 4 years of age fish no differences were indicated and the values of albumin concentration in serum were similar in both gobies.

No correlations between fish age and albumin concentration were observed in Black Sea fish, while in Azove Sea animals it was negative ($r = -0.85$).

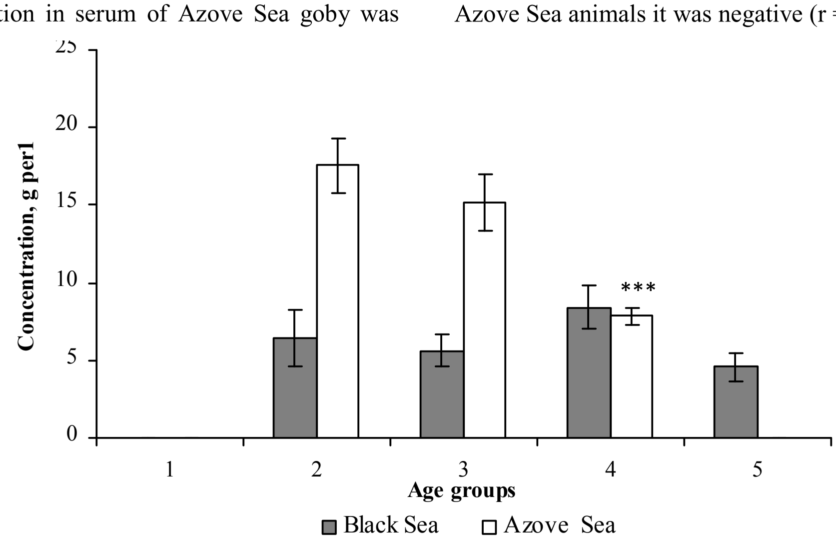

FIGURE 2. Age-related changes of serum albumin concentration (g l^{-1}, mean $\pm$ SEM) in fish from Black Sea and Azov Sea. Asterisk (*) indicates significant differences ($p \leq 0.001$) between the values of fish of 2 years of age; ** between the values of fish of 3 years of age.

Albumin concentration in blood serum of Black Sea fish male and female was the similar, while in Azove Sea goby albumin level was significantly higher ($p \leq 0.01$) in female as compared with male (Fig. 3).

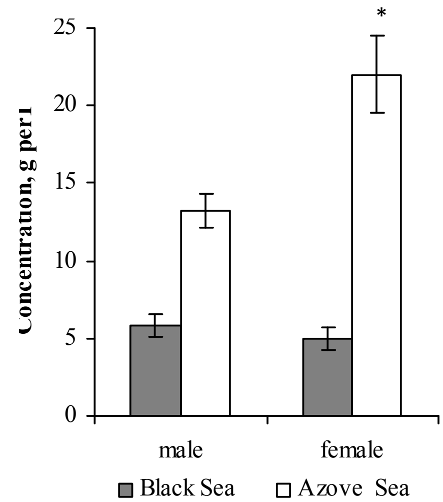

FIGURE 3. Sex-related changes of serum albumin concentration (g l^{-1}, mean + SEM) in fish from Black and Azov Sea. Asterisk (*) indicates significant differences between male and female ($p \leq 0.01$).

Sexual-maturation -dependent fluctuations of albumin level in serum in fish male and female from both locations are presented in figure 4.

In Black Sea males albumin level was significantly higher in IV-V stages as compared with the III-IV stages. In Azove Sea fish the opposite trend was observed: albumin level was greater in III-IV stage males as compared with IV-V stages fish. In Black Sea fish females the similar values of albumin concentration was detected in both cases while in Azove Sea animals the values were higher in VI-II stages females than in IV-V stages fish.

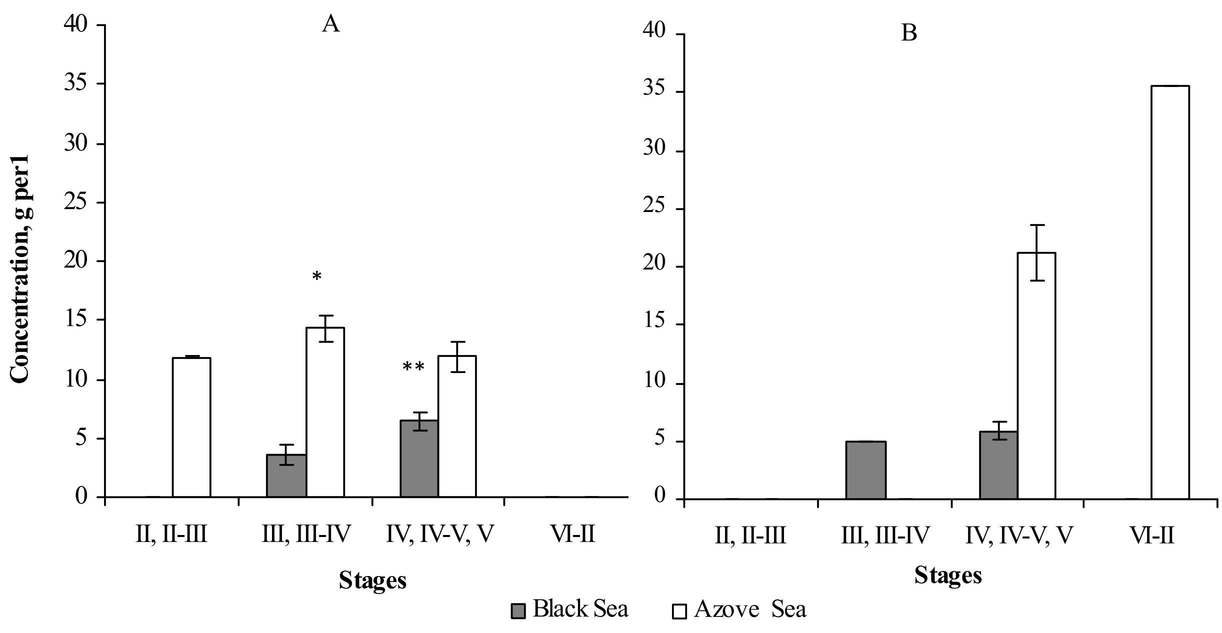

FIGURE 4. Sexual maturation stage- related changes of serum albumin concentration (g l^{-1}, mean + SEM) in male (A) and in female (B) from Black Sea and Azov Sea.

Asterisk (*) indicates the significant differences ($p \leq 0.05$) between the values of II, II – III stages; ** - between the values of III, III - IV stages.

Season-related fluctuations of albumin concentration in fish serum were not observed in Black Sea fish while in Azove Sea gobies the values were significant higher in warm period (summer and autumn) as compared with cold time (winter and spring) (Fig. 5).

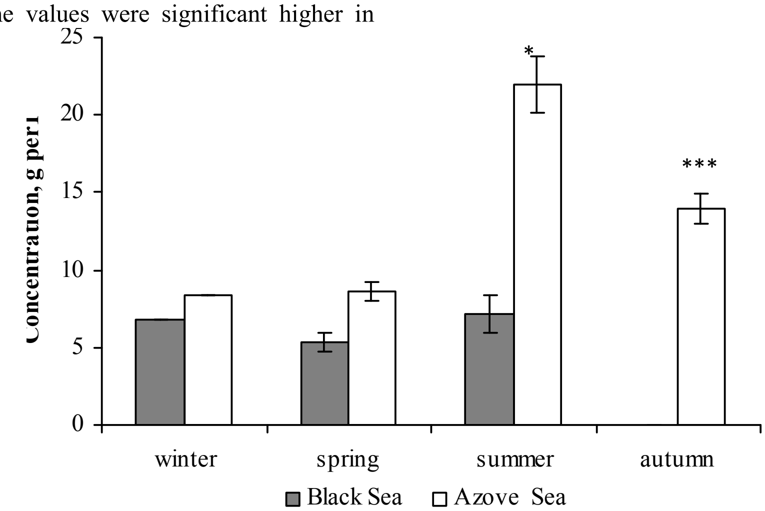

FIGURE 5. Seasonal-related changes of serum albumin concentration (g l^{-1}, mean + SEM) in fish from Black Sea and Azov Sea. Asterisk (*) indicates significant differences ($p \leq 0.001$) between the values of spring; **- between the values of summer.

DISCUSSION

Serum proteins composition and levels of their separate components depend on fish species, age, life cycle and sexual maturity, diet, health and environmental factors. Albumin in fish involves in plastic metabolism and plays an important role in transport functions of exogenous chemicals and endogenous metabolites (De Smet *et al*., 1998; Baker, 2002). Thus albumin determination in fish plasma or serum is considerable diagnostic tool which reflects the health of the animal, liver function, metabolic status and stress conditions.

Our findings demonstrated the high values of albumin concentration in both gobies which agrees with the data obtained for *Sphyrno tiburo* in which albumin was present 4 g l^{-1} (Harms *et al*., 2002) and for *Clarias gariepinus* which serum albumin level was 4.1 g l^{-1} (Yekeen & Fawole, 2010). In our study 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 were higher that was noted by the other researchers (Metcalf & Gemmell, 2005). However, the main idea of the present study was to show the trends of albumin concentration in fish from two separate locations and their dependence on animal physiological status, age and season.

Albumin concentration in serum of Azove Sea fish was approximately 3-fold higher than the value of Black Sea animals which may be explained by the different geographical and food conditions between both locations, as well as genetic factors. Food organisms biodiversity in Azove Sea is more preferable diet for goby than in Black Sea, hence this factor could influence on albumin concentration increase in fish serum. The researchers have shown that albumin concentration strongly depends on diet composition and it is able to modulated serum proteins spectra both qualitatively and quantitatively (Chukwuma *et al*., 2010; McQueen *et al*., 2011). At the other hand the genetic differences between fish species from examined locations could play a role in albumin concentration in blood serum (Marco *et al*., 2011).

We documented some age-related changes of albumin concentrations in gobies blood serum from both locations. The lowest albumin level was indicated in the group of 5 years of age which agree with the data obtained for mammals. Blood samples from rats at two different age groups observed that with advancing age there was a general decrease in serum albumin concentration (McQueen *et al*., 2011). However, in Black Sea fish age-related fluctuations of albumin concentration were less than in Azov Sea animals, and high negative correlation between fish age and albumin concentration was shown (r=-0.85). It was attributed with the age-related changes of metabolism, liver function which is the site of albumin production, gene expression and albumin transport function. Previously we documented the age-related changes of blood antioxidant enzymes in few Black Sea fish species including round goby, and in some species we showed the decrease of enzyme activities during age (Rudneva *et al*., 2010). Besides that the modified forms of albumin may increase during aging and their level could be elevated in elder fish as compared with younger group (Hasnain *et al*., 2004).

Albumin binds and transports steroid hormones, including sex hormones (Baker, 2002), but no differences of albumin electrophoretic mobility in male and female were shown in our previous study (Rudneva & Kovyrshina, 2011). At present investigation we have shown the similar values of albumin concentration in male and female serum of the goby caught in Black Sea. However, albumin level in fish from Azove Sea was significantly greater in female than in male. Our results were contradicted the data obtained Yousefian *et al*., (2010), who demonstrated the higher serum albumin concentration in male of rainbow trout as compared with female. At the other hand the differences in albumin level in male and female depend on the time of reproduction and sexual maturation stages (Marco *et al*., 2011). At spawning time (stage IV - V) albumin concentration was significantly higher in female as compared with male in Azove Sea goby while in Black Sea fish we have noted the opposite trend: albumin concentration in male increased at the time of reproduction and its level was higher than in female. It could be explained by the induction of albumin synthesis in spawning time because it plays an important role in transport function of various components that need for gonads formation and eggs development. In addition, as we marked previously at the period of fish maturation and reproduction the physical and chemical properties of albumin including electrophoretic mobility were changed (Rudneva & Kovyrshina, 2011).

Fish physiological status strong correlated with seasonal fluctuations depending on water temperature, oxygen concentration, food composition, anthropogenic impact and algae bloom (Kamal & Omar, 2011). Our findings demonstrated insignificant fluctuations of albumin level in Black Sea fish, while seasonal-related changes were shown in Azov Sea animals. Albumin concentration was significantly higher in fish caught in summer and autumn than that in winter and spring. We could propose that the main reason of this fact is the diet of Azove Sea fish and high level of eutrophication of this location in warm period of the year. The researchers documented that in fish from eutrohped habitats their physiological status was differed which was accompanied with the changes of the basic biochemical indices, including albumin concentration in plasma (Kopp *et al*., 2010). Fish consume microalgae with water and food and they are accumulated in their tissues which is attributed with the enhance of total protein and albumin levels (Abasali & Mohamad, 2010) as a result of the organism response on toxic effects of microalgae accumulation in tissues (Zacharia *et al*., 2003). In addition, in autumn and summer time recreation increases in sampled Azove Sea area. High level of biogens and endocrine disruptors enter in marine water which also modify fish physiological, endocrine and biochemical status. It agrees with the data of some investigators who documented the changes of serum proteins composition in fish from chemical polluted areas (Metcalf & Gemmell, 2005; Michelis *et al.,*2010; Osman *et al*., 2010). Thus, our findings have shown that fish serum albumin may be used as good biomarker for monitoring of fish ecological, physiological and health status.

CONCLUSION

Fish physiological status, age, season and habitats play an important role in serum albumin concentration. The variations of albumin level in plasma of the round goby from Black Sea and Azove Sea reflect the habitat specific conditions such as food composition, seasonal factors, age and sex structure of populations as well as genetic status of both gobies. Serum albumin level determination is a good tool fro the evaluation of fish physiological, biochemical and ecological status.

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