

**Shalgimbaeva S.M.¹, Ibragimova N.A.², Jumakhanova G.B.¹,
Popov N.³, Omarova Zh.S.¹, Kairat B.K.¹, Rakybayeva A.A.³**

¹Al-Farabi Kazakh National University, Kazakhstan, Almaty,

e-mail: Gaukhar.Dzhumakhanova@kaznu.kz

²Kazakh-German University, Kazakhstan, Almaty

³Kazecoproject LLP, Kazakhstan, Almaty

**BIOMARKERS OF EFFECT:
GROSS AND HISTOPATHOLOGICAL INDICATORS
ATHERINA BOYERI OF THE CASPIAN SEA, KAZAKHSTAN SECTOR**

Feature of ichthyofauna North-eastern part of the Caspian Sea is the presence of it includes not only the valuable commercial fish species, but also of low value species who participate in the formation of the food chain. Fish, as the top trophic unit of the aquatic ecosystem, are considered indicators of pollution, therefore pathological studies allow us to estimate and predict effects of finding toxic substances in water. This article presents the results of the morphometric and histological study Caspian Atherina (*Atherina boyeri*), inhabiting the North-Eastern part of the Caspian Sea. As a result of the study, were found that the biomarkers-effect are pathological changes gill tissue and liver. So, in the gills are observed destructive lamella changes, phenomena of hyperplasia of the primary and secondary gill epithelium and degeneration of cartilage elements. The liver changes manifested in the form of parenchymal hepatocytes dystrophy and vascular reaction. The observed pathological changes gills and liver may affect the survival *Atherina boyeri* and these organs can serve as biomarkers of effect. Histological study of muscle and gonad it did not reveal any changes pamorphological and observed processes are reversible. In these studies, substantiates use of Caspian Atherina (*Atherina boyeri*) as a test type of monitoring the water of the Caspian Sea basin and including to predict the number of and quality of harvested species.

Key words: Caspian atherina, *A. boyeri caspia* (Eichwald, 1838), Caspian Sea, histological changes, gills, liver.

Шалгимбаева С.М.¹, Ибрагимова Н.², Джумаханова Г.Б.¹,
Попов Н.³, Омарова Ж.С.¹, Кайрат Б.К.¹, Ракыбаева А.А.³

¹әл-Фараби атындағы Қазақ ұлттық университеті, Қазақстан, Алматы қ.,
e-mail: Gaukhar.Dzhumakhanova@kaznu.kz

²Қазақ-Неміс университеті, Қазақстан, Алматы қ.

³«ҚазЭкоЖоба» ЖШС, Қазақстан, Алматы қ.

**Эффект биомаркерлері: Каспий теңізінің
Қазақстан аймағындағы Каспий атеринасы (*Atherina boyeri*)
балықтарына жасалған жалпы және гистопатологиялық көрсеткіштері**

Каспий теңізінің солтүстік-шығыс аумағының ихтиофаунасының ерекшелігіне өнеркәсіптік маңызы бар балықтармен қатар, қорек тізбегін қалыптастыруға қатысатын құнсыз балық түрлерінің болуын айтуға болады. Балықтар су экожүйесіндегі трофикалық қатардың жоғары өкілдері ретінде ластанудың индикаторы болып табылады. Сыртқы ортадағы өзгерістерді анықтаудың бір әдісі – балықтардың физиологиялық күйін зерттеу. Сол себептен патоморфологиялық зерттеулер судағы токсинді заттардың таралуының зардаптарын бағалауға және болжауға болады. Мақалада Каспий теңізінің солтүстік-шығыс бөлігін мекендейтін атерина балығына жүргізілген морфометриялық және гистологиялық зерттеудің нәтижелері келтірілген. Зерттеу нәтижесінде желбезек ұлпасы мен бауырдағы патоморфологиялық өзгерістер эффект-биомаркері бола алатындығы анықталды. Сонымен қатар балықтардың желбезектерінде ламеллалардың деструктивті өзгерістері:

бірінші реттік және екінші реттік желбезек эпителийлерінің гиперплазиясы мен шеміршекті элементтердің дегенерациясы байқалды. Балықтардың бауырындағы өзгерістер гепатоциттердің паренхиматозды дистрофиясы және тамырлар реакциясы түрінде байқалды. Байқалған желбезек пен бауырдағы патоморфологиялық өзгерістер *Atherina boyeri* балықтарының өміршеңдігіне әсер етуі мүмкін және зерттелген мүшелерді эффект-биомаркерлері ретінде қолдануға болады. Бұлшық ет ұлпасы мен гонадаларды гистологиялық зерттеу барысында патоморфологиялық өзгерістер анықталмады және байқалған процестер қайтымды сипатқа ие. Берілген зерттеулерде Каспий атеринасы (*Atherina boyeri*) балықтарын Каспий теңізінің суына мониторинг жүргізуде, кәсіптік маңызы бар балықтардың сапасы мен санын болжауда тест-объект ретінде қолдану ұсынылады.

Түйін сөздер: Каспий атерина *A. boyeri caspia* (Eichwald, 1838), Каспий теңізі, гистологиялық өзгерістер, желбезек, бауыр.

Шалгимбаева С.М.¹, Ибрагимова Н.², Джумаханова Г.Б.¹,
Попов Н.³, Омарова Ж.С.¹, Кайрат Б.К.¹, Рақыбаева А.А.³

¹Казахский национальный университет имени аль-Фараби, Казахстан, г. Алматы,
e-mail: Gaukhar.Dzhumakhanova@kaznu.kz

²Казахстанско-Немецкий Университет, Казахстан, г. Алматы

³ТОО «Казэкопроект», Казахстан, г. Алматы

Биомаркеры эффекта: валовые и гистопатологические показатели атерины каспийской (*Atherina boyeri*) казахстанского сектора Каспийского моря

Особенностью ихтиофауны Северо-Восточной части Каспия является наличие в ее составе не только ценных промысловых видов рыб, но и малоценных видов, которые участвуют в формировании пищевой цепочки. Рыбы как верхнее трофическое звено водной экосистемы считаются индикаторами загрязнения, поэтому патоморфологические исследования позволяют оценить и прогнозировать последствия нахождения токсических веществ в воде. В данной статье приведены результаты морфометрического и гистологического изучения атерины каспийской (*Atherina boyeri*), обитающей в Северо-Восточной части Каспийского моря. В результате исследования были обнаружено, что биомаркерами-эффекта выступают патоморфологические изменения жаберной ткани и печени. Так, в жабрах наблюдаются деструктивные изменения ламелл, явления гиперплазии первичного и вторичного жаберного эпителия и дегенерации хрящевых элементов. В печени изменения проявлялись в виде паренхиматозной дистрофии гепатоцитов и сосудистой реакцией. Наблюдаемые патоморфологические изменения жабр и печени могут сказываться на выживаемости *Atherina boyeri* и эти органы могут служить биомаркерами эффекта. Гистологическое изучение мышечной ткани и гонад не выявило патоморфологических изменений и наблюдаемые процессы имеют обратимый характер. В данных исследованиях обосновывается использование атерины каспийской (*Atherina boyeri*) в качестве тестового вида мониторинга водного бассейна Каспийского моря, и в том числе для прогнозирования численности и качества промысловых видов.

Ключевые слова: атерина каспийская, *A. boyeri caspia* (Eichwald, 1838), Каспийское море, гистологические изменения, жабры, печень.

Introduction

Currently increasing relevance have research related the identification of ecosystem responses and (or) its individual components to the action of unfavorable factors [1]. Constant changes environmental quality lead to the need for continuous monitoring, for better environmental management. One of the widely used methods of biological type of monitoring is bioindication. A considerable interest has been acquiring search for test objects (biomonitors) and biomarkers – molecular, cellular, physiological, organismic and population parameters for monitoring the quality of

the aquatic environment [2-4]. Quite obvious that for each ecosystem can be used several indicator species and biomarkers that are sensitive to environmental factors, including anthropogenic.

The study of the enzymatic system of aquatic organisms can be used to monitor level of water pollution by tetrabromobisphenol. So, serum research silver crucian (*Carassius auratus*) showed that the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glutathione reductase increases when using tetrabromobisphenol at a concentration of 0.35 mg / l already from 4 days of toxicant addition and stored during the experiment (32 days) at the same time,

there is a significant increase in AST. Provided that the concentration of tetrabromobisphenol exceeds 0.50-0.71 mg / l, after its cancellation levels AST, ALT, glutathione-S-transferase (GST), glucuronosyltransferase (UDPGT) and glutathione reductase in liver homogenates remain enlarged for a long time. Therefore, measurement of AST and ALT can be used in the normal monitoring water quality in areas at risk of tetrabromobisphenol and determination of glucuronosyltransferase (UDPGT) and glutathione reductase in liver homogenates for use in emergency situations in which this toxicant is emitted [5].

The effect of aqueous solutions of sulfonamides in micromolar concentrations for 96 h on nematodes (*Caenorhabditis elegans*) was studied and it was found that what they inhibit behavior indicator (including body bending frequency) and growth rates (reducing body length), behavioral changes were more pronounced. In addition, behavioral disturbance was noted in the descendants [6].

Triclosan (2,4,4'-trichloro-2'-hydroxy-diphenyl ether; TCS) is an antibacterial agent and is part of a variety of household and personal care products. Now found in natural waters and bottom sediments. In addition, having a high hydrophobicity, triclosan is able to accumulate in the fatty tissue of various aquatic organisms, methylated to methyl triclosan (2,4,4'-trichloro-2'-methoxy diphenyl ether). So, it was shown triclosan and its metabolite change the morphology and density of blood cells, causing violations of the respiratory gill epithelium abalone (*Haliotis tuberculata*) [7].

The search for available methods for monitoring water pollution is relevant, with organisms that are dominant in aquatic communities of greatest interest. The present studies substantiate the use of the Caspian atherine (ordinary atherina, small atherina) of the *Osteichthyes* class as a biomonitor for the quality of water in the Caspian Sea. The species was described as *A. mochon* Cuvier, 1829 [8,9], but then reduced to synonymy *A. boyeri* [10], is a marine euryhaline fish [11], inhabiting both brackish and strongly saline regions [8,9] and in the desalinated waters of the Caspian Sea in the pelagic zone. It should be noted that the *Atherina boyeri* is regarded as a secondary target of the craft.

Materials and research methods

Atherina boyeri females were selected for the study in the amount of 72 specimens from trawling material from the North-Eastern part of the

Caspian Sea during the 2017 autumn ichthyological expedition. Morphometric and pathomorphological studies were performed on 15 female year-olds (Figure 1).



Figure 1 – *Atherina boyeri*, selected for study

Fish were anesthetized in ice-cold water, killed by a slit behind the gill cover and fixed in 10% formalin for subsequent morphometric and histological examination. All studies were conducted at the Department of Biodiversity and Bioresources of the Al-Farabi Kazakh National University. Morphometric measurements of fish were carried out according to I.F. Pravdin [12]. The general condition factor (CF) was calculated as the ratio of body weight to total length and the Fulton condition index (K index), which is an indicator of fish health [$K = 100 \times (\text{weight} / (\text{total length})^3)$].

Histological studies of gills, liver, muscles, and gonads were performed by standard methods [13]. Stained sections were studied using a Leica DM 6000M optical microscope. Statistical data processing was performed using standard methods and statistical programs Microsoft Excel 2013.

Results and discussion

Table 1 presents growth and weight indices of Caspian atherine (*Atherina boyeri*). The weight of the females-yearlings was $7,03 \pm 0,35$ g and the length was $93,5 \pm 2,42$ mm.

In the table 2 show the general state (CF) and Fulton state factors (K index). The average indicators of the general condition (CF) are $61,00 \pm 3,05$ and the Fulton state factor is $0,63 \pm 0,08$.

Table 1 – Height and weight indicators *Atherina boyeri*, females

№	Weight, g	Mass without internal organs, g	Overall length, mm	Body length without tail, mm
1	6,34	5,73	104	97
2	5,64	5,19	97	91
3	5,03	4,51	96	81
4	6,17	5,62	98	93
5	7,13	6,43	103	96
6	7,71	7,14	103	97
7	7,01	6,44	101	97
8	7,42	6,51	112	96
9	10,33	9,22	118	101
10	6,62	6,13	99	84
11	5,71	5,22	98	83
12	6,74	6,21	103	98
13	7,02	6,39	102	97
14	7,13	6,51	99	93
15	9,45	8,72	116	102

Table 2 – Condition factor (CF) and Fulton's condition index (K index) of *Atherina boyeri*

№	Condition factor (CF)	Fulton's condition index (K index)
1	55,1	0,56
2	53,5	0,62
3	47,0	0,57
4	57,3	0,66
5	62,4	0,65
6	69,3	0,71
7	63,8	0,68
8	58,1	0,53
9	78,1	0,63
10	53,3	0,68
11	53,3	0,61
12	60,3	0,62
13	62,6	0,66
14	65,8	0,73
15	75,2	0,61

When histological examination structures of the respiratory epithelium of gill lobes all fish marked focal changes, manifested in the form of hyperplasia, which affects both the primary gill epithelium and the secondary. The individual lamellae are shortened or typical symmetry is lost due to the destruction of blood vessels and necrobiotic changes

in respiratory cells. Over the entire length of the lamellas, an expansion is noted intercellular space between the layers of respiratory cells. Mucous cells are found along the entire length. Diffuse blood supply of supporting cells. Focal adhesion of adjacent lamellas. Focal depletion of the cellular composition of the primary gill epithelium and an

increase in cellularity, mainly due to mucous cells, the nuclei of which are shifted to the periphery of the cell (figures 2-4).

Histological examination of the liver of *Atherina boyeri* traced two features in the microscopic picture of the organ parenchyma. The first is manifested in the “openwork”: hepatocytes appear empty with a centrally located large nucleus or displaced core on the periphery of the cell with

a reduced nucleus. At high magnification are found in the cytoplasm of large vacuoles. Second, hepatocytes appear enlarged in volume with fine-grained contents in the cytoplasm, while the nucleus is large, lightened with heterogeneously located chromatin. Picture of diffuse expansion of the Disse space. In general, in all individuals, the lobular structure of the liver is preserved (figures 5-7).

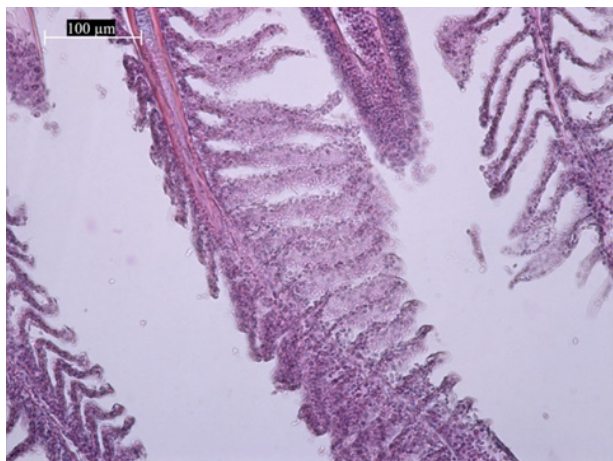


Figure 2 – Hyperplasia of the secondary gill epithelium and destruction of capillaries. Hematoxylin-eosin. Increasing $\times 10$



Figure 3 – 1 – expansion capillaries lamella, expanding intercellular spaces, mucous cells along the entire length of the lamella; 2 – the flattening of the respiratory epithelium of secondary gill epithelium. Separate lamellae on tops like maces thickened. Hematoxylin-eosin. Increasing $\times 10$

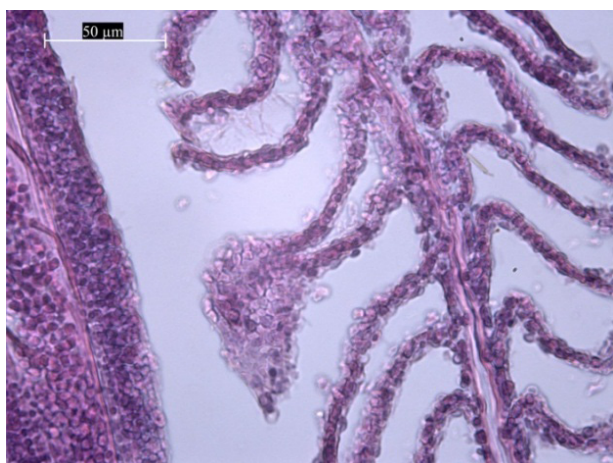


Figure 4 – Lamella shape change: thickening like a mace, fusion of lamellae tops. Hematoxylin-eosin. Increasing $\times 20$

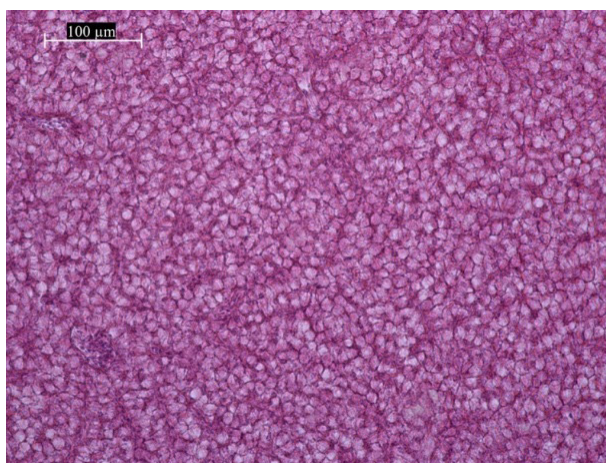


Figure 5 – The nuclei of hepatocytes shifted to the periphery of the cell. Vascular congestion. Hematoxylin-eosin. Increasing $\times 10$

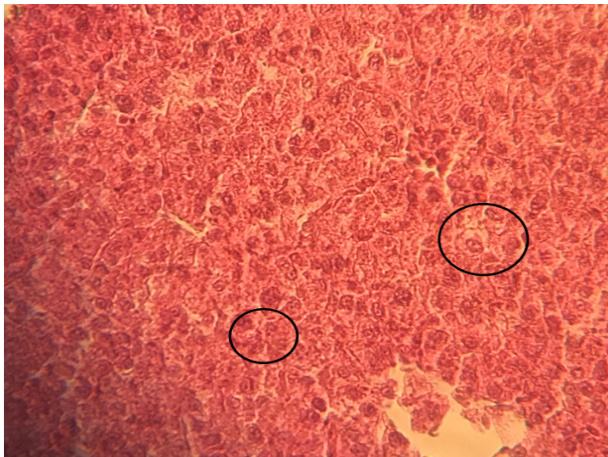


Figure 6 – Granular dystrophy of hepatocytes.
Individual hepatocytes loaded vacuoles.
Hematoxylin-eosin. Increasing $\times 20$

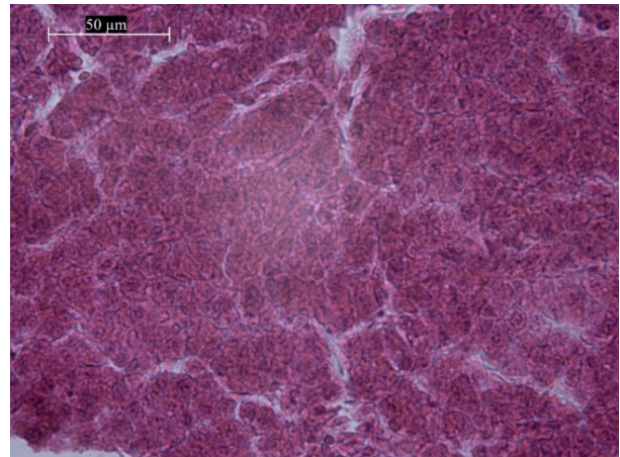


Figure 7 – Diffuse expansion of sinusoids.
Swelling of the stroma. Cell walls appear thickened.
Hematoxylin-eosin. Increasing $\times 20$

In muscles, transverse striation is preserved, fibers with peripherally arranged nuclei. In some individuals focal loss of muscle fibers, individual fibers with small vacuoles (Figures 8 and 9).

In the reproductive system of studied fish not observed destructive changes in germ cells.

On histological sections of the ovaries are located different sized oocytes of cytoplasmic growth, oocytes of the first order on the phases of vitellogenesis with the phenomenon of vacuolization of peripheral cytoplasm and an increase in the number of nucleoli in the nucleus (Figure 10-12).



Figure 8 – Loss of muscle fibers.
Hematoxylin-eosin. Increasing $\times 20$



Figure 9 – Disorganization of muscle fibers.
Hematoxylin-eosin. Increasing $\times 40$

Fish used for assessing the quality of the aqueous medium and serve as bioindicators of environmental pollution. Biomarkers act as “early warning” signals that can detect an adverse effect in biota before it is observed at the population, community or ecosystem level [14]. The results of these studies demonstrate that the use of various potential biomarkers allows you to set the most sensitive indicators to detect water pollution. It is known that the gills provide

breathing processes osmoregulation and excretion in fish. It is the gills that are sensitive to changes in water quality, due to the greater area of contact with the external environment through thin epithelium [15]. The observed pathological changes indicate about lowering respiratory capacity and the occurrence of osmotic imbalance. The liver has been recognized as a target organ for various pollutants. In our study, a histopathological examination of the

liver revealed a circulatory disorder associated with the pathological conditions of the parenchyma of the organ and regressive changes. The observed pathological effects can have a negative impact on the sustainability of fish to stressful influences and

the ability to react to changes in the environment, and including susceptibility to disease that eventually affects survival. In this case, the gills and liver can serve as a biomarker of the effect [16].

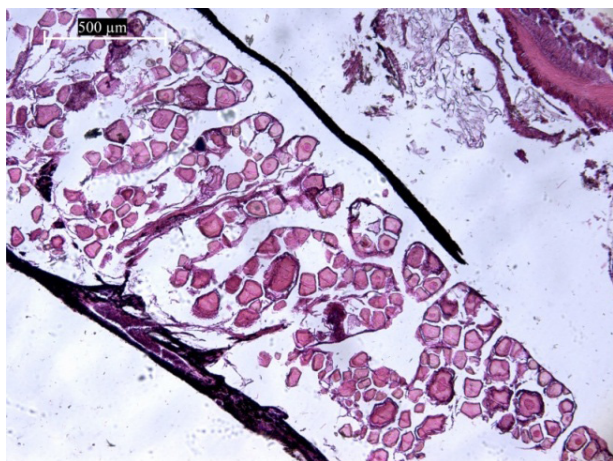


Figure 10 – Histostructure gonads of *Atherina boyeri*
Hematoxylin-eosin. Increasing $\times 10$



Figure 11 – Different dimensions oocytes cytoplasmic growth
Hematoxylin-eosin. Increasing $\times 40$

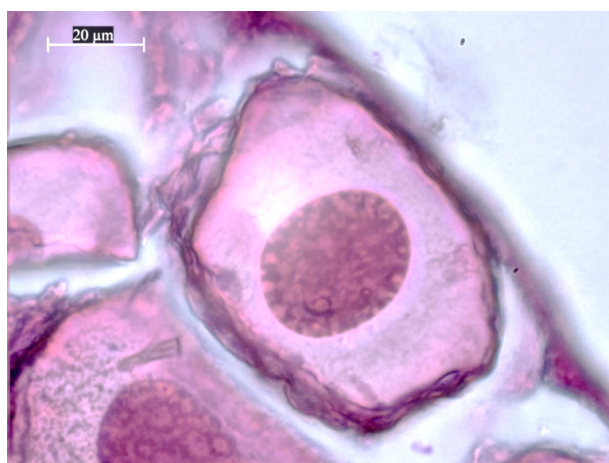


Figure 12 – The oocyte first order.
Hematoxylin-eosin. Increasing $\times 1000$

Conclusion

It is well known that changing environmental conditions cause the appearance of a large number of females in fish. In our studies, all the females in the gonads were no destructive changes.

We propose to use *Atherina boyeri* as a test species for monitoring the Caspian Sea, which provides for the expansion of knowledge about the reaction of fish to various pollutants under experimental conditions, including for validation.

Therefore, it can be argued that the focal view of *Atherina boyeri* can serve as a biomarker of the state of the water basin of the Kazakh sector of the Caspian Sea and to predict the number and quality of commercial species.

Conflict of interest

All authors have read and are familiar with the content of the article and do not have a conflict of interest.

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