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Effects of heavy metals on sex inversion of the mussel *Mytilus galloprovincialis* Lam., 1819 in coastal zone of the Black Sea

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ABSTRACT

Sex inversion in the mussel *Mytilus galloprovincialis* under the influence of heavy metals as one of the reasons for the shift in the sex ratio in the mussel population on the Black Sea coastal zone of Crimea were considered in the work. The present study is the first to directly show that heavy metals can cause females of the mussel *M. galloprovincialis* to change into males during post-spawning development of gonads. The degree of impact of heavy metals on the sex change in mussel females was different and decreased in the following sequence: $Cu^{2+} \rightarrow Cd^{2+} \rightarrow Hg^{2+} \rightarrow Pb^{2+} \rightarrow Zn^{2+}$. Copper ions had the greatest effect, which caused a sex inversion in 54 % of females. The heavy metals Hg^{2+} and Pb^{2+} were also quite toxic causing mortality in 13 % and 10 % of individuals, respectively. It is possible to use *M. galloprovincialis* as a model organism in the study of mechanism of environmental sex reversal in bivalves.

1. Introduction

Aquatic pollution is a global environmental problem (Shulman and Soldatov, 2014; Nobles and Zhang, 2015). In recent decades there has been a tendency to increasing sea surface temperature (Chelyadina et al., 2017), acidification of sea water (Doney et al., 2009; Rodrigues et al., 2015; Polonsky and Grebneva, 2019), increasing concentration of detergents (Katunina and Smyrnova, 2015) and an increase in the concentration of heavy metals (HM) in the Black Sea (Silkina et al., 2016; Fazio et al., 2020; Bakan and Ozkoç, 2007; Zakharikhina et al., 2022).

Changes in the basic physical and chemical parameters of the marine environment affect aquatic organisms, including the mussel *Mytilus galloprovincialis*, which is one of the main cultivated species in the Azov-Black Sea basin. A shift in the sexual structure in the *M. galloprovincialis* populations, specifically an increase in the proportion of males has been observed (Machkevsky et al., 2011; Chelyadina et al., 2018). It is hypothesized that the change in sex ratio is influenced by adverse environmental conditions and anthropogenic impact, including exposure to higher levels of heavy metals.

Sexual structure (sex ratio) is one of the main parameters of the populations, as well as the main link in the mechanisms of population regulation and reproduction rate (Geodakian, 1991). Also, with an increase in the violation of the sex ratio of breeding individuals, the ratio of the effective population size to the number of mating individuals decreases (Anthony and Blumstein, 2000). This can lead to a loss of genetic diversity in the mussel population. Therefore, the study of the influence of adverse environmental conditions, in particular HM, on the shift in the sexual structure of mussel settlements is relevant.

One of the reasons for the shift in the sexual structure of *M. galloprovincialis* is the inversion of the sex from females to males in mollusks, which can occur under the influence of pollution of the marine environment (Chelyadina et al., 2018). It should be noted that only females have been observed to change sex (Chelyadina et al., 2018). The effect of HM on the shift in the sexual structure in colonies of cultivated Black Sea mussels has not been studied. However, it is known that even essential trace elements, such as Cu, Ni, and Zn, at elevated concentrations in the aquatic environment can have deleterious effects (Dethloff et al., 1999; Mason and Jenkins, 1995) like other contaminants of anthropogenic origin (Pagano et al., 2016). They can impede the integrity of physiological and biochemical processes and cause changes in metabolic reactions in hydrobionts (Basha and Rani, 2003). Analysis of the Black Sea coastal waters of recent decades indicates that the concentration of Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+} Cu^{2+} and other HM coming from atmospheric deposition and as a result of anthropogenic activity is constantly growing (Bufetova, 2019; Silkina et al., 2016).

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1.1. Research aim

To determine whether sex change from females to males of the mussel *M. galloprovincialis* cultivated in the coastal waters of Crimea increases under the influence of the heavy metals Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+} Cu^{2+} during the period of post-spawning restructuring of gonad.

2. Material and methods

2.1. Material sampling area

The mussels *Mytilus galloprovincialis* (shell length = 55.3 ± 3.1 mm; mean ± SD) were sampled from collector ropes (depth 6 m) on a mussel-oyster farm located on the outer road of Sevastopol city during the spring mass spawning season in 2018. The farm, which was established in 2005, is located 200–300 m from the coast between the South breakwater of Sevastopol Bay and Karantinnaya Bay. The length of the farm is about 400 m, the width in the widest part is about 200 m. The depths under the farm are 16–18 m. The soil under the farm is represented by sand, shells and silt (Fig. 1).

The flow velocities, the food base and concentrations of nutrients in the farm water area favorable for the development of suspended conchioculture (Kuftarkova et al., 2006; Popov and Erokhin, 2017; Ryabushko et al., 2017a). The trophic index (TRIX) value of the water in the area estimates range from 2.42 (=oligotrophic) to 4.18 (=mesotrophic) (Ryabushko et al., 2017b; Vollenweider et al., 1998). Feeding conditions on this marine farm are favorable for the growth and development of mussels, since the microalgae (dinoflagellates, small cell diatoms and coccolithophores) included in the diet of cultivated mussels are constantly present in phytoplankton (Ryabushko et al., 2017a).

Interannual fluctuations in salinity of surface waters on the farm were in the range of 17.26–18.39 ‰ and pH varied from 8.14 to 8.88 (Kuftarkova et al., 2006). The concentrations of the metals (C_M) (in $\mu g{\cdot}L^{-1}$) studied in this work in the farm water were: $C_{Zn} = 27.9 \pm 5.4$, $C_{Cu} = 15.2 \pm 3.5$, C_{Pb} 2.8 ± 1.01, $C_{Cd} = 1.5 \pm 0.4$ and $C_{Hg} = 0.1 \pm 0.07$ (Kapranov et al., 2021). They did not exceed the MPC (maximum permissible concentration) according to the Federal Russian Standards (FRS) for waters used for fishing are (SanPiN 2.1.5.980-00, 2017), except for C_{Cu} (Table 1).

2.2. Focal species

Mollusk *M. galloprovincialis* (Mollusca: Bivalvia) is a widespread species in the shelf zone of the Black Sea and in the fouling of various hydraulic structures. Mussels form an independent biocenosis and are part of other communities. *M. galloprovincialis* has a significant potential for variability, which allows them to adapt to different living conditions and be found in almost all biotopes of this reservoir. Mediterranean mussel has become a promising and popular cultivation object in the Black Sea coastal zone due to the high nutritional value of meat and the ability to create huge mass settlements (Kholodov et al., 2017; Massa et al., 2017). This is a moderately warm species, the range of which includes the Black, Adriatic and Mediterranean Seas. Currently, it has spread to temperate waters across Mediterranean, west coast of Europe, West coast of North America, East coast of Asia, part of western South America, and southern Africa, southeastern Australia and much of New Zealand (Atasaral et al., 2015; FAO, 2020; Gosling, 2003; Kholodov et al., 2017; Lutz et al., 1991). The optimum temperature for this species is 12–20 °C and salinity 12–25 $g{\cdot}L^{-1}$ (Ivanov et al., 1989; Kapranov et al., 2021). The mussel from the Black Sea has two spawning peaks in autumn and in spring. Under favorable temperature conditions, mussels can spawn also in winter. The spawning occurs when the surface water warms up to 9–12 °C in spring or when the water temperature drops to 11 °C in autumn. The interplay of internal and external factors regulates the reproductive cycle of mussels (Kholodov et al., 2017; Pirkova et al., 2019). *M. galloprovincialis*, being an active filter feeder, filters water at a rate of 0.5 to 7.5 $L{\cdot}h^{-1}$ and more and, as a result, accumulates trace elements from the incoming water and food, phytoplankton and detritus (Coombs and Keller, 1981; Temerdashev et al., 2017). Mussel is also widely used as environmental biomonitor. Substantial progress in this application has been achieved through the implementation of the “Mussel Watch” program (Cantillo, 1998; Rainbow and Phillips, 1993;

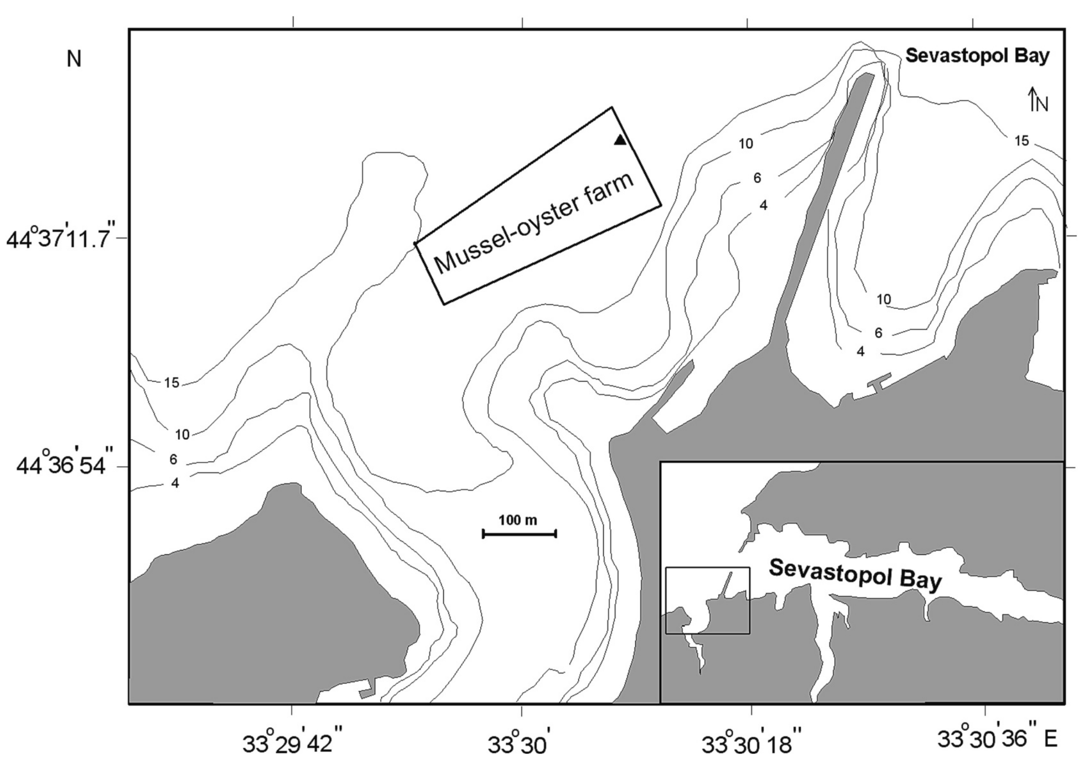

Fig. 1. The map of the study and sampling location.

Table 1
Concentrations of heavy metal salts in experimental containers and MPC of heavy metal ions in the Black Sea water (SanPiN), μg· l[1].

Concentration	Salts of heavy metals				
	$CuSO_4{\cdot}5H_2O$	$ZnCl_2$	$Pb(COOCH_3)_2{\cdot}3H_2O$	$Hg(NO_3)_2{\cdot}H_2O$	$CdSO_4{\cdot}8H_2O$
Salt concentration in containers, $\mu g{\cdot}l^{-1}$	19.5	104.5	15.7	0.2	31.0
MPC of HM ions in sea water, $\mu g{\cdot}l^{-1}$	5.0	50.0	10.0	0.1	10.0

Stankovic and Jovic, 2012).

2.3. Experimental research

Mussels were sampled during the spring mass spawning season in 2018. The choice of sampling time is due to the fact that the sex change of mussels can occur during the post-spawning restructuring of gonads, which is controlled by a neuroendocrine mechanism involving hormones (Ivanov et al., 1989).

In laboratory conditions, the sex of mussels was determined individually by examination of gametes after inducing spawning by temperature stimulation (Chelyadina et al., 2018). Mussels were placed in separate containers (V = 250 ml), where the water temperature was increased to 18–22 °C. The sex of mussels after spawning was determined visually in containers with males and females using an Olympus CX43 microscope. The reproductive products of males looked like a white suspension whereas that of females consisted of orange-colored eggs that settled to the bottom. An aliquot of the suspension was taken from each container with a pipette and examined using a microscopically at 250 x magnification to confirm presence of eggs or sperm. Following identification of sex, 30 females were placed in each of six separate containers with sea water (V = 40 l). Each container also contained one of the five heavy metals being tested. An additional container was used as a control. Heavy metal salts ($CuSO_4{\cdot}5H_2O$; $ZnCl_2$; $Pb(COOCH_3)_2{\cdot}3H_2O$; $Hg(NO_3)_2{\cdot}H_2O$; $CdSO_4{\cdot}8H_2O$) were added to containers with female mussels daily for a month). Divalent Cu, Zn, Pb, Hg, Cd ions concentrations selected for experimental work correspond to their MPC according to the FRS for marine waters used for fishing (in $\mu g{\cdot}l^{-1}$) (SanPiN 2.1.5.980-00, 2017). At higher concentrations, mussels slow down the movement or close the shell valves and switch to an anaerobic type of nutrition. The required amount of HM salts to obtain concentrations of Cu^{+2}, Zn^{+2}, Pb^{+2}, Hg^{+2}, Cd^{+2} in separate containers at the MPC is given in Table 1.

All experiments were performed in duplicate. The water in each tank was changed daily. Before the start of the experiment, the mussels were acclimated for 5 days to the tank conditions which included aeration and daily water changes. The mussels were fed with a mixture of live microalgae consisting of chrysophytes (*Isochrysis galbana* Parke 1949 and *Diacronema lutheri* (Droop) Bendif & Véron, 2011, chlorophytes (*Tetraselmis viridis* (Rouchijajnen) R.E. Norris, Hori & Chihara 1980 and *Dunaliella viridis* Teodoresco 1905), and diatoms (*Chaetoceros calcitrans* (Paulsen) H. Takano 1968 and *Phaeodactylum tricornutum* Bohlin 1898). The microalgae were grown in a batch cultivator (Trenkenshu et al., 2017). An algal suspension (100 ml of culture each species) was added to each tank three times a day. The water temperature in the experimental containers was maintained at the level of 17.0–18.5 °C (measured using a meteorological thermometer). The oxygen concentration in the water of the experimental containers was determined by the Winkler method (Sapozhnikov, 1988) and maintained with the help of aerators at the level of 5.0–6.0 $mg{\cdot}l^{-1}$. The noted conditions (temperature, food intake, water change, concentration of dissolved oxygen) were saved during the subsequent experimental work for a month.

The mussels were exposed to the studied HM ions for 1 month in the laboratory, since during this period the post-spawning restructuring of the gonads takes place and at this time sex changes can occur (Dolgov, 1985; Ivanov et al., 1989). Then the mussels were placed in separate marked cages and hung on a mussel-oyster farm for maturation of the gonads and subsequent determination of sex.

After 3 months of growth at sea, cages were delivered to the laboratory and sexual state (male, female, hermafrodite) of each individual was determined by visual examination of lethal gonad smears using the microscope (Pirkova et al., 2019). An exposure of 3 months in the sea was necessary for the maturation of the mussel gonads and accurate sex identification. During the laboratory experiment, a record of dead mussels was kept. The mortality of mussels was estimated as a fraction of dead mussels relative to in the total number of mussels.

2.4. Data analysis

In total, 360 mussels were studied. The significance of differences in sex inversion between the control and experimental groups were tested using the chi-square test, Chi-square tests with Yates' correction and Fisher's exact test. A *P*-value of <0.05 was considered as statistically significant for all tests. Data analysis was performed in Microsoft Excel 2010.

3. Results

Sex change was noted in all groups of *Mytilus galloprovincialis* by the end of the experimental period in 2018 (Table 2). The number of hermaphrodites, which are a transitional stage between females and males, in experienced cages after 3 months of exposition in the sea were different (Table 2). There were no significant differences ($p < 0.05$, Chi-test) in the proportion of females that were transformed the hermaphrodite after exposure to metals. It was assumed that sex inversion is the sum of males and hermaphrodites.

Despite the relatively short duration of the experiment, the inversion of sex in mussel females exposed to HM was 3–5 times higher compared with the control group. It was noted that Cu^{+2} had the greatest impact on sex change (Chi-square with Yates correction = 13.1, $p < 0.001$) (Tables 2, 3). The high value of the Criteria for assessing the significance of differences also showed the influence of Cd^{2+} (Chi-square with Yates correction = 7.5, $p < 0.007$), Pb^{2+} (Chi-square with Yates correction = 4.9, $p < 0.027$) and Hg^{2+} (Chi-square with Yates correction = 4.6, $p < 0.033$). There appears to be no effect of Zn^{+2} on sex inversion of mussel females: a statistically significant effect did not find (Chi-square with Yates correction = 3.5, $p < 0.062$). However, Fisher's exact test showed significant differences groups effected Zn^{+2} compared with the control group (Table 3).

The mortality of mussels in the laboratory experiment within one month was not noted. The dead mussels were recorded only after a three-month marine exposure in cages. The impact of all studied metals caused the mortality of mussels. However, the degree of HM toxicity varied (Fig. 2). Of the HMs tested, Cu^{2+} caused the highest rate of mortality – 20 % of mussel (from the whole number of mussels in the experiment). Hg^{2+} and Cd^{2+}, which caused mortality in 13 % and 10 % of individuals respectively, were also highly toxic to female mussels.

4. Discussion

4.1. The impact of heavy metals on mollusks

The choice of heavy metals used in the experiment was determined by their constant presence in the Black Sea coastal waters of Crimea in

Table 2
Sex inversion and mortality of experimental settlement of *M. galloprovincialis* treated with heavy metals (after a month-long laboratory experiment and 3-month of growth at sea in a mussel-oyster farm).

Experimental proceeding		Sex			Sex inversion, %	Mortality, specimen
		♀, specimen	♂, specimen	⚥, specimen		
Before experiment		30/30*	0/0	0/0	–	–
After experiment	Control	26/27	2/2	2/1	13.3/10.0	0
	Zn^{2+}	18/17	10/9	1/0	39.3/31.0	2/1
	Cd^{2+}	14/12	13/15	1/1	50.0/57.1	2/2
	Pb^{2+}	16/15	10/10	2/2	42.9/44.4	2/3
	Hg^{2+}	15/14	11/10	0/1	42.3/44.0	4/5
	Cu^{2+}	7/8	14/15	3/2	70.8/68.0	6/5

* Above the line - sample 1, below the line - sample 2.

Table 3
Criteria for assessing the significance of differences between the median values of sex inversion in mussels after exposure to heavy metals compared to control.

Statistically criterion	Heavy metal ions				
	Zn^{+2}	Cd^{+2}	Pb^{+2}	Hg^{+2}	Cu^{+2}
Chi-square test	4.7 (0.031)*	9.1 (0.030)	6.3 (0.012)	5.9 (0.015)	15.4 (<0.001)
Chi-square test with Yates correction	3.5 (0.062)	7.5 (0.007)	4.9 (0.027)	4.6 (0.033)	13.1 (<0.001)
Fisher's exact test (two-tailed)	0.039 ($p <$ 0.05)	0.0041 ($p <$ 0.05)	0.0182 ($p <$ 0.05)	0.0184 ($p <$ 0.05)	0.0002 (<0.05)

* The level of significance of the statistical test is given in parentheses.

concentrations that often reach and exceed the MPL (Kuftarkova et al., 2006). It is known that HM have a mutagenic effect (Liu et al., 2014; Solovykh et al., 2015; Breton et al., 2018). They can affect cellular processes, connected with change of sex, mainly on hormonal level, and in disorders of the endocrine system (Breton et al., 2018). It has been shown (Kramarenko, 1989; Slesarev, 2005), that HM ions form metallothionein-like protein complexes after passing cellular membranes. Accumulation of metals in the composition of such complexes can reach high levels, which leads to mutagenic effect (Sabirov, 2015; Guerra et al., 2016; Xu et al., 2018). A number of researchers have shown a sex change in bivalves including *Mya arenaria* Linnaeus, 1758 (Gagne et al., 2005), *Gomphina veneriformis* (Lamarck, 1818) and *Sinonovacula constricta* (Lamarck, 1818) (Ju et al., 2009) under influence of HM. Results of our research has shown, that the females of *M. galloprovincialis* change sex after action of all HM tested (Tables 2, 3). This leads to a shift in the sex ratio in a settlements of *M. galloprovincialis*, which can lead to degradation (depletion in numbers) of the mussel population in the future. The proportion of hermaphrodites that appeared after laboratory experiments (Table 2) allows us to assume that hermaphrodites are a transitional stage during the sex change from females to males. Previously, we have shown that sex change goes in one direction - from females to males, males do not change sex (Chelyadina et al., 2018).

The experiment showed that sex change (10–13 % of females changed sex) also occurred in the control group (Table 2). One of the reasons for this is the impossibility to create natural conditions in the laboratory, as well as using of natural sea water from the farm's water area, where Cu^{2+} concentrations of 3 MPC were previously noted (Kapranov et al., 2021).

Of all the studied divalent HM ions, only Zn showed an ambiguous effect on mussel sex change: Chi-square with Yates correction did not show a statistically significant difference compared to the control group, while Fisher's exact test showed significant differences (Table 3). Apparently, this is due to the fact that Zn is an important trace element for mollusks, taking part in enzymatic catalytic reactions. It is involved in the synthesis of DNA and proteins, acts as an antioxidant and is important for supporting the functions of the reproductive system. (Pearson et al., 2018; Kapranova et al., 2021).

The control and maintenance of environmental quality is important to maintain the stability of the population in which aquaculture products have been produced or harvested.

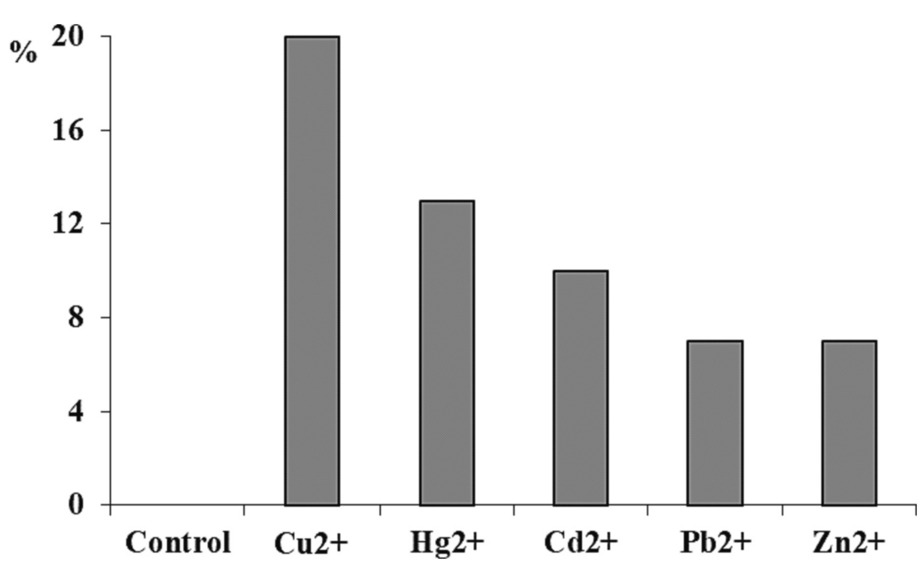

Fig. 2. Mortality of mussel females *M. galloprovincialis* after one-month laboratory experiment and 3-month of growth at sea in a mussel-oyster farm, 2018.

4.2. Toxic effect of HM

High level of sex inversion after the influence of Cu^{2+} and Cd^{2+} was noted (Tables 2, 3). The highest level of difference from the control group was in the group exposed to copper ions (Table 3). It is known that those elements affect genetic apparatus and have gonadotoxic and embryotoxic effects (Golovanova and Frolova, 2005; Shevtsova et al., 2011; Davydova et al., 2014; Zitoun et al., 2019). Copper has biological activity, so it accumulates in excess in the body of aquatic organisms (Silkina et al., 2016). Compared to other studied HM, ionic forms of copper form more stable organic complex compounds by binding with nitrogen, oxygen, sulfur, increase the activity of enzymes, are part of proteins, and have the ability to stabilize sulfur-containing radicals (Gorinstein et al., 2006). However, copper ions can also block many biochemical reactions, displacing and replacing some trace elements, such as zinc, from the active sites of enzymes and have a cytotoxic effect (Franca et al., 2005; Solomonova et al., 2022; Casas et al., 2008). Such interactions can result in disturbances in cellular metabolism, the structure and permeability of cell membranes, increased lipid peroxidation, and inhibition of oxidative phosphorylation (Wilson and McMahon, 1981; Yaroslavtseva and Sergeeva, 2005).

Zn^{2+}, Pb^{2+}, Hg^{2+} had almost the same effect on the inversion of the sex of mussel females (Tables 2, 3). As trace elements, HM studied in this work affect the growth and reproduction of organisms, redox processes, oxygen binding by tissues, take an active part in metabolic processes, being a constructive part and catalyst of many enzymes (Dethloff et al., 1999; Golovanova and Frolova, 2005; Marie et al., 2006). However, Cd^{2+}, Pb^{2+}, Hg^{2+} and Cu^{2+} are toxic (Basha and Rani, 2003). Accumulating in the tissues and organs of *M. galloprovincialis* (Pospelova, 2008), they damage cell membranes, interact with amine nitrogen and SH-groups of proteins, causing coagulation and inactivation of enzymes and disrupting cellular hypersensitivity, which causes its death (Bebianno and Langston, 1992; Marie et al., 2006).

There was observed high mortality of mussels (20 %) under the influence of Cu^{2+} during laboratory and natural studies (Fig. 2).

Higher toxicity of Cu^{2+} in comparison with Cd^{2+} and Pb^{2+} was noted under effect of HM on mussels *Tegillarca granosa* (Linnaeus, 1758) (Liu et al., 2014). Cu^{2+} in concentration of 0.01 and 0.02 $mg \cdot l^{-1}$ decreased development and growth of embryos and larvae of *Mytilus trossulus* Gould, 1850 (Karaseva and Medvedeva, 1993).

The study of the concentration of various HMs in the soft tissues and shells of mussels sampled along the southwestern and southern coasts of Crimea, the Caucasian and Turkish Black Sea coastal zones showed that the level of HMs accumulation reflects anthropogenic pollution of ecosystems in coastal water areas (Zakharikhina et al., 2022; Nekhoroshkov et al., 2022; Bakan and Ozkoç, 2007; Bat and Oztekin, 2016).

Toxic and mutagenic activity of Cu^{2+} on the mollusks is dependent on form of its presence in seawater – ionic or bound in organic and inorganic complex compounds (Zitoun et al., 2019). Currently, there is a tendency to increase the amount of more toxic ionic form of copper in coastal waters which is associated with an increase in the temperature of sea water and its acidification (Millero et al., 2009; Richards et al., 2011). Hg^{2+} is classified as a priority hazardous substance under the Environmental Quality Standards Directive (2013/39/EU) and is highly toxic and one of the most dangerous metals in the aquatic environment. In this work Hg^{2+} was less toxic to mussel females than the ionic form of Cu^{2+} (Tables 2, 3). It is known that organic mercury compounds (methylmercury and dimethylmercury) are the most toxic for living organisms (Solovykh et al., 2015). In our experiment, we used an inorganic form of mercury (Table 1). The toxic effect of Zn^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+} is associated with their ability to cause destruction of cell membranes, disrupt the functioning of enzymes, leading to tissue dysfunction and ultimately to death of an organism (Basha and Rani, 2003; Liu et al., 2014; Silkina et al., 2016). Among these elements, Cd^{2+} is the most toxic (Bufetova, 2019). The danger of Cd^{2+} is explained by the fact that it's atomic radius almost completely coincides with the radius of Ca^{2+}. As a result, "… cadmium, 'camouflaging' as calcium, which is the most important regulator of many intercellular processes, has a destructive, toxic effect, causes the death of cells, tissues and cells and an organism as a whole" (Silkina et al., 2016).

5. Conclusions

For the first time, the causal influence of heavy metals (Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+} Cu^{2+}) on the sex inversion of female mussels of *M. galloprovincialis* cultivated in the coastal waters of Crimea has been shown. As a result of the study, it was demonstrated that HM affect the sex change of mussel females of *M. galloprovincialis* during the post-spawning restructuring of the gonads. The degree of exposure of HM on sex inversion of mussel females was different and decreased in the following sequence: $Cu^{2+} \rightarrow Cd^{2+} \rightarrow Hg^{2+} \rightarrow Pb^{2+} \rightarrow Zn^{2+}$. Under similar environmental conditions during experimental work, Cu ions had the greatest effect on mussels, resulting in the death of 20 % of individuals and causing sex inversion in 56.3 % of females in mean. By the strength of the toxic effect, simultaneously with Cu, it should be noted Hg and Pb, which caused mortality in 13 % and 10 % of individuals, respectively. It should be noted that the number of hermaphrodites, which are a transitional stage between females and males, in cages with mussels after intoxication with Cu^{2+} and Cd^{+2} was maximum and amounted to 9 % and 6 %, accordingly.

This is the first study directly showing that heavy metals can trigger sex reversal in an adult bivalve. *M. galloprovincialis* is possible to use as a model organism in the study of mechanism of environmental sex reversal in bivalves.

CRediT authorship contribution statement

Chelyadina Natalya: Conceptualization, Methodology, Data curation, Investigation, Original draft preparation; Pospelova Natalya: Data curation, Investigation, Validation, editing; Popov Mark: Methodology, Investigation, Original draft preparation; Smirnova Lyudmila: Investigation, Writing - Reviewing and Editing.

Author notes

All authors listed have significantly contributed to the development and the writing of this article.

Additional information

No additional information is available for this paper.

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Declaration of competing interest

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Data availability

Data will be made available on request.

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