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Biomonitoring of estuaries of the Peter the Great Bay (the Sea of Japan) using *Corbicula japonica* (Prime, 1864)

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Abstract. The estuaries of the Razdolnaya and Partizanskaya Rivers in the Peter the Great Bay were chosen to conduct the comparative studies of the effects of pollution on aquatic organisms. *Corbicula japonica*, a bivalve mollusk, was used as an indicator species. Molecular biomarkers in the digestive gland and gills of *C. japonica* were used to determine the indicators of oxidative stress, such as lipid peroxidation (malondialdehyde), antiradical activity, DNA damage and microelement composition of tissues. High concentrations of iron, zinc and lead were identified in the tissues of *C. japonica* from the Partizanskaya River. A high concentration of copper was found in the tissues of those from the Razdolnaya River. The level of lipid peroxidation products in the digestive gland of *C. japonica* from the Partizanskaya was higher than that of the other areas under study. Moreover, the level of antiradical activity and the genetic damage index in its tissues were two times higher. It is found that chronic oxidative stress is observed in all the estuaries under study. The proposed biomarkers clearly show the state of *C. japonica* in the biotopes of different pollution levels, indicating that the worst state is attributable to the estuary of the Razdolnaya River.

1. Introduction

Areas, such as estuaries and lagoons, where sea and river waters mix are unique and specific as far as their ecosystem properties are concerned. Estuaries are a so-called marginal filter, i. e. an area where sea and fresh waters mix and represent a biogeochemical barrier where there is a sharp change in the concentration of matters suspended and dissolved in water and there is an obstacle to the ingress of a giant amount of various matters into open sea water areas [1]. Depending on the type of estuaries, water from these areas is characterized by reduced salinity, but with a tendency to constantly change it, and by zones of increased turbidity and sedimentation of a large amount of organic matter. These areas can collect up to 90-95% of suspended and up to 30-45% of dissolved matters and river runoff pollutants [2]. In this regard, sediment accumulation rates in an estuary exceed those in the ocean by an average of 1,000-10,000 times and therefore the abundance of microelements and compounds in these water areas exceeds by tens of thousands times [3]. As a rule, estuaries are adjacent to zones where people carry out active economic and business operations that are manifested in strong anthropogenic effects on ecosystems, which leads to irreversible changes in these areas and gives rise to well-founded concerns for their future. A primary hazard to lagoons and estuaries is associated with an influx of various chemicals and pollutants from river runoffs, from the open sea and the continent. The consequences of anthropogenic impacts are most felt in estuarine ecosystems where variability in



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physical and chemical factors can affect bioavailability and, as a result, alter the toxicity of xenobiotics, thus synergistic and antagonistic combinations of anthropogenic factors may arise. Such variations in factors frequently and considerably complicate the assessment of negative changes in ecosystems using traditional hydrobiological methods since they provide no means for quick assess of the ecotoxicological situation in water areas under study. Therefore, the parameters that characterize the state of estuaries should be integrated through assessing the state of biota and reflect hydrochemical, biogeochemical and ecotoxicological conditions in this ecosystem. In light of this, the use of biochemical (molecular) markers is most appropriate to monitor sea coastal waters due to its high accuracy, sensitivity and rapid determination.

For the purpose of biomarker studies of the river estuaries and coastal lagoons of the Sea of Japan, the most accessible, convenient and informative subjects are considered to be bivalve mollusks of *C. japonica* as filter feeders since they are dominant in bottom communities and valuable from the industry point of view.

The purpose of this work is to biomonitor the river estuaries and sea lagoons of the Peter the Great Bay. At the same time, the main task of this study is to show (identify), using biochemical markers, any pathological changes in the organism of *C. japonica* experiencing the effect of complex pollution of the aquatic environment. To carry out biomonitoring, we specified the following water areas: estuaries of the Razdolnaya (Amurskysky Bay) and Partizanskaya (Ussuriysky Bay) Rivers that are characterized by different levels of anthropogenic load.

2. Materials and methods

The sexually mature individuals of *C. japonica* were selected from the estuary of the Partizanskaya River (St. 1), the estuaries of the Razdolnaya River (St. 2) and the Tikhaya Lagoon (St. 3) located 5 km from St. 2 (Peter the Great Bay, Sea of Japan) (figure 1).

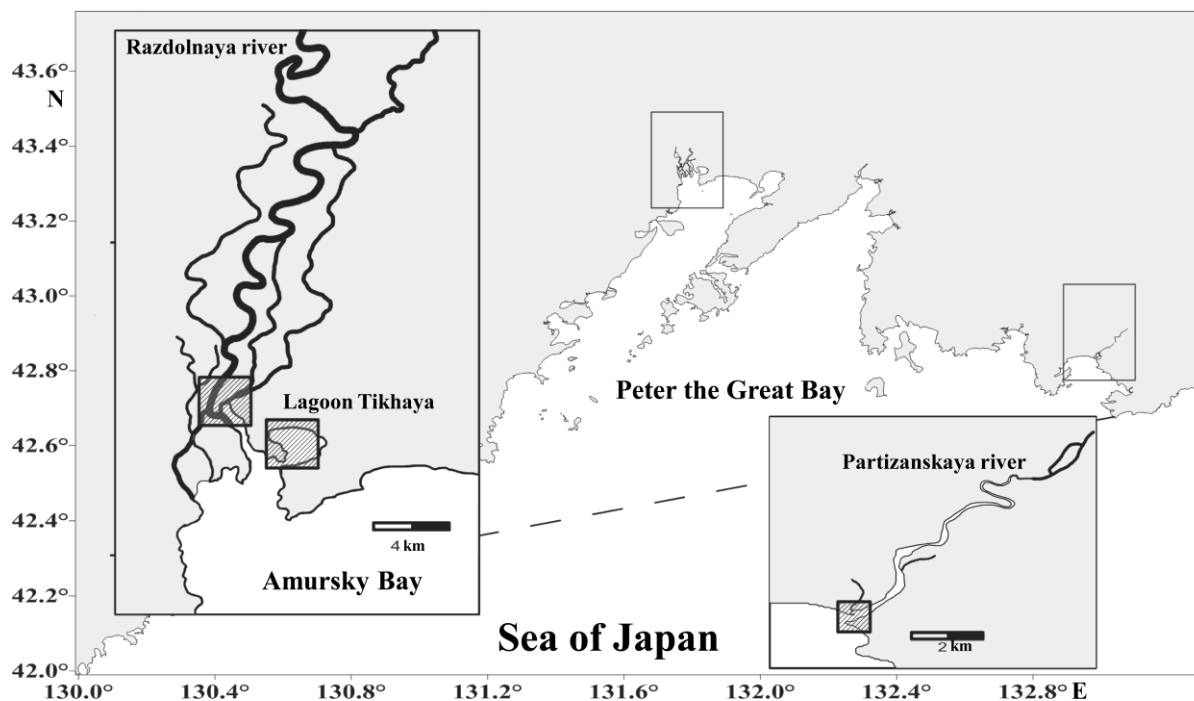


Figure 1. Study area and sites of *Corbicula japonica* sampling.

The Razdolnaya River originates in the territory of China (Northern Manchuria). The river enters the territory of the Russian Federation in the area of the village of Poltavka (Primorsky Krai) and flows into the northern part of Amursky Bay. The Razdolnaya River is the second largest river in the

catchment area of the Peter the Great Bay (the Sea of Japan). The estuary of the Razdolnaya River is of lagoon type: oligohaline, shallow-water, with poor water exchange and reduced oxygen dissolved in water. During summer floods, the river carries a large amount of biogenic elements so that hypoxia and eutrophication of the estuary and northern part of Amursky Bay are observed during this period [4].

The runoff of the Partizanskaya River is 2 times inferior to that of the Razdolnaya River and provides a river runoff for the eastern part of the Peter the Great Gulf [5]. The river is actively used as a source of domestic and technical water supply, as a fishing area and for recreational and industrial purposes. The estuary of the Partizanskaya River is of channel type: with good water exchange, high oxygen content and salinity of up to 34‰ [1]. The river is a collector of waste waters from the cooling reservoir and retention ponds of the Partizansk State District Power Station (SDPS). Water intended to cool generation equipment comes from a water reservoir and further enters the river. Used and runoff waters from the Partizansky Brewery are also discharged into the river. The town of Partizansk is located in the lower part of the river. Nakhodka, a large industrial center, is situated near the mouth of the river. Currently, the ecological state of the Partizanskaya River is considered to be transient, ranging from low to very high pollution levels [6].

The individuals of *C. japonica* were selected using a creeper in April 2019. During the catching, water temperature varied between 0 and 20°C.

Methods for determining biochemical indicators

The caught mollusks were prepared on ice, their gills as a main organ contacting their natural habitat and their digestive gland involved in the detoxification and deposition of toxic substances were removed. The collected tissues were immediately frozen in liquid nitrogen and delivered to the laboratory for analysis. The gills and the digestive gland were homogenized in a phosphate buffer, and the homogenate was used to determine: protein content by the Lowry method [7], malondialdehyde as an end product of lipid peroxidation (LPO) [8] and integral antiradical activity (IAA) of antioxidants according to their ability to inhibit the ABTS radical cation (ABTS⁺) [14]. Measurements were made using the Shimadzu UV-1650 PC and Shimadzu UV-2550 spectrophotometers with a thermostated cell.

The atomic absorption method was used to quantify heavy metals (HMs) in the tissues of *C. japonica* [9, 10].

To determine the genetic damage index of the DNA molecule of the cells of the digestive gland and gills of *C. japonica*, we used the alkaline version of comet assay [11] adapted to marine organisms [12, 13]. DNA comets were recorded using the scanning fluorescence microscope (Zeiss, AxioImager A1), with the AxioCamMRc digital camera. The CometScoreFreeware v1.5 program (http://www.autocomet.com/products_cometscore.php) was used to calculate the digital images of the DNA comets. The genetic damage index (GDI) was calculated according to the formula $(C1+2*C2+3*C3+4*C4)/(C0+C1+C2+C3+C4)$, for this purpose, based on the results of comet calculation through the computer program, they were divided into 5 classes as per the level of the DNA fragmentation [13].

The following programs were used for the statistical processing of biomonitoring results: STATISTICA 6.0 and Microsoft Excel 2016; the Dennett's nonparametric criterion ($P \leq 0.05$) was used to compare mean-group indicators.

3. Results and discussion

The comparative analysis of the results shows considerable differences in the concentrations of the studied microelements in the tissues of *C. japonica* from different collection places, as well as in the tissue specificity of their accumulation (tables 1, 2).

Table 1. Microelement composition of the gills of *C. japonica* collected in different areas of the Peter the Great Bay (mean $\pm$ standard deviation, $n = 15$).

Element Collection place	Cd	Mn	Cu	Zn	Fe	Pb
	mkg/dry matter					
St. 1 Partizanskaya	1.44 $\pm$ 0.07	10.5 $\pm$ 1.0	5.18 $\pm$ 1.7	72.3 $\pm$ 11.2	991.5 $\pm$ 14.2	5.18 $\pm$ 0.6
St. 2 Razdolnaya	2.38 $\pm$ 0.06	26.27 $\pm$ 9.5	12.12 $\pm$ 0.2	41.4 $\pm$ 0.5	258.8 $\pm$ 4.5	2.63 $\pm$ 0.3
St. 3 Tikhaya Lagoon	2.01 $\pm$ 0.05	8.16 $\pm$ 0.9	2.48 $\pm$ 0.3	39.4 $\pm$ 1.1	385.4 $\pm$ 12.9	2.48 $\pm$ 0.3

The highest concentrations of iron, zinc and lead were observed in the tissues of *C. japonica* from the Partizanskaya River. In the gills, the content of iron was 991.5 $\pm$ 14.2 mkg/g dry matter, zinc – 72.3 $\pm$ 11.2 mkg/g dry matter, lead – 5.18 $\pm$ 0.6 mkg/g dry matter, which was 2-2.5 times higher than the concentrations of these elements in the gills of *C. japonica* from the Tikhaya Lagoon and the estuary of the Razdolnaya River. At the same time, the highest concentrations of cadmium and copper were observed in the tissues of St. 2. According to Shulkin [5], the content of dissolved copper in the Razdolnaya River was slightly higher than the maximum permissible concentration (1.05 mkg/l), while concentrations in the Partizanskaya River were 2 times lower than the MPC (0.50 mkg/l). It should be noted that, according to Kalinina, in 2010 in the gills of *C. japonica* from the Razdolnaya River the level of iron varied from 1,333 mkg/g dry matter to 2,324 mkg/g dry matter, and the content of copper in the digestive gland and gills was within the obtained concentrations (18 - 27 mkg/g dry matter) [14].

Table 2. Microelement composition of the digestive gland of *C. japonica* collected in different areas of the Peter the Great Bay (mean ($\pm$ standard deviation, $n = 15$).

Element Collection place	Cd	Mn	Cu	Zn	Fe	Pb
	mkg/dry matter					
St. 1 Partizanskaya	1.64 $\pm$ 0.07	5.47 $\pm$ 1.1	6.83 $\pm$ 1.4	135.73 $\pm$ 27.2	993.34 $\pm$ 19.4	13.22 $\pm$ 2.6
St. 2 Razdolnaya	0.39 $\pm$ 0.05	8.15 $\pm$ 0.7	22.92 $\pm$ 2.2	56.5 $\pm$ 10.3	363.33 $\pm$ 10.0	3.83 $\pm$ 1.9
St. 3 Tikhaya Lagoon	0.53 $\pm$ 0.01	8.2 $\pm$ 0.9	3.26 $\pm$ 0.6	45.9 $\pm$ 7.7	482.85 $\pm$ 8.26	3.26 $\pm$ 0.6

When compared to the other stations, elements, such as iron, zinc, lead and cadmium (993.34 $\pm$ 13.8 mkg/g dry matter, 135.73 $\pm$ 6.75 mkg/g dry matter, 13.22 $\pm$ 0.99 mkg/g dry matter, 1,641 $\pm$ 13.8 mkg/g dry matter (table 2), were found in large quantities in the digestive gland and gills of *C. japonica* from the Partizanskaya River. Manganese concentrations in the tissues of the digestive gland of St. 2 and 3 were at the same level and exceeded values for St. 1 by 1.5 times.

As far as is known, one of the most important ways for metals to enter aquatic organisms is through the contact of water with the body's surface and nourishment and, as a result, organisms living in this environment concentrate and accumulate elements from different sources (water, food and soils) [15]. Mollusks are believed to sort particles suspended in water into organic components (bacteria, plankton) and inorganic suspensions. Organic components in which copper, zinc and cadmium are dominant elements are consumed by mollusks as food and therefore these metals are concentrated and accumulated in their organism. Suspensions (inorganic part) are dominated by such microelements as iron, manganese, nickel and lead that are removed from the organism with pseudo-feces, so the number of these elements along the trophic chain decreases [15]. However, the high content of iron, as compared to the other stations, is observed in the tissues of *C. japonica* from the estuary of the

Partizanskaya River. This is probably due to the fact that mollusks live in soft soils where the content of microelements in the near bottom layer and pore waters is considerably higher than in the water column, so the concentration of these elements in the soft tissues of mollusks considerably increases through filtering these waters. We also believe that the main external source of iron and lead ingress into the soils of the Partizanskaya River is ashes from the Partizanskaya SDPS, the macroelement composition of which is dominated by aluminum, iron, lead, cadmium, etc. [16]. The ecological state of the river has deteriorated markedly in recent decades, especially after an emergency discharge in the ash dump of the Partizanskaya SDPS in 2004, which resulted in the river receiving more than 80,000 tons of ash slurry [6]. The river is also affected by mine waters coming from liquidated coal mining enterprises in the Partizansky basin. The total amount of naturally flowing mine waters in the Partizanskaya River basin is 304 m³/h and has high contents of iron, zinc and manganese exceeding the MPC. In the place where mine waters enter the river the chemical composition of surface waters considerably changes and iron, manganese and other microelements and chemical compounds are actively deposited due to geochemical barriers [17]. Thus, the maximum amount of these elements is concentrated in the soils, the near bottom layer of water and living organisms of the estuarine zone where the mollusks were selected. It was also noted that the iron content in the tissues of *C. japonica* from the Tikhaya Lagoon exceeded that of the individuals collected at the distance of 5 km upstream of the Razdolnaya River (table 1). Probably, in this part of the estuary there are more intensive sediment and microelement accumulation processes as compared to St. 2.

The use of molecular markers makes it possible not only to assess general changes in the physiological state of a living organism affected by adverse factors, but also to establish cause-and-effect relations when the organism interacts with the environment, as well as to predict possible changes in the aquatic ecosystems of the water areas under study. The biochemical data are consistent with the microelement composition in the tissues of *C. japonica*. The lowest MDA values are observed in the gills of *C. japonica* from the Tikhaya Lagoon (0.975 ± 0.05 nmol/mg protein), whereas those from the Razdolnaya River are notable for the highest ones (1.365 ± 0.08 nmol/mg protein) (figure 1). The highest LPO products are observed in the digestive gland of *C. japonica* from the Partizanskaya River (2.685 ± 0.134 nmol/mg protein), whereas the MDA content in the individuals from the Razdolnaya River and the Tikhaya Lagoon was 2 times lower (1.412 ± 0.071 nmol/mg protein and 1.325 ± 0.069 nmol/mg protein respectively) (figure 2A).

The level of integral antiradical activity (IAA) characterizes the general antiradical ability of low molecular antioxidants to neutralize peroxy and alkoxy radicals ($RO_2^\bullet$ - and $RO^\bullet$) in the cell [18]. The highest IAA values are found in the digestive gland of *C. japonica* from the Partizanskaya River (132.12 ± 6.9 trollox unit/mg protein), whereas the lowest ones are observed in those from the Tikhaya Lagoon (60.88 ± 3.1 trollox unit/mg protein) (figure 2B).

The DNA molecule's degradation in the cells of the gills was practically the same for every station (figure 2B). In the digestive gland, the highest GDI values were obtained for *C. japonica* from the Razdolnaya River (0.52 ± 0.025) and the Tikhaya Lagoon (0.47 ± 0.023), whereas those from the Partizanskaya River were 2 times lower (0.25 ± 0.012).

The roles of the digestive gland include detoxification, deposition and accumulation and therefore, according to the biochemical parameters obtained for the digestive gland, we can assess a long-term effect of various factors, including metals. Moreover, we primarily associate the high MDA values in the digestive gland of *C. japonica* from the Partizanskaya River with the high content of iron and other microelements in the tissues as a cause of oxidative stress. Iron is known to be a potentially toxic element as its reactivity manifests itself in variable valency. In the cells of living organisms, particularly in mollusks, there is a labile pool of iron (Fe^{2+} and Fe^{3+}) in complexes with different ligands of low molecular weight, the accumulation and storage of which are controlled by transport and binding proteins. This pool constitutes a minor part of the total iron content in the cell, and surpluses lead to the development of oxidative stress through the iron-dependent conversion of the superoxide oxygen radical and hydrogen peroxide into the toxic hydroxyl radical (the Haber-Weiss

reaction). Thus, the labile iron content causes the intensive accumulation of lipid peroxidation products (such as MDA) in tissues [19, 20].

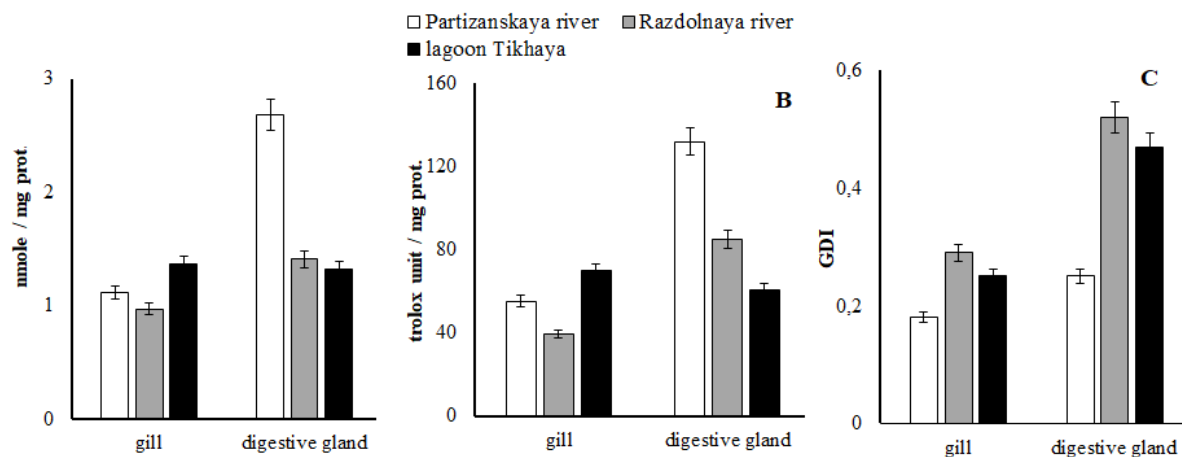


Figure 2. Molecular biomarkers of oxidative stress in the gills and digestive gland of *C. japonica* from the estuaries of the Partizanskaya River, the Razdolnaya River and the Tikhaya Lagoon. Units of measurement: malondialdehyde – nmol MDA/mg protein; integral antiradical activity (IAA) – nmol trolox/mg protein; genetic damage index (GDI).

The results entitle us to believe that mollusks from all the areas under study experience chronic oxidative stress. We believe that *C. japonica* from the Partizanskaya River experiences oxidative stress induced by high iron and lead contents. However, the results show the high antioxidant potential (IAA) and the low genetic damage index (IGP) in the tissues from the Partizanskaya River, which is evidence of the stable operation of all body systems under stressful conditions and their adaptation to such conditions. The authors made similar observations through the example of *Crenomytilus grayanus* living in the chronically polluted Desantnaya Cove (Peter the Great Gulf, Sea of Japan) where the long-term complex stimulation of biochemical systems effectively adapts protective antioxidant systems and stabilizes the level of final LPO products, including MDA [21].

Mollusks living in the Razdolnaya River and the Tikhaya Lagoon experience both oxidative stress caused by the complex pollution of organic and inorganic compounds and oxygen deficiency. Among pollutants, biogenic substances (inorganic phosphorus, nitrates) and metals dissolved in water (Fe, Zn, Cu, Ni, etc.) are in first place [5]. Agriculturally used areas in the river basin located in both the Russian and Chinese territories is dominant in relation to other sectors of the national economy (up to 41% in the Russian territory). In addition, selitebic areas adjacent to the river basin contribute significantly to its pollution. Waste waters carried by the Razdolnaya River to Amursky Bay are 24.5% of the total amount, whereas pollutants make 41% (20·103 t/year) of their total quantity [2, 22 23].

By analyzing the results, we conclude that the antioxidant protective system of St. 2 and 3 is in the most depressive state when compared to that from the Partizanskaya River. The low activity of an antiradical component and the high genetic damage index in the tissues of *C. japonica* are due to complex organic and inorganic types of pollution caused to the Razdolnaya River, coupled with the eutrophication of waters and hypoxic phenomena specific to this area.

Thus, the results show that *C. japonica* from all the stations under study experience chronic oxidative stress caused by pollution of the rivers of the Peter the Great Bay. The proposed markers clearly reflect the state of *C. japonica* in the biotopes of different pollution nature, thereby confirming their suitability as indicators in the assessment of estuarine pollution.

4. Conclusion

The studies show that the highest concentrations of iron, zinc and lead are observed in the tissues of *C. japonica* from the Partizanskaya River, the highest concentrations of copper and cadmium are found in the tissues of the individuals from the Razdolnaya River. It is found that chronic oxidative stress is observed in all the estuaries under study. In the Partizanskaya River *C. japonica* experiences oxidative stress induced by high iron and lead contents; in the Razdolnaya River and the Tikhaya River the stress is due to complex organic and inorganic types of pollution coupled with the eutrophication of waters and hypoxic phenomena specific to this area. The proposed biomarkers clearly show the state of *C. japonica* in the biotopes of different pollution levels, indicating that the worst state is attributable to the estuary of the Razdolnaya River.

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