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BIOACCUMULATION OF Na, Mg, Ca, K, AND P IN FISH LARVAE OF THE GENUS *ATHERINA* L. COLLECTED IN THREE BAYS IN THE REGION OF SEVASTOPOL

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Abstract

The monitoring of pollution in bodies of water is an important part of both environmental protection policy and natural resources management policy. The use of larvae and young fish in the evaluation of the toxicity of the water environment is a more sensitive index compared to the bioindication methods using adult organisms. The aim of the research was to assess the content of Na, Mg, Ca, K, and P in water and in fish larvae of the genus *Atherina* L., caught in three Sevastopol bays. The research was carried out in 2012 in three Sevastopol bays: Golubaja, Omega, and Karantinna. The larvae were caught with a fishing net in July 2012, in shallow coastal waters at a depth up to 1 m. Water samples were also collected from the same locations. The concentration of the studied elements in the samples was determined by inductively coupled plasma atomic emission spectrometry. The content of magnesium and calcium was higher in the water collected from Golubaja Bay than in the other sample collection sites, whereas water from Karantinna Bay was found to have the most phosphorus. The concentration of the studied elements in the larvae of *Atherina* L had the following order, starting from the greatest: P>K>Na>-Ca>Mg. The sodium content ranged between 7.081 and 19.06 g kg^{-1}, magnesium between 1.033 and 2.79 g kg^{-1} potassium between 14.41 and 34.80 g kg^{-1}, calcium between 2.043 and 4.9671 g kg^{-1}, whereas phosphorus was between 15.23 and 44.73 g kg^{-1}. The highest content of all the studied elements, except for calcium and phosphorus, was found in the organisms caught in Golubaja Bay, whereas the lowest accumulation of these elements, except for phosphorus, was observed in larvae caught in Karantinna Bay which is located in the area with the highest anthropogenic impact. The decreased content of macronutrients in fish, particularly in their early developmental stages, may be the result of the toxic effect of pollutants.

Keywords: fish larvae, *Atherina*, Black Sea, macronutrients, bioaccumulation, Sevastopol bays.

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INTRODUCTION

The peculiarity of marine ecosystems lies in the naturally high content of sodium, potassium, sulfate, and chloride ions as well as in the low content of phosphorus and some micronutrients. The salinity level in sea water is higher than the level in body fluids of living organisms. That is why the processes associated with osmoregulation are directed to eliminate some ions (with the intensified absorption of other ions) from the organism. Maintaining a proper ionic balance in body fluids under conditions of high environmental salinity requires great inputs of energy. The change of environmental conditions as a consequence of introducing xenobiotics, or changes in water salinity or the invasion of pathogens leads to a disorder of the mechanisms associated with maintaining osmoregulation. That is why the content of macronutrients and micronutrients in the tissues of sea organisms is an important indicator of ecosystem quality (Moelleret al. 2003, Fisher al et. 2013, Tellis al et. 2013). Pollution of the aquatic environment with heavy metals or organic compounds may lead to changes in the chemism of animal tissues, therefore the content of macronutrients in the tissues of marine animals is an important indicator of the quality of the environment. The content of macronutrients in fish tissues is also of great importance in terms of the possibility of using them as food for humans. Fish and fish products are an important source of macronutrients such as phosphorus, calcium or magnesium, that is why the amount of these elements determines the quality of these products.

The assessment of the quality of the environment by using bioindication methods has great cognitive value as the information on the content of elements in individual, biotic parts of the ecosystem allows us to assess the actual effect of environmental pollution on living organisms. Among bioindication methods of assessment in a marine environment, methods using algae or certain fish species are the most frequently used (Bervoets et al. 2009, Brito et al. 2012). Using larvae and juvenile fish specimens in monitoring research on the marine environment has particular value due to the very fast anatomical changes as well as the physiological disturbances of young organisms under the influence of unfavorable environmental conditions (Gopalakrishnan et al. 2008, Kienle et al. 2008, McKinley et al. 2011, Kong et al. 2013). Most often, ecotoxicological studies dealing with fish larvae focus on developmental changes in embryos, the histopathological changes in certain organs, and mortality (Hallare et al. 2005, Arambourouet al. 2014). Larvae and juvenile specimens also have a greater capacity to accumulate trace elements, and they are the first to show toxicological effects associated with the disturbance in the uptake and accumulation of macronutrients. Heavy metals and some organic xenobiotics may, among other things, limit the absorption of calcium and magnesium by binding with their receptors (Meinelt et al. 2001). This leads to a deficiency of macronutrients in organisms, regardless of their amount in the abiotic parts of the environment (Varsamos et al. 2005).The content of macronutrients in fish tissues is a consequence of ionic regulation which is connected with the species, physiological state, health of the organism, sex, and also the quality of the environment in which the organisms live. Ionic balance in fish is maintained by the gills, kidneys, and intestines (Varsamos et al. 2005). Proper functioning of these organs ensures the osmotic balance of the organism. Sea water is a hypertonic solution for aquatic organisms, therefore proper quantitative relations among elements that occur in a relatively great number in water (e.g. potassium, sodium or magnesium) and elements whose numbers are low, require great inputs of energy from the fish organism (Fisher et al. 2013). Water acidification is one of the most important anthropogenic effects on the marine environment. Acidification of sea water is observed in all salt water regions and is considered to be one of the most important causes of their degradation (Walther et al. 2011). Changes of the level of water acidification often result in a disordered system of ionic regulation in the organism (Weng, Wang 2013).

The aim of the research was to assess the content of Na, Mg, Ca, K, and P in water and in fish larvae of the genus *Atherina* L., fished out of three Sevastopol bays. The second goal was to determine the level of bioaccumulation of these elements in organisms used in the research.

MATERIAL AND METHODS

The research was carried out in 2012 in three Sevastopol bays: Golubaja, Omega and Karantinna (Figure 1). The area of Sevastopol has for years seen the negative impact of pollution on the aquatic ecosystems. Atherina fish are commonly found in the research area, which makes them good bioindicators. The fish larvae of the *Atherina* genus were caught in July 2012. Fishing took place in the morning hours (between 7 and 8 o'clock). The larvae were caught using a fishing net in coastal shallow waters at a depth of up to 1 m. The

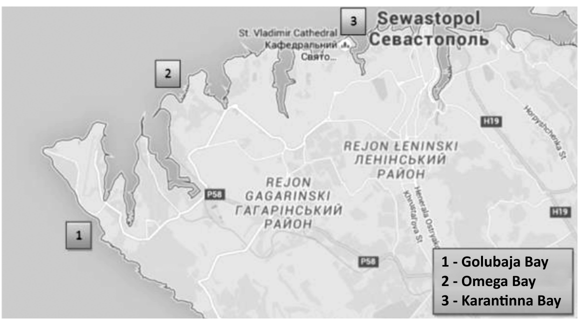

Fig. 1. Points of sampling

laboratory sample was the sum of increments taken from coastal zones around the bays. The mass of the laboratory sample was approximately 1 g of dry matter and was created from approximately 20 increments. The mass of one larva was between 0.001 and 0.005 g. Water samples were collected from the same places. The laboratory sample, with a volume of 1 dm^3, consisted of approximately 10 increments. The cumulative sample was the sum of the increments. Five laboratory samples of water and fish were taken from every point. The collected water was conserved at the collection site by adding nitric acid in the amount of 2 cm^3 per each 100 cm^3 water, then the samples were transported to the laboratory. Immediately after collection, the material was transported to the laboratory, where it was conserved by drying. Prior to drying, the fish samples were thoroughly rinsed in distilled water. In the laboratory, the samples of fish larvae were subjected to wet mineralization in a closed system with the use of microwave energy. An Anton Paar Multiwave 3000 microwave system was used for mineralization. The analytical sample amounted to approximately 0.5 g in terms of dry matter. The biological material was digested in a mixture of HNO_3 and H_2O_2, in the 5:1, v/v ratio. Concentration of the studied elements in the solutions was determined by inductively coupled plasma atomic emission spectrometry on an Optima 7600 DV manufactured by Perkin Elmer. The content of elements in larvae are presented on a dry matter basis (DM). The obtained results of the average contents of the element were compared using the *t*-Student test at a significance level of $p = 0.01$. On the basis of the obtained results, bioaccumulation factors were calculated for the individual elements. The bioaccumulation factors were calculated by dividing the concentration of an element in dry matter of the larvae used in the experiment by the content of these elements in water. The lengths of waves which were used to determine the concentration of the studied elements and limits of determination for the utilized methods are presented in Table 1. Certified reference material IAEA-407 was used to check the correctness of the analyses of the studied elements. Table 1 presents results of analyses of the reference material and an estimated value of recovery, based on the analyses conducted in the 4 replications.

Table 1

Paremeters of analytic method

Parameters	Wavelenghts	Limit detection	Content in certificated material	Measured	Recovery
	(nm)	(g dm^{-3})	(g kg^{-1})	(g kg^{-1})	(%)
Na	589.592	0.069	13.1	12.88	98.32
Mg	285.208	0.0016	2.72	2.904	106.8
Ca	317.933	0.01	27	28.33	104.9
K	766.490	-	13.1	12.91	98.55
P	213.617	0.076	-	-	-

On the basis of the obtained results, bioaccumulation factors were calculated for individual elements. The bioaccumulation factors were calculated by dividing the concentration of an element in dry matter of the larvae used in the experiment by the content of these elements in water.

RESULT AND DISCUSSION

The magnesium content in the water ranged between 511.9 and 598.7 mg dm^{-3}, the potassium content ranged between 376.8 and 399.5 mg dm^{-3}, calcium between 211.2 and 273.9 mg dm^{-3}, phosphorus between 0.086 and 0.113 mg dm^{-3}, and the sodium content ranged between 11.61 and 11.74 mg dm^{-3} (Table 2). The macronutrient contents determined in the authors' own

Table 2

Content of elements in water from bays (mg dm^{-3})

Sampling points	Na	Mg	Ca	K	P
Karantinna	11611*a*	511.9*a*	211.2*a*	376.8*a*	0.113*b*
Omega	11.744*a*	523.6*a*	246.0*a*	399.5*a*	0.099*a*
Golubaja	11.676*a*	598.7*b*	273.9*b*	385.5*a*	0.086*a*

Different letters beside the mean values indicate statistically significant differences among the samples from different bays at a significance level of $p = 0.01$.

research were at a level found in sea water from different regions of the world (Culkin, Cox 1966, Sánchez-Rodríguez et al. 2001). No statistically significant differences in the content of potassium and sodium in samples collected from individual bays were detected. The content of magnesium and calcium was higher in the water collected from Golubaja Bay than in the other sample collection sites, whereas water from Karantinna Bay was found to have the most phosphorus. Sodium, magnesium, calcium, and potassium contents in sea water depend on to what degree sea water mixes with inflowing fresh water as well as on the trends and intensity of biogeochemical cycles. The phosphorus content in sea water is associated mainly with the inflow of this element from land areas. The main source of phosphorus in sea water is the inflow of this element with as well as dry and wet deposition. An important source of this element in coastal zones of the seas is municipal and industrial sewage discharged into the sea directly from coastal areas. Magnesium, calcium and phosphorus contents determined in the authors' own research in water from individual bays located within one city are considerable and are indicative of the limited mixing of waters and the considerable impact of local conditions on the shaping of the chemism of the waters.

In general, potassium content in fish shows considerable diversity depending on the species, age, and environment of life. Constitutional management associated with this element in sea fish is connected with the efficiency of its elimination from the organism, because this element occurs in sea water in higher concentrations than in body fluids (EDDY 1985). Potassium content in sea water is approximately 400 to 500 mg K dm^{-3}, whereas fresh water contains approximately a hundred times less of this element. The mean content of this metal in the studied fish larvae of the genus *Atherinidae* L. was 25.19 g K kg^{-1} and varied from 14.41 to 34.40 g K kg^{-1} DM (Table 3). Signifi-

Table 3

Statistic parameters of results mean, minimum value, maximum value, standard deviation (g kg^{-1}), Relative standard deviation (%)

Specification	Na	Mg	Ca	K	P
Karantinna					
Mean	12.03*a*	1.831*a*	2.732*a*	17.84*a*	36.76*a*
Min	7.081	1.033	2.043	14.41	15.23
Max	16.29	2.766	3.079	20.56	44.73
SD	3.548	0.685	0.400	2.47	12.31
(%)*	29.49	37.39	14.63	13.82	33.49
Omega					
Mean	17.06*b*	1.999*a*	4.310*c*	28.07*b*	28.83*a*
Min	13.63	1.689	3.064	24.65	25.73
Max	20.70	2.431	4.967	31.49	32.37
SD	3.025	0.340	0.770	2.949	2.932
(%)*	17.73	17.03	17.86	10.50	10.17
Golubaja					
Mean	19.06*b*	2.433*b*	3.638*b*	29.66*b*	33.76*a*
Min	16.34	2.131	3.065	26.36	31.10
Max	24.21	2.790	4.155	34.80	36.94
SD	3.256	0.285	0.489	3.51	2.121
(%)*	17.09	11.71	13.43	29.66*b*	6.28

* Relative standard deviation.
Different letters beside the mean values indicate statistically significant differences among the samples from different bays at a significance level of $p = 0.01$.

cant differences in contents of this element in the larvae collected from individual bays were found. At the same time, differences among individual samples within the same bay were insignificant. The coefficient of variation for the potassium content in the fish was only approximately 10% for individual bays. The content of this element in organisms caught in Karantinna Bay was over 60% lower than the amounts of potassium found in the larvae from Omega and Golubaja bays. No statistically significant differences in the content of this element in fish larvae from Omega and Golubaja bays were detected. FISHER et al. (2013) provide potassium contents in sea fish larvae at a level of approximately 8 g kg^{-1} DM. These authors prove that potassium content in fish larvae may change very quickly over time, depending on availability of this element in water as well as physico-chemical properties of water. The value of the bioaccumulation factor for potassium in the studied organisms ranged between 4.749 and 6.458 (Table 4). The highest value of

Table 4

Bioaccumulation coefficient in larval bodies

Points of sampling	Na	Mg	Ca	K	P
Karantinnaja	1.036*a*	20.10*a*	6.684*a*	4.749*a*	15784*a*
Omega	1.453*b*	56.35*b*	14.04*b*	5.004*a*	28354*b*
Golubaja	1.630*b*	60.59*b*	13.60*b*	6.458*b*	34494*b*

Different letters beside the mean values indicate statistically significant differences among the samples from different bays at a significance level of $p = 0.01$.

this parameter was found in larvae caught in Golubaja Bay, and the lowest bioaccumulation factor for potassium was observed in larvae from Karantinna Bay.

The magnesium content in the studied organisms showed insignificant differences based on the sample collection site. No differences in the content of this element in larvae caught in Karantinna and Omega bays were found. By contrast, statistically significantly more magnesium was found in larvae caught in Golubaja Bay. Mean contents of magnesium in fish larvae of the genus *Atherina* caught in Karantinna, Omega and Golubaja bays were, respectively, 1.831; 1.999 and 2.433 g kg^{-1} DM (Table 3). FISHER et al. (2013) provide magnesium contents in sea fish larvae within the range from 1.2 to 2 g kg^{-1}, depending on environmental conditions. MOELLER et al. (2003) provide magnesium contents in sea fish caught in the area of Malibou Bay are similar to the ones obtained in the authors' own research. These authors found considerable differences in contents of this element based on the species of fish. They also found significant differences within specimens of one species based on the length of specimens as well as the place of origin. Magnesium contents determined in the authors' own research are characteristic for healthy organisms in which no deviation associated with ionic regulation is observed (HAMRE et al. 2008, WOODCOCK et al 2012).

The mean phosphorus content in the studied fish samples was 33.12 g P kg^{-1} DM and varied from 15.23 to 44.73 g P kg^{-1} DM (Table 3). No statistically significant differences in the content of this element in the organisms caught in the individual bays were found. Phosphorus contents determined in the studied larvae were several times higher than amounts of this element determined by MOELLER et al. (2003) in a few species of sea fish from the region of California. The value of bioaccumulation factor for phosphorus in the studied larvae varied from 15.78 to 34.49 (Table 4). The highest value of this parameter was observed in the studied organisms caught in Golubaja Bay, and the lowest in organisms caught in Karantinna Bay. Phosphorus contents determined in the authors' own research in the fish larvae do not indicate a disturbance of management of this element. However, considerable differences in the content of this element in organisms from individual bays indicate different living conditions of these organisms influencing the uptake of this element.

Calcium is an element which is directly connected with the development of the skeletal system and is involved in numerous physiological processes. Its uptake is mostly via the fish gills directly from the water. The uptake of this element from the alimentary system is of secondary importance. When contents of this metal in water are lower or when absorption of this element is disturbed due to the presence of xenobiotics, a lower calcium content in individual fish organs can be observed (Hossain, Furuichi 2000). The calcium content in the studied samples ranged between 2.273 and 4.967 g Ca kg^{-1} (Table 3). The mean content of this element in all the samples was 3.56 g Ca kg^{-1}. The determined contents were several times lower than the calcium contents in organisms of several sea fish species caught in the region of California (Moeller et al. 2003). Calcium content in fish larvae organisms is an important index of the quality of their environment. In marine organisms that live in a polluted environment, a lower calcium content is often observed (Lall, Lewis-McCrea 2007). It particularly applies to specimens in the larval stage which do not yet have a fully functional system of ionic regulation (Walther et al. 2011). In the authors' own research, considerable differences in contents of calcium in the fish larvae were found. The lowest amount of calcium was found in fish larvae from the genus *Atherina* L. caught in Karantinna Bay. The highest amount of calcium was found in those organisms from Omega Bay. Statistically significant differences in contents of this element in the larvae from individual bays were found. The value of bioaccumulation factor for this element ranged between 6.684 and 14.04 (Table 4). The values of this parameter in Omega and Golubaja bays were at a similar level, whereas in organisms caught in Karantinna Bay the bioaccumulation factor for calcium was twice lower. Limited calcium uptake from water by aquatic organisms is one of the causes of stress due to pathogenic factors or the presence of xenobiotics in a biotope.

Sodium is the element which occurs in the highest amount in sea water, and out of all elements it affects water salinization the most. Due to the fact that sea water is a hypertonic solution in relation to body fluids of the organisms, maintaining osmotic balance is possible thanks to constant excretion of electrolytes from the organism. Marine animals drink the water, and elements are excreted by the gills, thanks to which it is possible to maintain salt concentration at a steady level. Appearance of a stressor in the environment very often leads to disturbance of ionic balance in the organism, which is caused by damaged gills as the most responsible organ for osmotic balance or decreased functionality of the organism caused by pathogenic factors (Zhou et al. 2011, Sathya et al. 2012). Sodium content in an organism in a quantitative relation to potassium is a parameter that is often used in the assessment of the health condition of sea organisms and quality of the environment they live in (Jeon et al. 2010). The sodium content in the studied fish larvae was in a wide range from 7.081 to 24.21 g Na kg^{-1} DM (Table 3). In general, organisms caught in Karantinna Bay contained the lowest amount of sodium. The content of this element in larvae from the other bays was approximately 50% higher and no statistically significant differences were found. The value of bioaccumulation factor for sodium was between 1.036 and 1.630 (Table 4).

The highest content of all the studied elements, except calcium and phosphorus, was found in the organisms caught in Golubaja Bay, whereas the lowest accumulation of these elements, except phosphorus, was observed in larvae caught in Karantinna Bay which is located in the region with the highest anthropogenic impact. The decreased content of macronutrients in fish, particularly in their early developmental stages, may be a result of toxic effect of pollutants, which is confirmed by literature reports (Castro-González, Méndez-Armenta 2008, Jeon et al. 2010, Zhou et al. 2011). The research was carried out in the area of bays which are located close to one another. Habitat conditions arising out of geographical and climatic conditions are similar (Gordina et al. 2001, Pavlova et al. 2007, Kuzminova et al. 2014), that is why differences in chemical composition of the organisms used in the research in all probability are the result of the presence of stressors associated with different levels of anthropopression.

CONCLUSIONS

1. The content of potassium and sodium in the water from individual bays of Sevastopol did not differ statistically. Statistically more calcium and magnesium was found in water from Golubaja Bay, whereas the highest amount of phosphorus was found in Karantinna Bay.

2. Concentration of the studied elements in the larvae of Atherina had the following order, starting with the greatest: P > K > Na > Ca > Mg.

3. The value of bioaccumulation factor for the studied elements in the larvae of Atherina had the following order, starting with the greatest: P > Mg > Ca > K > Na. The highest values of bioaccumulation factor for all the elements, except calcium, was found in organisms caught in Golubaja Bay. The lowest values of bioaccumulation factor for all the elements were found in the larvae from Karantinna Bay.

4. The highest contents of Na, Mg and K were found in fish larvae from Golubaja Bay. The highest calcium content was found in larvae caught in

Omega Bay, whereas larvae from Karantinna Bay had the highest amount of phosphorus.

5. Considerable differences in contents of the studied elements in fish larvae of the genus *Atherina* L from individual bays can be indicative of the effect of environmental conditions on the growth and development of these organisms.

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