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Age- Dependent Changes of Mediterranean *Trachurus mediterraneus* Male and Female from Coastal Waters of Sevastopol (Black Sea, Ukraine)

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Abstract

Age-and sex-dependent characteristics of Mediterranean horse mackerel *Trachurus mediterraneus* from Sevastopol coastal waters (Black Sea, Ukraine) at the period of 2008-2010 were studied. The alanin- and aspartataminotransferase (ALT and AST) activities in the liver and oligopeptides concentration in blood were measured in male and female at the age of 1-3 years. Significant age-dependent changes were evidenced, while the sex differences were not significant. The results can be applied for development of monitoring management and for perspectives of conservation ecology and biodiversity.

Keywords: Horse mackerel, Black Sea, age, sex, size, liver, blood, aminotransferases, oligopeptides.

Introduction

Status of fish population is a reflection of the ecological status of the aquatic ecosystems. Fish population characteristics are used as indicators of environmental health (Adams, 2005; Goksoyer *et al*., 1996), because age and sex structure can be modified under impact of some negative factors including anthropogenic pollution. Age-dependent changes of fish size and mass, morphological and physiological characteristics can be attributed to endogenous (development, genetic defects, hormones fluctuations) and exogenous, including environmental unfavorable factors and diseases. Accumulation of the negative changes and loss of the ability to repair or detoxify them increase the risk of death of individuals, loss of population size and biodiversity. The study of aging strategy, both endogenous and exogenous factors is very important for the understanding of the key mechanisms of aging and its early prevention.

Alanine transaminase (ALT) and aspartate transaminase (AST) catalyze the interconversion of amino acids and α-ketoacids by transfering of amino groups. ALT catalyses the transfer of the amino group from alanine to α-ketoglutarate to form glutamate and pyruvate. AST catalyses the transfer of the amino group from aspartat to α-ketoglutarate to form glutamate and oxaloacetate (van der Oost *et al*, 2003). Both aminotransferases function as a link between carbohydrate and protein metabolism. They are detected in blood serum, in the cytoplasm and mitochondria in various tissues. An increase of enzyme activity in the blood serum, plasma and other extracellular fluids was documented in many investigations of the organisms impacted unfavorable conditions and it is related to organ disfunction or internal lesions in tissues. The cells damaged release their contents (including aminotransferase) towards the blood stream and the level of these enzymes enhances in serum (Martinez-Porchas *et al.*, 2011).

AST is used as clinic diagnostic tool and it is associated with cell necrosis of the liver and skeletal or cardiac muscle, starvation and lacking of vitamin E. Plasma ALT is an acute hepatic damage good marker (Coppo *et al.* 2001-2002). The increase of serum ALT activity was demonstrated in tilapia after injection of benzo[α]pyrene (BaP) (Martinez-Porchas *et al.,* 2011). The increase of both aminotransferases activity was documented in common carp impacted heavy metals (cadmium, lead, nickel and chromium) (Rajamanickam, Muthuswamy, 2008) and exposed in herbicide pendimethalin (Abd-Algadir *et al.,* 2011). The ratio of AST/ALT has been proposed by de Rytis (Rytis coefficient) for the analysis of transaminases activity in organs and tissues. This index allows us to estimate the functional load on the liver and heart. Sudden changes in the coefficient of relative discriminatory value as indicators of pathological processes occurring in these organs, which cause displacement synthesis, release, and metabolic

conversion of aminotransferases. Lowering this value is indicative of massive hepatocytes, increase - of myocardial infarction (Titov and Bichkova, 1990).

Oligopeptides are short sequence peptides ranging from 2 to 20 amino acids. This class of proteins includes potent biologically active compounds, which can modulate various cellular and molecular processes. Among them, the biological mediators are identified which play an important role in affecting biological activity. Currently, the use of protein peptide fragments is a rapidly growing field of research. Studies suggest that various biologically active peptides can result in favorable or negative outcomes on organism and the role of biologically active short sequence peptides as potential agents through the modulation of age-and sex-dependent biochemical pathways is very important (Grune, 2000; Karyakina and Belova, 2004).

The main part of fish in the Black Sea coastal catches is presented by red mullet, horse mackerel, scorpion fish and high body pickerel (Oven *et al.* (2008); data of Sevastopol inspection of protection and reproduction of fish stocks and the fishing regulation). The first two species are commercial. Recently horse mackerel and red mullet accounts 9 to 15%, 27 to 42% respectively of all the species in catches in the bottom snares installed by Institute of Biology of the Southern Seas.

Based on our recent study of some characteristics of Black Sea horse mackerel, it was shown that at fish growing small of individuals in older age groups the size and weight of juvenile (0±1 y.o.) *Trachurus mediterraneus* from the coastal zone of the Black Sea from 2008 began to rise. However, still low values of the indexes of the spleen, condition factor, gonadosomatic index and falling of catches characterize the stress functioning of this species population (Kuzminova, 2011, 2012a). It was confirmed, in particular, by biochemical analyzes of the gonads of *T. mediterraneus*: the activity of antioxidant enzymes in these organs was higher in the 2000s compared with the 1990s (Rudneva and Kuzminova, 2011).

Previously, the analysis of the size- mass, age and sex structure of Mediterranean horse mackerel caught in different areas of the Crimean coast in 2011 – 2013 was carried out. According to this, we can conclude that near Sevastopol and Balaklava, two distinct groups *T. mediterraneus* are inhabiting and belonging to the northern herd. After study of horse mackerel from autumn and winter catches in the areas of the southern coast of Crimea, Sudak, Alushta and cape Takil, we have fond some differences in values of size, weight and in the distribution of fish of different ages, which is associated primarily with the seasonal migration of this species (Kuzminova, 2013).

Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner, 1868) is widely distributed throughout the Black Sea and it is important commercial fish species. Hence, study of age composition, age- and sex-dependent changes of size, mass, morphological physiological and biochemical characteristics of this species is the important part of the monitoring of Black Sea marine ecosystem. The aim of the present work was to study the age composition and age-dependent trends of *T. mediterraneus* caught in the coastal waters of Sevastopol region (Black Sea, Ukraine).

Materials and Methods

Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner, 1868) (male n=282, female n =349) was caught in autumn-winter period 2008-2010 in Sevastopol coastal waters (Sevastopolskaya and Karantinnaya bays) (Black Sea, Ukraine) (Figure 1). The animals were transported to the laboratory in the containers with marine water and constant aeration.

Characteristics of the Sampling Sites

Data described earlier point to the improvement of the state of the environment as well as Black Sea

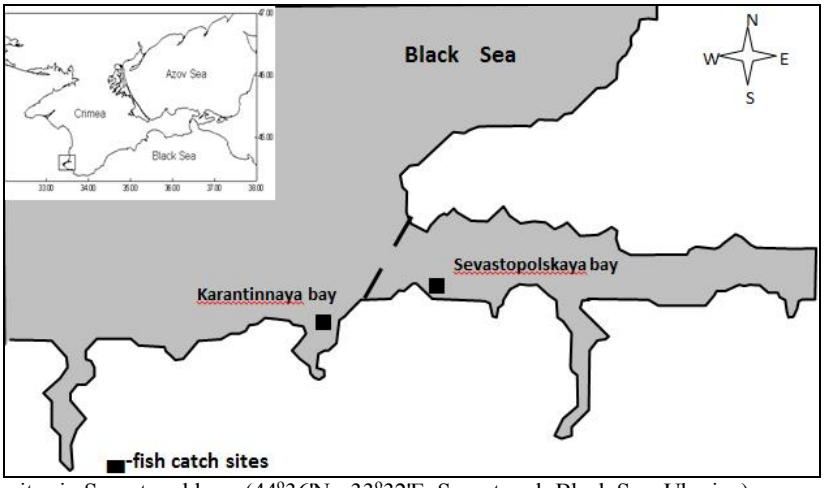

Figure 1. Sampling sites in Sevastopol bays (44°36'N - 33°32'E, Sevastopol, Black Sea, Ukraine).

biota (Kuzminova, 2012a). Nevertheless, differences in the level of contamination of some bays in the coastal area of Sevastopol are still observed. On the basis of last research data that we have described below it can be concluded that despite the proximity of Sevastopolskaya bay and Karantinnaya bay, there are some differences between them in physico-chemical and biological status.

Sevastopolskaya bay is the largest bay in the Black Sea, its length is more than 7 km, the maximum width is about one kilometer, the depth is 18 m; it is open to the west winds (Figure 1). It consists of about 20 bays. In the upper reaches of the Sevastopolskaya bay the Black River falls into it, while natural quasi-stationary estuary in the mixing zone is formed. At this site during the year the water temperature of the surface varies from 0°C to 26.6°C, and of the bottom does not drop lower than 4°C, salinity ranges from 3.25 to 16.13‰ on the surface and 14.74-17.36‰ in the bottom layer (Boltachev and Karpova, 2012).

Sevastopolskaya bay is semi-closed area with heavy water exchange, which was exposed to anthropogenic impact for a long time. Sevastopol bay sediments were classified in 2008 according knowledge about oil pollution as a very dirty, however the level of petroleum hydrocarbons (PHC) compared with 2003-2004 decreased tenfold (Kopitov *et al.,* 2010). There are data that the concentration of PHC and chloroform extracted substances (CES) in the bottom sediments of the Sevastopolskaya Bay were, according to recent information, 676 and 1231 mg x 100 g^{-1} (Eremeev *et al.,* 2008).

The copper concentrations in sediments to 2008 increased 10-350 times, and zinc – 4-times. The content of As and such toxic metals such as Cr, Cu, Cd, Ni, Pb, Zn in grounds of bay is 1.5-2.5 times higher than background concentrations of these elements (which is typical for Black Sea shelf which is not exposed to direct contamination caused by human activity) in other bays of the Crimea. For bay investigated high content of cadmium (7 of permitted concentration) and the local maximum of copper (5 of permitted concentration) in the bottom sediments are recorded, that associated with the accumulation of heavy metals near the artificially formed orographic barrier - breakwater.

The maximum values of the index of total contamination of bottom sediments of Sevastopolskaya bay (PHC, arsenic and heavy metals) in 2008 were determined in the Jugnaya Bay (17.3), near the thermal power plant (7.9), Ingenernaya (7.5) with the value 5.3 for Sevastopolskaya bay on average (Kopitov *et al.,* 2010). The high contamination of Sevastopol Bay grounds (hydrocarbons, mercury, cadmium, lead, zinc, nickel and chromium) in 1999 – 2005 was reported earlier, which, according to the opinion of the authors led to changes in the morphometric characteristics of the shellfish gills. The maximum degree of dissection of the gills was registered in filter-feeding shellfish *Mytilus galloprovincialis, Chamellea gallina, Cerastoderma glaucum* sampled at the end of Sevastopolskaya bay and Jugnaya bay (Timofeev *et al.,* 2009).

Karantinnaya bay is half a kilometer to the west of Sevastopolskaya bay, it is about 1.5 km long and 0.5 km wide at the out of the bay, and in the middle it narrows to 0.1 km or less, the depths at the bottom are 12-16, in the middle – 4-6 m (Boltachev and Karpova, 2012). Karantinnaya bay in the cold season is affected by northerly winds prevailing during this period.

The index of total contamination of bottom sediments for Karantinnaya bay (PHC, arsenic and heavy metals) in 2008 was 7.8, and at the out of the bay background value of this index was 1.3-1.5. In 2008 in the place where the bottom trap of IBSS is installed the concentrations of pollutants in the surface layer of the sediments were: Zn - 108.10. Cu - 28.80. Pb - 14.20. Cd - 2.95. Cr - 17.20. Co - 47.10. Ni - 18.30. As - 18.80. PHC - 3048 mg * kg^{-1} (Kopitov *et al.,* 2010). There is data that the concentration of CES in the bottom sediments of Karantinnaya bay was, according to recent research, 165 and 65 mg * 100 g^{-1} (Eremeev *et al.,* 2008).

The coastal waters of Karantinnaya bay is a zone of ecological rehabilitation of environment, because, despite the existence of an emergency release of domestic sewage and "neighborhood" with badly contaminated Sevastopolskaya bay, it has active water exchange with the adjacent part of the sea, as well as the presence of mussel farms that provide the intense processes of destruction of organic matter (Kuftarkova, 2006). Probably, these factors are responsible for considerable biodiversity of macrozoobenthic community of hard substrates (rocks) in the bay (Makarov *et al.,* 2010).

Data set of all indices of fish caught from two bays were combined due to the proximity of water areas, as well as the fact that the Mediterranean horse mackerel - sample of the pelagic longline and highly mobile swimmer that smoothes the difference of effect of habitat conditions for this specie.

Sample Collection and Preparation

Fish were individually measured, weighed and immediately processed for biochemical analysis. The total (TL), standard (Sl) lengths, total weight (TW) and weight without viscera (W), sex, stages of sexual maturity, weight of gonads and liver were measured. Liver, spleen and gonads were weighted for somatic indices calculations. The growth rate (GR) was estimated as the ratio of fish size or mass of the individuals belonging to previous age group to the next one x 100% according the formula (Anisimova and Lavrovsky, 1983):

$$GR\ \% = (W_{i+1} - W_i) \cdot 100 / W_i,$$

where W_{i+1} – weight of fish in age i+1, W_i – weight of fish in previous (i) age

Hepatosomatic indices were calculated according the formula (Shwarz *et al.,* 1968):

HSI ‰ = (Wl / W) x 1000

Gonadosomatic and spleen indices were calculated according the formula (Pravdin, 1966; Anisimova, Lavrovsky, 1983):

GSI % = (Wg / W) x 100
SI % = (Wsp. / W) x 100

Condition Factor was calculated according to formula of Clark (Anisimova, Lavrovsky, 1983):

CF % = (W x 100) / (Sl^3),

Where Wg, Wl, W sp., W – weight of gonad, liver, spleen and fish without viscera in grams.

The age structure of fish was determined by analyzing otolits. Annual rings on the otoliths of Black Sea horse mackerel are seen quite clearly in the microscope, as shown in Figure 2.

The blood was taken by caudal arteria puncture and serum was separated. The red blood cells were processed as we described previously (Rudneva, 1997). The sediment was washed three times with 0.85% NaCl solution and then lysed by addition of 5 vol of distilled water for 24 h at the refrigerator. Liver was excised, had been washed three times in cool 0.85% solution of NaCl and immediately homogenized in it (1:5 w/v) and centrifuged at 8,000 g during 30 min at +4°C. The supernatants were used for further biomarkers assay immediately after preparation. The enzyme activity was determined in the lysates immediately after preparation.

Biochemical Assays

Aminotransferases (ALT and AST) activity was determined spectrophotometrically with 2,4-dinitrophenylhydrazine at 500-530 nm used the standard kit (Felicit - Diagnosis, Ukraine). 0.2 ml of substrate-buffer solution was added to 0.04 ml of liver extract and incubated at the room temperature 1 h. The reaction was stopped by 0.2 ml of 1,4-dinitrophenylhydrazine and the solution was incubated during 20 min. 2 ml of 0.4 N NaOH was

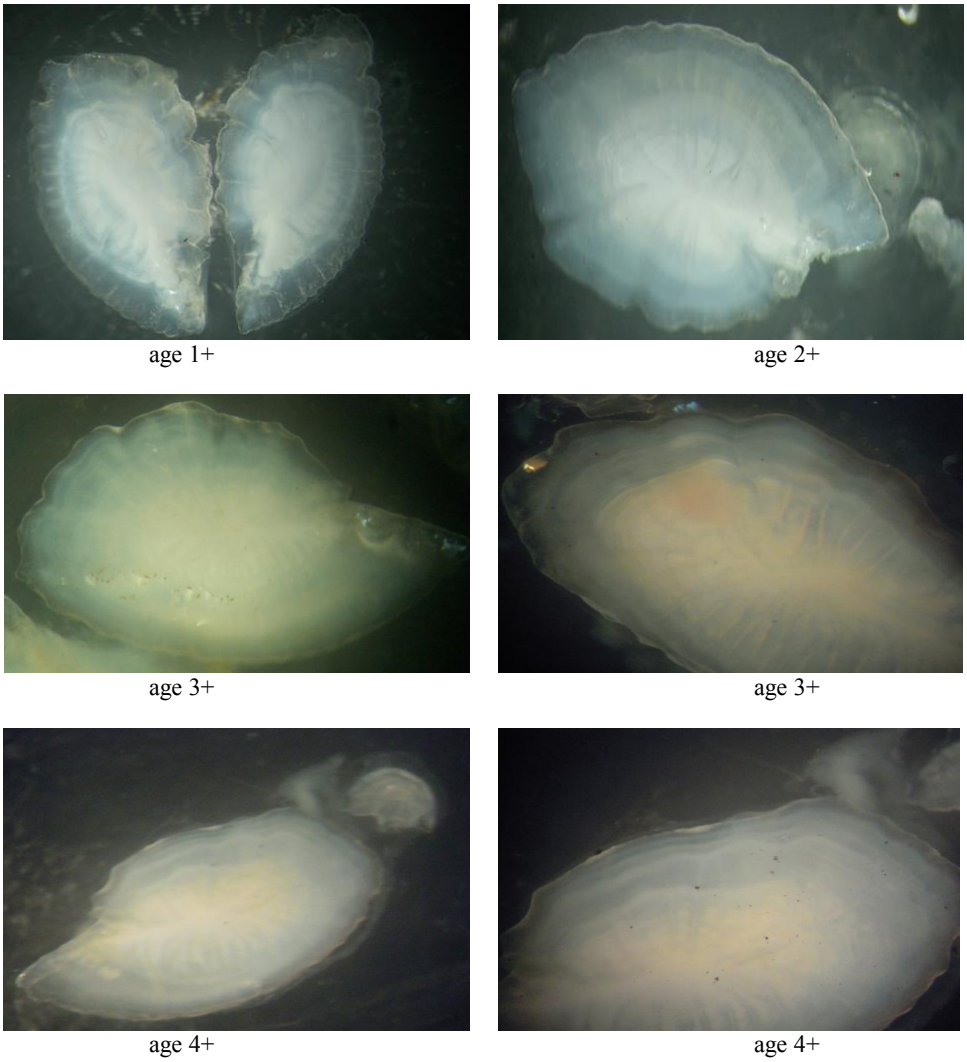

Figure 2. Otholits of Mediterranean horse mackerel from coastal area of Sevastopol (Black Sea, Ukraine).

added in the mixture and the optical density of the sample was measured at 500-530 nm at the spectrophotometer Specal-211 (Carle Zeiss, Germany). The ratio AST/ALT (de Rytis coefficient) was calculated also.

The concentration of oligopeptides was detected in fish blood. 0.25 ml TCA was added to 0.5 ml of the sample and centrifugated at 9,000 g 30 min. 0.3 ml supernatant was mixed in 3.7 ml of 3% NaOH and 0.2 ml of Benedict reagent was added. The mixture was incubated 15 min and then optical density was measured at 330 nm. The protein concentration in the tissues homogenates was estimated by the method of Lowry *et al.* (1951) using human serum albumin as the standard protein.

Statistical Analysis

The results were processed to statistical evaluation with ANOVA one-way test. All numerical data are given as means ± SEM (Halafyan, 2008). The significance level was 0.05. The correlation coefficients were calculated by the least-squares method between tested biochemical parameters. The correlation coefficient $0.3<r<0.5$ was selected as moderate, $0.5<r<0.7$ was selected as significant and $0.7<r<0.9$ was strong.

Results

Age and sex composition of examined fish is presented in Figure 3.

At the examined period the age of Black Sea horse mackerel varied from 0 to 5 years old. Middle age group was dominated. Female prevailed beginning with 2 years old in the population, however in juveniles (0^+-1) the opposite tendency was indicated (Figure 3). Generally, the ratio of male and female in population was approximately similar (0.8:1), that is usual for this species (Salekhova, 2007). Pronounced sexual dimorphism (size, weight) was not detected for this species (Table 1). Only at the age of 3^+-4 years mass of the male was larger as compared with the female. The Sl of 0-2 years old male group was significantly lower ($P \leq 0.01$), than the corresponding female parameters (Table 1). Non-availability of sexual dimorphism agrees with the results of other researcher (Salekhova, 2007). However, we could note the fluctuations of male and female size in different age groups and especially in their growth rate (Table 1). The growth rate of male (both size and mass) was greater than in female (with the exception of mass increasing of age class 1^+-2).

Due to the dependence of morphophysiological parameters on age, sex and stage of maturity, hepatosomatic (HSI), gonadosomatic (GSI), spleen (SI) indices and condition factor (CF) were calculated for fish of the identical sex, age and stage of maturity. Taking into account that generally commercial caught of horse mackerel takes place in autumn and in winter and in these periods fish gonads are on II stage of maturity, we have investigated morphological and physiological parameters of the individuals in cold period.

HSI and GSI of female of examined age groups were higher than in male. The values of both parameters were slightly differed in male of tested age classes. Female HSI was increasing from 1^+-2 years, tended to maximum at 4 years. The highest GSI for female was noted at age group of 1^+-2 years. The greatest value of SI was indicated in the young and old male, but the highest SI for female was showed only in old fish. CF of *T. mediterraneus* was slightly different for male and female of examined age classes. However, at the age group of 3^+-4 years this parameter was increased in female, while in male, in contrast, it was declined (Figure 4).

Hence, we could note the significant age-dependent changes of tested morphological parameters in horse mackerel and their trends were identical both in male and female. The physiological parameters varied less and in some cases (GSI, CF) their age-dependent trends were differed in male and female.

Aminotransferase activity in the liver of horse mackerel is presented in Figure 5.

The general trend of ALT and AST activity in both male and female liver was the similar: enzymatic activity was decreased at the middle-age group and increased in old fish. No significant differences were observed in female and male values in the middle-age group (both AST and ALT) and old age group (AST), while hepatic ALT activity was significant higher in male than in female. The greatest ration of AST/ALT was shown in the liver of fish belonging to 1^+-2 years age class.

Oligopeptides level progressively decreased in female blood with the age increasing while in male it varied insignificantly in tested age groups (Figure 6). Hence, we could conclude that the age-dependent trends of oligopeptides concentrations in fish blood of male and female was differed each from other.

Discussion

Our findings did not show significant differences between the examined age groups, but the values of HSI and GSI were the highest for old fish, while the great values of GSI were detected in the individuals of the predominated reproductive group of age of 1-3 years (Figure 3). At this case sex-related differences of GSI and HSI were significant, while the CF was differed for male and female belonging to different age groups. The obtained trend was indicated for majority of fish species, such as Black Sea *Atherina hepsetus, Sciaena umbra, Spicara flexuosa, Mullus barbatus ponticus, Scorpaena porcus and Gaidropsarus mediterraneus* (Smirnov, 1959; Kuzminova, 2005 a, b; Kuzminova, 2006; Kuzminova *et al.,* 2011). It's a common knowledge that the

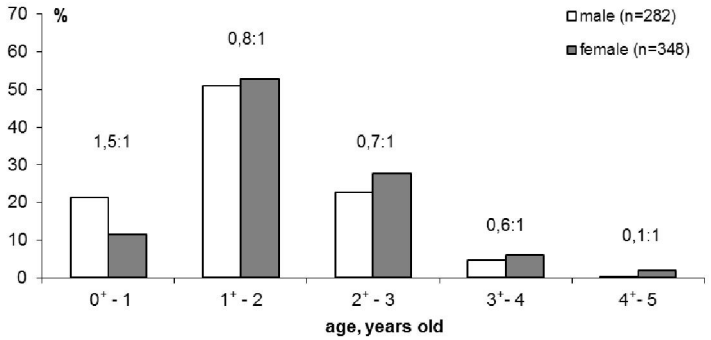

Figure 3. Age and sex composition of horse mackerel *Trachurus mediterraneus* from coastal area of Sevastopol (Black Sea, Ukraine) in 2008 - 2010.

Table 1. Size and weight of horse mackerel *Trachurus mediterraneus* from coastal area of Sevastopol (Black Sea, Ukraine) in 2008 - 2010

age, years old	parameters	female		male		GR			
		SL(cm)	TW(g)	SL(cm)	TW(g)	female SL(%)	male TW(%)	female SL(%)	male TW(%)
0^+ - 1	lim	7.3 – 12.0	5.1 – 20.36	7.0 – 12.1	4.78 – 23.68				
	M±m	**10.03±0.15**	12.79±0.48	**9.73±0.14**	12.51±0.53				
	n	41	41	60	60				
0→1						11.7	12.3	44.8	38.53
1^+ - 2	lim	8.2 – 14.4	7.43 – 40.72	7.6 – 13.7	5.74 – 37.16				
	M±m	**11.21±0.08**	18.52±0.40	10.93±0.09	17.33±0.48				
	n	184	184	144	144				
1→2						10.1	13.3	35.5	40.97
2^+ - 3	lim	9.9 – 14.9	13.3 – 41.14	8.4 – 14.7	8.83 – 40.75				
	M±m	12.35±0.10	25.10±0.61	12.45±0.14	24.43±0.83				
	n	96	96	64	63				
3→4						8.34	15.7	30.2	48.30
3^+ - 4	lim	10 – 16	13.45 – 52.49	11.9 – 19.4	19.02 – 46.52				
	M±m	13.38±0.26	32.69±1.92	14.41±0.54	36.23±2.12				
	n	21	21	13	13				
3→4						6.35	13.8	17.2	59.45
4^+ - 5	lim	13.7 – 15.4	32.36 - 47.96						
	M±m	14.23±0.22	38.33±2.09	16.4	57.77				
	n	7	7	1	1				

Note: Bold means significant differences between values of female and male of same age group.

growth of the fish continues during all live cycle and mass growth more pronounced than size growth (Anisimova and Lavrovsky, 1983). Possibly, in fish male the rate of growth of muscle mass is greater than in female in which the annual increasing of body mass associated with the growth of the liver and gonads.

We could propose that phenomena that the growth rate of male (both size and mass) was greater than in female (Table 1) may be caused the differences of hormonal status of the fish because female need to mobilize the energetic and constituents sources for gonad and eggs formation. It's agree with the fluctuations of the activities in the fish liver and oligopeptides concentration in the blood. However, it's well-known that female′s growth rate is higher than male′s in the majority of fish species including Black Sea teleosts (Information materials, 2006; Kelley, 1988).

It's interesting to note that in fish belonging to age group of 1^+-2 years SI was increased to senescence, while in young individuals male and female the values of this index were significant differed each from other (Figure 4). The similar trend was demonstrated in male and female of *Scorpaena porcus, Spicara flexuosa* and *Mullus barbatus ponticus* (Kuzminova, 2012b). The main function of spleen is the blood storage, old erythrocytes export and erytropoesis. The formation of this function in round goby and golden grey mullet develop during two first years of ontogenesis. In elder fish the processes are stable (Soldatov, 1992). Perhaps, the similar trends are characterized in majority of fish, which agrees with the obtained results of the dynamics of SI values in tested age groups of horse mackerel. In addition, *T. mediterraneus* is pelagic fast swimmer, which requires high concentration of erythrocytes, red blood cells, which elevate the oxygen consumption in the blood in early development. Hence, high values of spleen mass in the young age group (especially in female) could be the adaptation of the organism to the specific life conditions in pelagic environment.

Several authors observed age- and sex-dependent fluctuations of morphological,

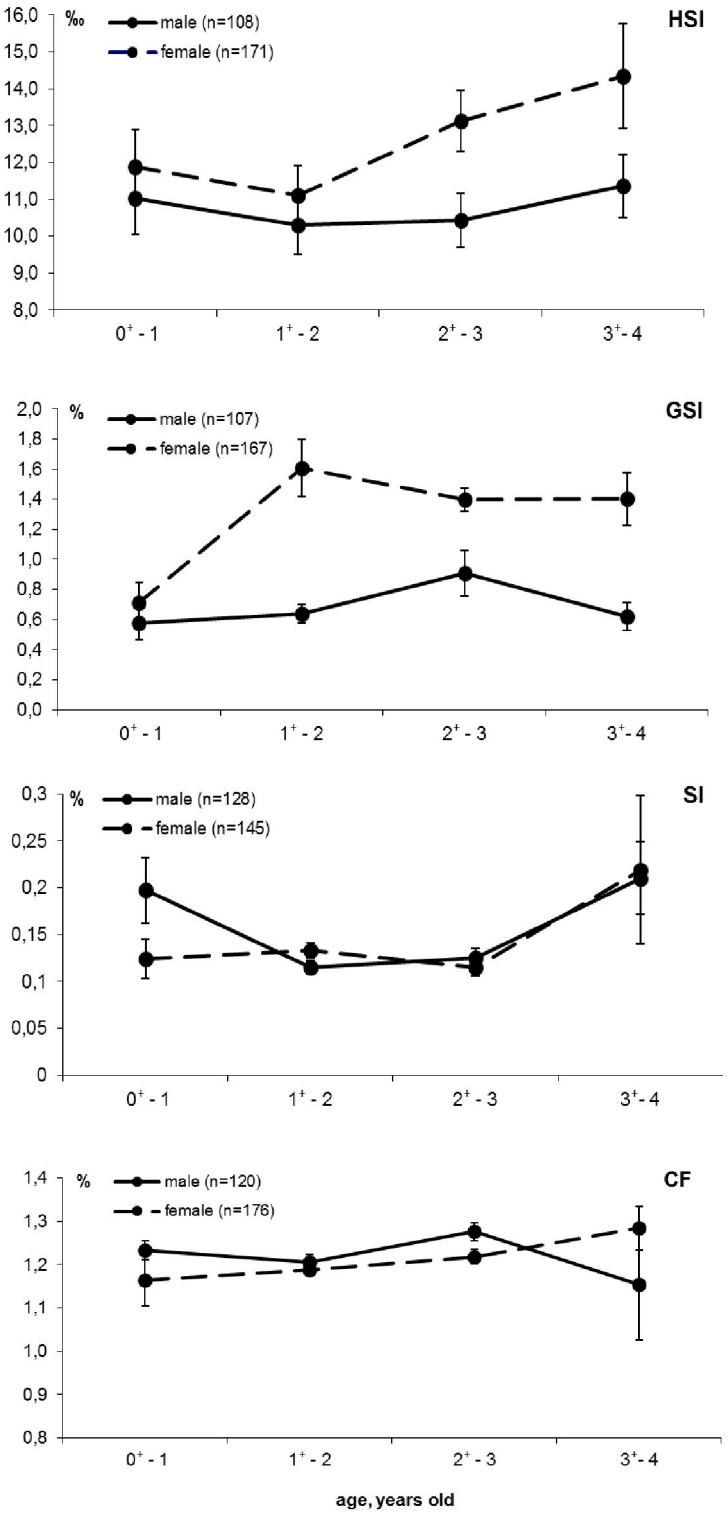

Figure 4. Morphological and physiological indices of horse mackerel *Trachurus mediterraneus* from coastal area of Sevastopol (BlackSea, Ukraine) in 2008 – 2010.

physiological and biochemical parameters of the animals, including fish. ALT and AST are non-plasma-specific enzymes located within the cells of the liver, heart, gills, kidneys, muscles, and other organs. In our studies the changes of transaminases activity were observed in the liver of fish belonging to different age classes. In male of the age group 0+ -1 enzyme activity was the greatest, it was decreased in the middle age class and then it grew in elder fish. Both in male and female the age-dependent trend of the ALT and AST activity was identical in the age groups 1^{+}-2 and 2^{+}-3 and the activity increased in elder individuals. We could propose that elevation of enzymatic activity with age could be associated with the induction of enzyme synthesis in the liver of elder animals. Increase of ALT and AST enzyme activity

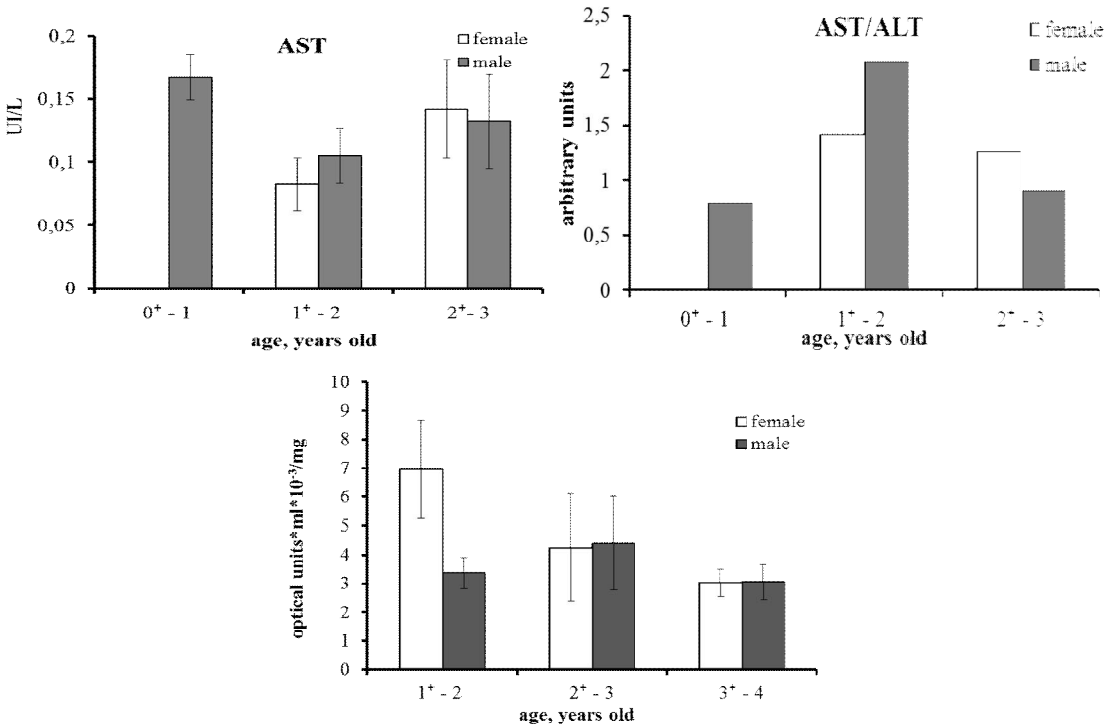

Figure 5. Age-dependent changes of aminotransferase activities in the liver of *T. mediterraneus.*

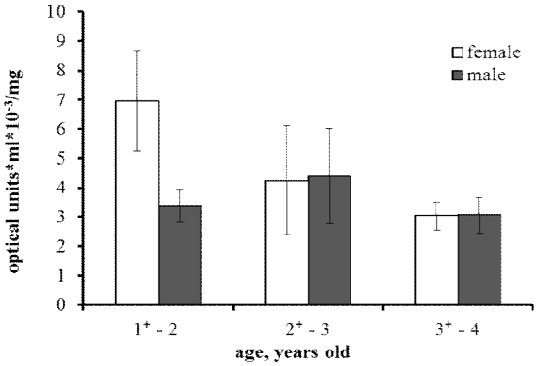

Figure 6. Age-dependent level of oligopeptides concentration in the blood of *T. mediterraneus.*

correlated with fish growth and differences in size, and may be related to changes in physiological status due to sexual maturation, feeding behavior and diet composition. Our findings agree with the results of Akrami1 *et al.* (2013), who documented that AST level in 7- and 8-year-old sturgeon of both sexes was significantly higher ($P<0.05$) than those in 4- and 6-year-old individuals. The mean ALT were significantly different ($P<0.05$) in both sexes of 4-, 6-, and 7-year-old sturgeon. Several researchers also demonstrated the age-dependent differences of aminotransferase activity in fish, amphibian and mammals tissues (Coppo *et al.,* 2001; Bicharova, 2011; MacQueen *et al*., 2011).

Priviousy, coefficient de Rytis was used for estimation of condition of Black Sea scorpionfish. It was shown that AST/ALT ratio changes depend on fish age, furthermore this changes correlate with bays contamination (Rochshina, 2010).

Accoding to Adeyemi *et al.* (2008), increased ALT/AST ratio may be indicator of the extent of cellular damage (Adeyemi *et al.,* 2008). The loss of AST and ALT activity in tissues may be interpreted as a compromise of the tissues integrity (Adeyemi *et al.,* 2009). Although ALT and AST are "marker" enzymes for the liver condition, it is believed that any alteration at the subcellular level may affect the activity of these enzymes in other tissues (Adeyemi *et al.,* 2008).

The decrease in the ALT and AST activity in the liver suggests that some of the effects led to inhibition of ALT and AST activity. Speaking of age, individuals of different age groups have different sensitivity to the factors of environment affected the different load on the liver, as the main organ of the body detoxification.

In addition, the concentration of oligopeptides which are the protein peptide fragments and their modifying forms fluctuated in fish blood with age. The level of it had tendency to drop in female and were not significantly changed in male. Hence, we could propose that the oligopeptides may be intensively utilized in female for gonad formation which agrees with high GSI index as compared with male.

At the same time the differences in enzyme activities could be cause the stress and damage of cell when the organism mobilizes its energy sources, including amino acids, to manage stress and repair damage caused by xenobiotics. The decrease activities of ALT and AST indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system in fish exposed to toxicants, it could be attributed to the high accumulation of metals in fish tissues (Abd-Algadir *et al*., 2011; Rajamanickam and Muthuswamy, 2008; Martinez-Porchas *et al.,* 2011).

Conclusions

Size, morphological and biochemical biomarkers variables seem to be useful indicators of the health status of fish in monitoring studies. The obtained results reflect the age-dependent fluctuations of fish life history and can be applied for development monitoring management and for perspectives of conservation ecology and biodiversity in impacted aquatic ecosystems. The analysis of biomarkers in fish blood and liver is important for the evaluation of fish abilities to protect against chemical pollution and keep their life in the pollute environments.

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