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Comparative Analysis of Subcellular Distribution of Heavy Metals in Organs of the Bivalve Mollusks *Crenomytilus grayanus* and *Modiolus modiolus* in a Continuously Polluted Environment

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Abstract—The distribution of Zn, Fe, Ni, Cu, Mn, Cd, and Pb in subcellular fractions, and of Cd, Zn, and Cu in cytoplasm proteins of the kidney and digestive gland of the mussels *Crenomytilus grayanus* and *Modiolus modiolus*, sampled from contaminated and conditionally clean areas, was studied. It was found that, in a contaminated environment, the organs of mussels were more highly enriched with metals. It was shown that essential trace elements were accumulated mostly in the cytosol of organs of both molluscan species from contaminated areas, whereas in the background areas the trace elements were associated mostly with membrane structures in Gray's mussel, *C. grayanus*, and with the cytosol in *M. modiolus*, the northern horse mussel. The lead was bound mostly to membrane structures in organs of both mussel species at all stations. The method of gel chromatography enabled us to isolate metallothionein-like proteins from the kidney of the northern horse mussel sampled in contaminated areas, whereas their concentration in the kidney of Gray's mussels was lower than the limiting error of the method. It is supposed that in the kidney of Gray's mussel the synthesis of metallothionein-like proteins was quenched by the integrated effect of the accumulated metals.

Key words: Gray's mussel, northern horse mussel, heavy metals, subcellular distribution, metal-binding proteins.

Heavy metals (HM), when accumulated in the organism of hydrobionts, can cause disturbances in cellular metabolism. Cytotoxicity of metals is caused mainly by three interconnected mechanisms: increase of lipid peroxidation, suppression of mitochondrial respiration, and disturbance of cellular calcium homeostasis [1]. Bivalves have some patterns of regulation and fixation of the accumulated metals for damage protection of their intracellular structures and biochemical systems [12]. As a rule, the excess metals accumulate in the cytosol, where they form resistant complexes with specific low-molecular proteins (metallothioneins) of unique amino acid compositions and a high affinity for HM ions [17, 23]. Hence, the subcellular distribution of metals indicates the efficiency of the process of translation of metals into nontoxic forms [27].

Most of the studies concerning analysis of the subcellular distribution of heavy metals and their binding to cytoplasm proteins in bivalves has been carried out in toxic experiments [11, 12, 18, 27]. However, the experimental conditions significantly differ from the natural conditions: the concentration of metals in water usually exceeded their content in nature, metals incoming with food were not taken into account, etc. Therefore, obtaining objective information requires field study of the subcellular distribution of metals in the tissues of mollusks living in natural conditions with a higher HM content in the ambient water [15]. Such works are still few, and almost none have been carried out in the Far East region. We stress also that such studies usually analyzed the soft tissues of mollusks in total [20, 22], the digestive gland [21] or gills, and the digestive gland and residue tissues [13], not taking into account that the kidney, as the primary organ of metal excretion, is most exposed to the effect of accumulated toxicants.

The objective of this work is a comparative analysis of the efficiency of the HM detoxication system in the mussels *Crenomytilus grayanus* (Dunker, 1853) and *Modiolus modiolus* (Linnaeus, 1758) (considered formerly as *Modiolus kurilensis* Bernard, 1983), continuously inhabiting a HM polluted biotope.

MATERIAL AND METHODS

The related bivalve species, *Crenomytilus grayanus* and *Modiolus modiolus,* individuals of which were sampled from Desantnaya Bay (st. 1) in summer 2002, were studied (Fig. 1). This bay adjoins the area of the coast dump of industrial–residential wastes of the city of Vladivostok (Gornostai Bay). That is one of the most highly HM contaminated areas of Peter the Great Bay

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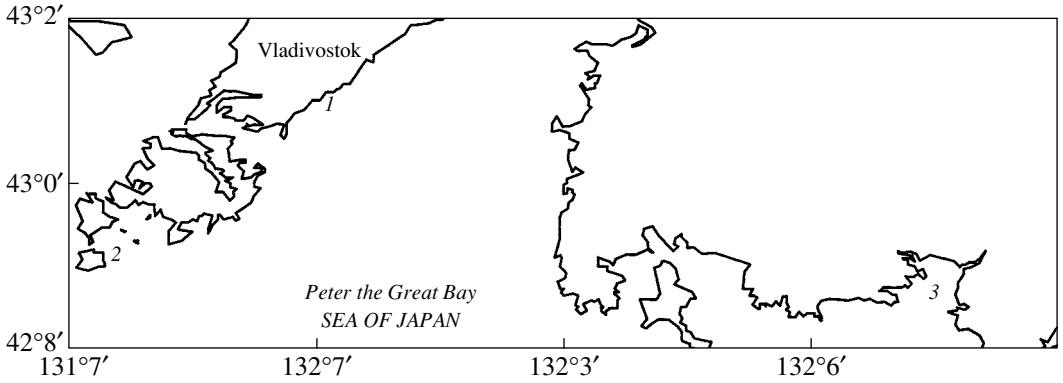

Fig. 1. Location of the sampling sites. (*1*) Desantnaya Bay, (*2*) Reineke Isl., (*3*) Vostok Bay.

due to the dump filtrate flux coming freely into the bay [9]. Gray's mussel and northern horse mussel form joint settlements in Desantnaya Bay, though normally they occupy different biotopes; Gray's mussel prefers hard bottoms while the northern horse mussel inhabits softer bottoms [4]. Gray's mussel from the inshore waters of Reineke Island (st. 2) and the northern horse mussel from Vostok Bay (st. 3) were used for the control, as those regions are conventionally background areas for the Sea of Japan [8].

At each station, 15 adult individuals of each species were sampled. The shell length of Gray's mussel varied within 10.3 ± 0.5 cm (st. 1) and 11.3 ± 0.5 cm (st. 2), that of the northern horse mussel, within 10.5 ± 0.3 cm (st. 1) and 10.9 ± 1.4 cm (st. 3). The depth of sampling was 5–6 m. The digestive gland and the kidney of mollusks, the primary organs that carry out accumulation, detoxication, and excretion of toxicants coming into the organism, were assayed. The concentration of heavy metals in the digestive gland and the kidney was determined in 5 individuals. The average values of the concentration of metals and the standard deviation were calculated by applying Microsoft Excel. The significance of differences between samples was determined by the Mann–Whitney test applying the STATISTICA program package.

The organs of the remaining 10 individuals were combined and used for analysis of the subcellular and cytoplasm distribution of metals. One part of the combined assay of the digestive gland and the kidney was homogenized at 0°C in a manual glass homogenizer adding 0.05 M tris-HCl buffer, pH 7.5, with 0.25 M of sucrose, 0.5 M NaCl and 0.01 M $MgCl_2$ ingredients for membrane stabilization. The homogenate was divided into two parts. Each part was centrifuged (20000 g, 1.5 h, 4°N) for separation of the membrane and cytosol fractions, the heavy metal content of which was determined.

A part of the combined assay was homogenized adding 0.05 M tris-HCl buffer, pH 7.5, and the homogenate was divided into two parts. Each part of the homogenate was centrifuged (20000 g, 1.5 h, 4°C) to obtain cytoplasm fractions, which were used for isolation of metal-binding proteins. The proteins were extracted with a Sephadex column, G100 (1.5 × 60 cm). The column was gauged by standard protein markers (bovine seralbumin, cytochrome C), the free volume was determined by applying dextran light blue. The elution of proteins was carried out with 0.01 M tris–HCl buffer, pH 7.5, the extracted fractions were collected in 3 ml volumes. It is known that metallothionein have special optical properties, thanks to the formation of mercaptide bonds. The maximum absorption occurs at a wavelength of 254 nm, and due to the low content of aromatic amino acids, light absorption occurs at the wavelength 280 nm [17]. Therefore, the optical density of the effluent was determined at these wavelengths applying spectrophotometer UV-260. The obtained fractions were combined by molecular mass: fractions of 0.8–1.4 Ve/Vo (Ve—elution volume, Vo—free volume) corresponded to high-molecular proteins (HMP, >60–150 kDa), fractions of 1.8–2.3 Ve/Vo, to metallothionein-like proteins (≈TLP, ca. 12 kDa), fractions of 2.3–3.3 Ve/Vo, to low-molecular proteins (LMP, <4 kDa).

The concentration of protein (mg/ml of assay) in the combined fractions of the effluent was determined by the microbiuret method [28]. The data were applied for determination of the metal concentration in fractions of the cytoplasm proteins (μg of metal/mg of protein).

The assayed organs, subcellular fractions, and combined effluent fractions were dried at 85°N and mineralized by nitric acid (*OSCh*, especially pure grade). The concentrations of metals, Fe, Zn, Cu, Cd, Mn, Pb, and Ni, were determined by the method of flame atomic absorptive spectrophotometry in an acetylene flame with a Hitachi 180-70 spectrophotometer. Measurement control included measuring the metal concentrations in used acids, in duplicates of the assays with metal salts, and in certificated molluscan specimens.

RESULTS

Analysis of the data on the metal content in the organs of the studied species of mollusks (Table 1) has

Table 1. Concentration of heavy metals (μg/g of dry mass) in organs of *Crenomytilus grayanus* and *Modiolus modiolus* (mean ± standard deviation; $N = 5$)

Species	Fe	Zn	Cu	Cd	Mn	Pb	Ni
Kidney							
Crenomytilus grayanus, st. 1	407 ± 77^{a}	3931 ± 1801^{a}	278 ± 75^{a}	123 ± 24^{a}	8.93 ± 2.77	3582 ± 1290^{a}	9.62 ± 4.23
C. grayanus, st. 2	182 ± 25	667 ± 151	11.7 ± 1.89	84 ± 25	7.32 ± 2.42	19.9 ± 3.91	17.3 ± 5.33
Medioliolus modiolus, st. 1	525 ± 654	$21238 \pm 111196^{b, c}$	$1783 \pm 545^{b, c}$	$203 \pm 75^{b, c}$	1455 ± 1245^{b}	4513 ± 2443^{c}	72 ± 36^{b}
M. modiolus, st. 3	980 ± 422	5021 ± 2129	598 ± 92	92 ± 17.7	2570 ± 653	134 ± 45	54 ± 22
Digestive Gland							
Crenomytilus grayanus, st. 1	245 ± 38^{a}	127 ± 13.1^{a}	258 ± 55^{a}	2.69 ± 0.36	5.02 ± 0.73^{a}	50 ± 22^{a}	2.46 ± 0.45^{a}
C. grayanus, st. 2	72 ± 37	46 ± 12.4	10.5 ± 2.16	1.61 ± 1.45	2.55 ± 0.86	4.02 ± 0.48	1.60 ± 0.37
Medioliolus modiolus, st. 1	535 ± 131^{b}	133 ± 18.8^{c}	$153 \pm 53^{b, c}$	$7.39 \pm 1.04^{b, c}$	$17.6 \pm 2.62^{b, c}$	$144 \pm 22^{b, c}$	2.00 ± 0.26^{b}
M. modiolus, st. 3	691 ± 416	85 ± 22	30 ± 7.89	2.06 ± 0.72	7.63 ± 3.34	4.59 ± 1.64	1.70 ± 0.99

Note: Significant difference ($p \leq 0.05$) comparing (a) organs of *C. grayanus* from station 2, (b) organs of *C. grayanus* from station 1, (c) organs of *M. modiolus* from station 3. Confidence of differences by Mann–Whitney test.

shown that concentration of Fe, Zn, Cu, Cd, and Pb in the kidney of Gray's mussel and of Zn, Cu, Cd, and Pb in the kidney of the northern horse mussel from station 1 was significantly higher than in the kidney of mollusks of the same species from stations 2 and 3. In the assayed organs of mussels, the highest level of accumulation of Pb, Cu, and Zn was recorded. Thus, in the kidney of Gray's mussel from station 1, the concentration of these metals was respectively 179, 24, and 6 times higher than in the kidney of individuals from control station 2. In the kidney of the northern horse mussel from station 1, the concentration of Pb, Cu, and Zn was respectively 34, 3, and 4 times higher than in the kidney of individuals from control station 3. The comparison of the trace element composition in the kidney of mussels from station 1 revealed that the concentration of Zn, Cu, Cd, Mn, and Ni was significantly higher in the kidney of the northern horse mussel than in the kidney of Gray's mussel.

The concentration of most of the assayed metals (except Ni in Gray's mussel, and Fe and Ni in the northern horse mussel) was significantly higher in the digestive gland of mussels from station 1 than that from stations 2 and 3. It was determined that, in digestive gland of Gray's mussel from station 1, the concentration of Pb, Cu, and Zn was respectively 12, 26, and 3 times higher than that at control station 2. In the digestive gland of the northern horse mussel, the concentration of Pb, Cu, and Zn was respectively 32, 5, and 1.5 times higher at station 1 than at control station 3. Comparison of the trace element composition in the digestive gland of the mussels from station 1 has shown that in Gray's mussel the concentration of Fe, Cd, Mn, and Pb was significantly higher, and that of Cu and Ni was significantly lower, than that in the northern horse mussel.

Analysis of the subcellular distribution of heavy metals (Table 2) in the kidney of mussels revealed that, in Gray's mussel from control station 2, Fe, Zn, Cu, Mn, and Ni, being essential metals, were mostly connected to membrane fraction (67–89%). In the northern horse mussel from control station 3, about 55% Fe and Ni, less than 50% Mn and Zn, and only 21% Cu occurred in the membrane structures. It was recorded that Gray's mussel inhabiting the contaminated area (st. 1) had a decreased ratio of all membrane–bound physiologically essential metals, especially Cu (50% decrease). At the same time, in the northern horse mussel inhabiting both the background (st. 3) and contaminated (st. 1) areas, the ratio of essential metals (except Mn) practically did not differ in the membrane fraction of the kidney. Pb was mostly bound to the membrane structures (62–78%), and Cd prevailed in the cytosol (73–89%) in the kidney of the mussels at all stations.

Analysis of the subcellular distribution of heavy metals in the digestive gland of the mussels (Table 2) inhabiting the background area (st. 2) has shown that the main part of Fe, Zn, Mn, and Pb (63–82%), about 50% Cu and Ni, and less than 50% Cd were connected to the membrane fraction in Gray's mussel. In the digestive gland of that species from the contaminated area (st. 1), the relative content of all membrane-bound physiologically relevant metals (except Ni) was decreased, and Cd was not revealed in that fraction. In the digestive gland of the northern horse mussel sampled at station 3 (background area), the main part of Fe, Ni, Cd, and Pb (65–79%), about 50% Cu and Mn, and only 31% Zn were connected to the membrane fraction.

Table 2. The heavy metal content (% from the total metal content in the cell) in subcellular fractions of the kidney and digestive gland of *Crenomytilus grayanus* and *Modiolus modiolus*

Fraction	Kidney				Digestive Gland			
	C. grayanus		*M. modiolus*		*C. grayanus*		*M. modiolus*	
	st. 1	st. 2	st. 1	st. 3	st. 1	st. 2	st. 1	st. 3
Fe								
Membrane	61	89	65	54	56	82	74	79
Cytosol	39	11	35	46	44	18	26	21
Zn								
Membrane	43	79	43	36	29	63	37	31
Cytosol	57	21	57	64	71	37	63	69
Cu								
Membrane	21	71	22	21	28	45	37	53
Cytosol	79	29	78	79	72	55	63	47
Cd								
Membrane	23	27	21	17	0	44	53	65
Cytosol	77	73	79	83	100	56	47	35
Mn								
Membrane	73	79	28	48	16	87	51	48
Cytosol	57	21	72	52	84	13	49	52
Pb								
Membrane	62	67	78	67	62	72	59	74
Cytosol	38	33	22	33	38	28	41	26
Ni								
Membrane	69	83	44	56	58	52	77	76
Cytosol	31	17	56	44	41	48	24	24

Note: Average values of the two parallels are given.

Comparative analysis revealed that the percentage of all membrane-bound metals (except Cu and Pb) practically does not differ in the digestive gland of the northern horse mussel, both at control station 3 and at station 1.

Gel-chromatographic separation of cytosol proteins of the assayed organs of mollusks (Figs. 2, 3) yielded two primary peaks, HMP and LMP. Beyond, another protein peak, which may be attributed to the family of metallothionein-like proteins (*LP*) of marine invertebrates by its molecular mass (ca. 12 kDa) and optical properties (maximum absorption at 254 nm and light absorption at 280 nm), was observed in the kidney of the northern horse mussel at stations 1 and 3. No MLTP peak was observed in the kidney of Gray's mussel and in the digestive gland of both species; however, a part of Cd, Cu, and Zn was eluted at the fraction corresponding to MTLP in the Ve/Vo index (1.8–2.3). Apparently, the level of the synthesized proteins was below the detection limit of the method in those cases.

Analysis of the Zn, Cu, and Cd distribution in the cytoplasm proteins of the kidney and digestive gland of the mussels showed that the Zn concentration in MTLP of their organs was lower than that of Cu and Cd, though the total concentration of Zn in the organs was

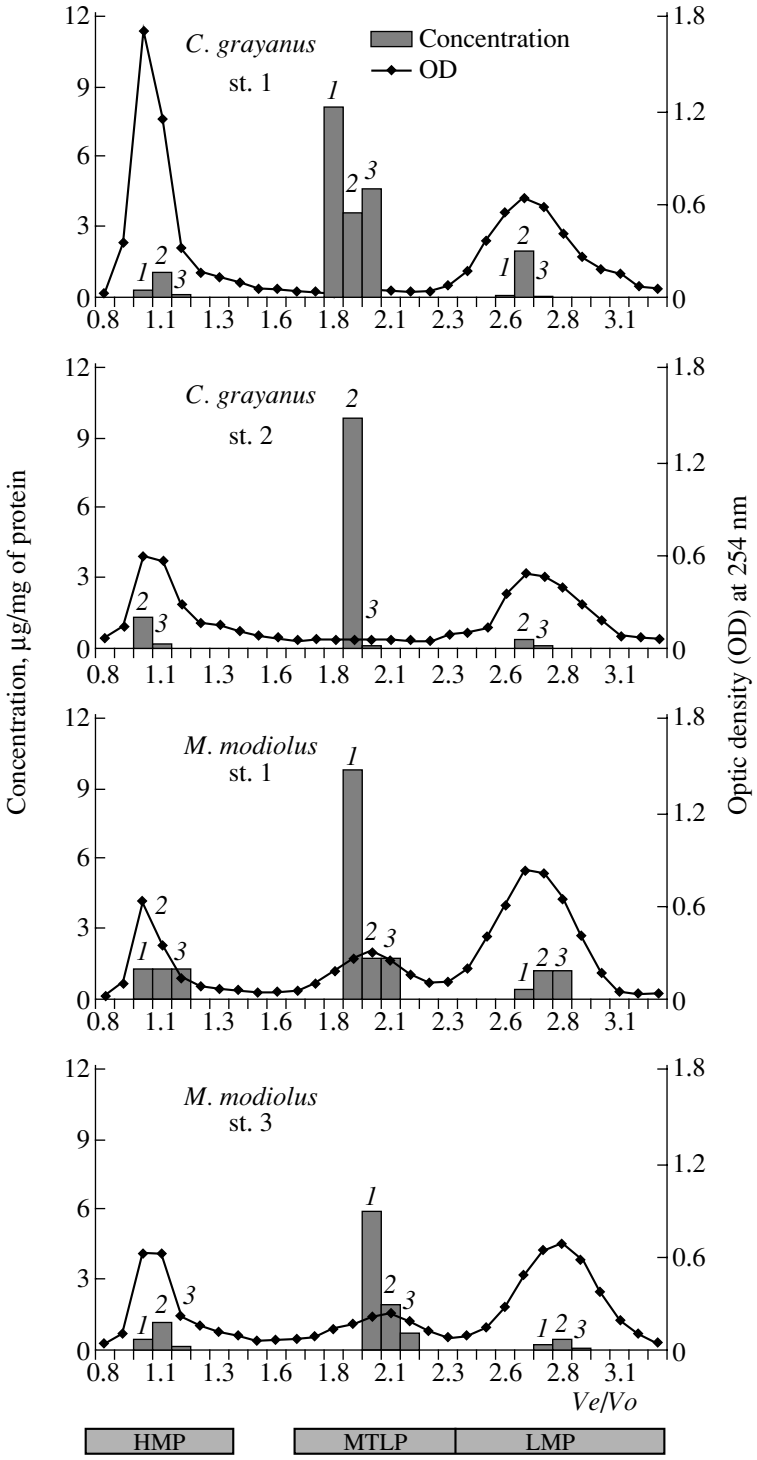

Fig. 2. Concentration of heavy metals in fractions of the kidney cytoplasm proteins in *Crenomytilus grayanus* and *Modiolus modiolus*. (*1*) Copper, (*2*) Zinc, (*3*) Cadmium. The respective figure was not given if the concentration of metal in the protein was below the limit of detection. Station 1—Desantnaya Bay, station 2—coastal waters of Reineke Isl., station 3—Vostok Bay.

higher (Table 1). It is worth noting that the Cu concentration in the cytoplasm proteins of the kidney and that of Cd in the cytoplasm proteins of the digestive gland of Gray's mussels from station 1 was below the detection limit (Figs. 2, 3).

DISCUSSION

It is known that Pb, Cu, and Zn are the primary contaminants in bottom sediments of areas adjacent to the city waste dump (Gornostai and Desantnaya Bay) [9].

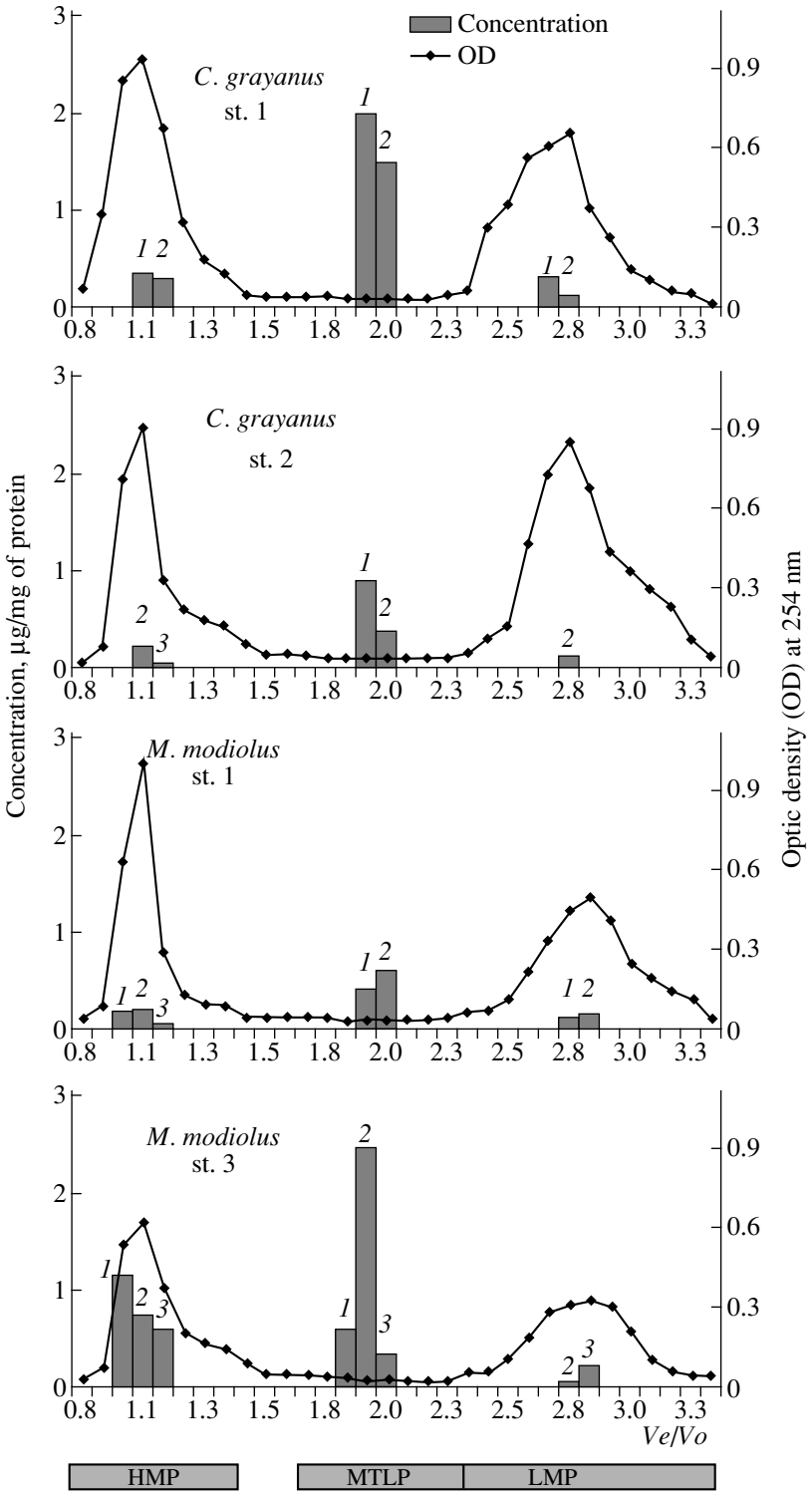

Fig. 3. Concentration of heavy metals µg/mg protein in fractions of cytoplasm proteins of the digestive gland in *Crenomytilus grayanus* and *Modiolus modiolus*. Designations are the same as in Fig. 2.

According to our data (Table 1), mussels from Desantnaya Bay accumulated significant concentrations of those elements.

A former study of the trace element composition in the bivalves *Modiolus modiolus* and *Crenomytilus grayanus* showed that the northern horse mussel accumulated heavy metals to a greater extent, obviously, thanks to adaptation to soft grounds enriched in trace elements [6]. Our data also evidenced that the kidney concentration of all metals was significantly higher in the northern horse mussel than

in Gray's mussel (Table 1). However, we should note that the degree of metal enrichment of the studied organs of the northern horse mussel was lower than that in Gray's mussel, e.g., the kidney concentration of Pb in individuals from Desantnaya Bay exceeded that of the control in Gray's mussel 179 times, and only 34 times in the northern horse mussel. This fact, apparently, evidences the more effective excretion of metals by *M. modiolus* in a continuously polluted environment.

It is known that a prevailing concentration of heavy metals in the kidney can indicate the degree of balance of their accumulation in the organism of mollusks [2]. In the northern horse mussel, by our data, the concentration of all assayed elements was significantly higher in the kidney than in the digestive gland at both stations. At the same time, the Cu concentration in the digestive gland and the kidney does not differ significantly in Gray's mussel from the Desantnaya Bay (Table 1). Taking in account that Cu can induce the occurrence of highly active radicals of oxygen (oxiradicals) in a cell, the protective systems of an organism should be tuned to detoxication and excretion of that metal [15]. As this situation was not observed in Gray's mussel inhabiting the contaminated area, apparently, the level of Cu accumulation in the organism exceeded the excreting capacity of its kidney. A similar dysfunction of the kidney was recorded in individuals of *C. grayanus*, transplanted from the background area to Desantnaya Bay [2]. In contrast to Gray's mussel, the Cu concentration in the northern horse mussel from the contaminated area was significantly lower in the digestive gland than in the kidney. This fact evidenced the efficient intertissual redistribution of this metal, and, respectively, the higher resistance of *M. modiolus* to a continuously higher level of HM in its environment.

To assess the efficiency of intracellular detoxication of accumulated metals in the organs of mussels, we studied the subcellular distribution of those elements. Heavy metals were segregated in two groups: essential trace elements, Zn, Cu, Mn, Fe, Ni, and toxic metals, Pb and Cd. Essential metals are part of some enzymes, hormones, and vitamins [3]; therefore, they usually mostly occurred in cellular membrane structures. We recorded such a distribution in the organs of Gray's mussel from the background area. However, an excess of essential trace elements is harmful for an organism. Therefore, accumulated elements were bound to the cytosol at an increased HM content in the environment. This process protects cellular structures from damage, as would happen in the organs of mussels from Desantnaya Bay (Table 2). Such a pattern of prevailed binding of essential trace elements to the cytosol under an increased HM content in the environment was displayed in laboratory experiments on the gastropod *Nassarius reticulatus* [18] and in a study of *C. grayanus* from upwelling areas [5]. In contrast to Gray's mussel, in the northern horse mussel from the background area (st. 3), essential metals were bound to the cytosol. In the digestive gland, the content of the membrane-bound metals also differed less in the northern horse mussel from both stations, than in Gray's mussel (Table 2). From the aforesaid, it is possible to suggest that, in organs of the northern horse mussel, the HM are accumulated redundantly even in the background environment. It is necessary to note that in the organs of mussels the toxic lead is bound mostly to membrane structures, as it is accumulated in colloid or suspended form and is stored in the form of sulfates or phosphates in the membrane fraction of tissues of marine mollusks [21].

Thus, in the organs of *M. modiolus* and *C. grayanus*, the excess of HM are accumulated in the cytosol, where they form complexes with metal-binding proteins. As is known, the essential metals are bound to HMP and LP, and their excess is bound by metallothioneins [17]. Unlike the given metals, Cd is bound mostly to metallothionein, with HMP being an intermediate ligand [5, 14, 20].

In the kidney of the mussels from Desantnaya Bay, the metallothionein-like proteins binding a part of the cytoplasm Cu, Zn, and Cd were synthesized (Fig. 2). MTLP were isolated from the kidney of the northern horse mussel, whereas in the kidney of Gray's mussel their concentration was below the limiting error of the method. However, it is known that the kidney of Gray's mussel can synthesize a greater amount of MTLP. Thus, in Gray's mussels inhabiting the effective area of powerful steady upwelling, these proteins that had an abnormally high concentration of Cd were isolated from the kidney [5]. Apparently, the synthesis of given proteins was quenched by the integrated effect of accumulated metals in Gray's mussel from heavy polluted Desantnaya Bay. Inhibition of the synthesis of metallothioneins in organs of mollusks with a high degree of HM accumulation was recorded in a number of experimental studies [10, 19]. However, the mechanism of inhibition of MTLP synthesis in mollusks naturally needs further study.

The inhibition of MTLP synthesis was not observed in *M. modiolus.* Furthermore, synthesis of a significant amount of these proteins was observed in individuals of this species inhabiting the background area (st. 3). Probably, as a result of evolutionary adaptation to life in soft bottoms, an induced tolerance to the environment with an increased HM content was established in *M. modiolus*. The ability of mollusks to increase resistance to the effect of HMs after their acclimation to a low content of toxicants was reordered in experiments [7, 24]. It is believed that the induced tolerance is established in the organism also in natural conditions with an increased content of metals [16, 23]. Apparently, the higher resistance of the mechanism of MTLP synthesis in *M. modiolus* may be explained by the induced tolerance.

It is known that the affinity of heavy metals to metallothioneins is moderated in series: Cu (I) > Cd (II) > Zn (II) [25]; hence, Cd and Zn may be displaced from MTLP at a high accumulated Cu. Most likely, that was exactly what was observed in mussels from Desantnaya Bay, in which the zinc concentration in the kidney was

higher than that of copper, but lower than the copper concentration in MLTP (Fig. 2). Displacement of Zn by ions of other metals was formerly observed in experimental studies. For example, it has been shown with *Mytilus galloprovincialis*, that 6–7 ions of Cu and Zn (also ions of Cd, Hg, and Ag) are normally bound to one metallothionein molecule, if these metals are available in the tissues. If a great number of Cd, Hg, or Cu ions penetrate the cell, they displace Zn from (Zn–Cu) thioneins, normally available in the cytosol; then the Zn ions induce the subsequent synthesis of metallothioneins [26]. Their concentrations in those metal-binding proteins are comparable at a low-level content of Zn and Cu, which was observed in the digestive gland of the studied mollusks. At a minimum Cu accumulation, the MTLP bind the maximum quantity of Zn in the cytosol, as occurred in the kidney of Gray's mussel from the background area (st. 2). It is necessary to note that, although the concentration of Cd, Zn, and Cu in the studied organs of the northern horse mussel from Desantnaya Bay was higher than that in Gray's mussel, the metal content in the cytoplasm proteins of organs of the first species was lower (Figs. 2, 3). Most likely, beyond MTLP synthesis, other mechanisms of HM regulation, impeding penetration of metals into the cell or stimulating their intense excretion, work intensely in *M. modiolus.*

Thus, in contrast to *C. grayanus*, an increased quantity of HMs is accumulated in the kidney and digestive gland of *M. modiolus* even in the background areas. Trace elements are bound in the cytosol, where a significant amount of MTLP is synthesized. Based on that, it is possible to suggest that an induced tolerance to a higher environmental HM content was established in the organism of *M. modiolus* as a result of adaptation to living in metal-enriched soft bottoms.

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REFERENCES

1. Aksenova, M.E. The Heavy Metals: Mechanisms of Nephrotoxicity, *Nefrologiya i dializ*, 2000, vol. 2, nos. 1–2. *(Translators note – Electronic journal, www.dialysis.ru/magazine).*
2. Kavun, V.Ya. and Shul'kin, V.M, Changes in the Microelement Composition in Organs and Tissues of the Bivalve *Crenomytilus grayanus* Acclimatized in a Biotope with Long-Term Heavy Metal Contamination, *Biol. Morya*, 2005, vol. 31, no. 2, pp. 123–128.
3. Koval'sky, V.V., *Geohimicheskaya ekologiya* (Geochemical Ecology), Moscow: Nauka, 1974.
4. Skarlato, O.A., *Dvustvorchatye mollyuski umerennykh shirot zapadnoi chasti Tikhogo okeana* (Bivalves of the Boreal West Pacific), Lengrad: Nauka, 1981.
5. Podgurskaya, O.V., Kavun, V.Ya., and Luk'yanova, O.N., Heavy Metal Accumulation and Distribution in Organs of the Mussel *Crenomytilus grayanus* from Upwelling Areas of the Sea of Okhotsk and the Sea of Japan, *Biol. Morya*, 2004, vol. 30, no. 3, pp. 219–226.
6. Khristoforova, N.K., Shul'kin, V.M., Kavun, V.Ya., and Chernova, E.N., *Tyazhelye metally v promyslovykh i kul'tiviruemykh mollyuskakh zaliva Petra Velikogo* (Heavy Metals in Commercial and Cultured Mollusks of the Peter the Great Bay), Vladivostok: Dal'nauka, 1993.
7. Chelomin, V.P., Bel'cheva, N.N., and Zakhartsev, M.V., Biochemical Mechanisms of Adaptation to Cadmium and Copper Ions in the Mussel *Mytilus trossulus*, *Biol. Morya*, 1998, vol. 24, no. 5, pp. 319–325.
8. Shul'kin, V.M., *Metally v ekosistemakh morskikh melkovodii* (Metals in Ecosystems of the Sea Shoal), Vladivostok: Dal'nauka, 2004.
9. Shulkin, V.M., Kavun, V.J., Tkalin A.V., and Presley, B.J., The Effect of Metal Concentration in Bottom Sediments on Accumulation of Metals by the Mytilids *Crenomytilus grayanus* and *Modiolus kurilensis*, *Biol. Morya*, 2002, vol. 28, no. 1, pp. 53–60.
10. Baudrimont, M., Andres, S., Metivaud, J., *et al.*, Field Transplantation of the Freshwater Bivalve *Corbicula fluminea* along a Polymetallic Contamination Gradient (River Lot, France) II. Metallothionein Response to Metal Exposure, *Environ. Toxicol. Chem.*, 1999, vol. 18, pp. 2472–2477.
11. Bebianno, M.J. and Langston, W.J., Metallothionein Induction in *Mytilus edulis* Exposed to Cadmium, *Mar. Biol.*, 1991, vol. 108, no. 1, pp. 91–96.
12. Bebianno, M.J. and Serafim, M.A., Comparison of Metallothionein Induction in Response to Cadmium in the Gills of the Bivalve Mollusks *Mytilus galloprovincialis* and *Ruditapes decussatus*, *Sci. Total Environ.,* 1998, vol. 214, pp. 123–131.
13. Bebianno, M.J. and Serafim, M.A., Variation of Metal and Metallothionein Concentration in Natural Population of *Ruditapes decussatus*, *Arch. Environ. Contam. Toxicol.*, 2003, vol. 44, pp. 53–66.
14. Carpene, E. and George, S.Y., Absorption of Cadmium by Gills of *Mytilus edulus* (L.), *Mar. Physiol.*, 1981, vol. 1, pp. 23–34.
15. Giguere, A., Couillard, Y., Campbeel, P.G.C., *et al.*, Steady-state Distribution of Metals Among Metallothionein and Other Cytosolic Ligands and Links to Cytotoxicity in Bivalves Living along a Polymetallic Gradient, *Aquat. Toxicol.*, 2003, vol. 64, pp. 185–200.
16. Hoare, K., Beaumont, A.R., and Davenport, J., Variation among Populations in the Resistance of *Mytilus edulis* Embryos to Copper: Adaptation to Pollution?, *Mar. Ecol. Progr. Ser.*, 1995, vol. 120, pp. 155–161.
17. Isani, G., Andreani, G., Kindt, M., and Carpene, E., Metallothionein in Marine Mollusks, *Cell. Mol. Biol.,* 2000, vol. 46, no. 2, pp. 311–330.
18. Kaland, T., Andersen, T., and Hylland, K., Accumulation and Subcellular Distribution of Metals in the Marine Gastropod *Nassarius reticulatus*, *Ecotoxicology of Metals in Invertebrates: A Special Publication of SETAS*, Lewis Publishers, 1993, pp. 37–55.

19. Langston, W.J. and Zhou, M., Evaluation of the Significance of Metal-binding Ptoteins in the Gastropod *Littorina littorea*, *Mar. Biol.,* 1986, vol. 92, pp. 505–515.

20. Mouneyrac, C., Bertnet, B., and Amiard, J.-C., Cd Distribution in the Tissues of Oysters *(Crassostrea gigas)* Exposed Chronically *in situ*, *Water, Air Soil Pollut.*, 1999, vol. 112, pp. 187–196.

21. Regol, F. and Orlando, E., Accumulation and Subcellular Distribution of Metals (Cu, Fe, Mn, Pb and Zn) in the Mediterranean Mussel *Mytilus galloprovincialis* During a Field Transplant Experiment, *Mar. Pollut. Bull.*, 1994, vol. 28, pp. 592–600.

22. Riveros, A., Zuniga, M., and Larrain, A., Copper Metallothionein-like Proteins as Exposure Biomarker in Native and Transplanted Intertidal Populations of the Mussel *Perumytilus purpuratus* from San Jorge Bay, Antofagasta, Chile, *Bull. Environ. Contam. Toxicol.*, 2003, vol. 70, pp. 233–241.

23. Roesijadi, G., Metallothioneins in Metal Regulation and Toxicity in Aquatic Animals, *Aquat. Toxicol.,* 1992, vol. 22, no. 2, pp. 81–114.

24. Roesijadi, G. and Fellingham, C.W., Influence of Cu, Cd and Zn Preexposure on Hg Toxicity in the Mussel *Mytilus edulis*, *Can. J. Fish. Aquat. Sci.*, 1987, vol. 44, pp. 680–684.

25. Romero-Isart, N. and Vasak, M., Advances in the Structure and Chemistry of Metallothioneins, *J. Inorg. Biochem.*, 2002, vol. 88, pp. 388–396.

26. Viarengo, A., Biochemical Effects of Trace Metals, *Mar. Pollut. Bull.*, 1985, vol. 16, pp. 153–158.

27. Wallace, W.G., Lee, B.-G., and Luoma, S.N., Subcellular Compartmentalization of Cd and Zn in Two Bivalves. I. Significance of Metal-sensitive Fractions (MSF) and Biologically Detoxified Metal (BDM), *Mar. Ecol. Progr.*, Ser. 2003, vol. 249, pp. 183–197.

28. Ytzhaki, R.F. and Gill, D.M., A Micro-biuret Method for Estimating Proteins, *Analyt. Biochem.*, 1964, vol. 9, pp. 401–407.