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ОПЫТ ЭКОЛОГИЧЕСКОЙ ОЦЕНКИ СТАТУСА АКВАТОРИЙ НА ОСНОВЕ ДИАГНОСТИКИ АДАПТИВНОГО ПОТЕНЦИАЛА НЕКОТОРЫХ БЕСПОЗВОНОЧНЫХ ЖИВОТНЫХ ИЗ СУБ-РЕГИОНОВ БАЛТИЙСКОГО МОРЯ

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Цель данной работы состояла в том, чтобы сделать обзор наших результатов по оценкам загрязнения некоторых проблемных акваторий суб-регионов Балтийского моря, полученных в ходе проведенных ранее полевых и лабораторных исследований на основе использования разработанной в НИЦЭБ РАН биоэлектронной системы мониторинга кардиоактивности беспозвоночных животных. Статья также посвящена опыту развития и апробации предложенного подхода к оценке биологических эффектов химического загрязнения окружающей среды на основе оценки адаптивного потенциала местных видов беспозвоночных, обитающих в разных по антропогенной нагрузке пресноводных, солоноводных и морских акваториях Балтийского моря, а также Невской губе и реке Неве. Оценка адаптивных возможностей животных выполнялась, используя метод функциональной нагрузки на двустворчатых моллюсков (*Anodonta anatina, Mytilus edulis, Mytilus trossulus и Macoma* (*Limecola) balthica*) и высших раков (*Carcinus maenas и Astacus leptodactylus*), на основе измерения времени восстановления сердечного ритма после снятия нагрузки. Как показали исследования, быстрое восстановление (меньше чем 50—60 мин) соответствует высокому уровню адаптивного потенциала этих видов животных, их хорошее функциональное состояние, указывая на хороший экологический статус места исследования, в котором живут эти животные. В работе рассмотрен ряд примеров, демонстрирующих, как предложенная авторами методология оценки функционального состояния беспозвоночных может использоваться в экологической оценке состояния (здоровья) экосистем акваторий Балтийского региона.

Ключевые слова: Суб-регионы Балтийского моря, хроническое загрязнение, биоэлектронные системы, восстановление частоты сердечных сокращений, двустворчатые моллюски, крабы, раки.

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EXPERIENCE ON ECOLOGICAL STATUS ASSESSMENT BASED ON ADAPTIVE POTENTIAL DIAGNOSTICS IN SELECTED INVERTEBRATES OF THE BALTIC SEA SUB-REGIONS

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The aim of the present paper was to review the results obtained in our previous field and laboratory studies using cardiac monitoring system worked out in SRCES RAS for assessing of environmental pollution in a few problem aquatoria of the Baltic Sea Region. Paper is concerned also to the experience of development and approbation of proposed approach to the assessment of biological effects of environmental chemical stress based on evaluation of adaptive potential of indigenous species of the invertebrates from different in anthropogenic pressure fresh water, brackish water or marine areas, with the emphasize on sub- regions of the Baltic Sea (including estuary of the Neva River). The assessment of

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adaptivity was performed using method of physiological loading on bivalve mollusks (*Anodonta anatina, Mytilus edulis, Mytilus trossulus and Macoma* (*Limecola*) *balthica*) and anti-orthostatic test in crustacean (*Carcinus maenas* and *Astacus leptodactylus*), based on measuring the heart rate recovery time after removal of stress load. Rapid recovery (less than 50—60 min) signifies a good adaptive potential in different species, indicating good ecological status of the study site they inhabit. The paper presents a number of examples demonstrating how methodology for the evaluation of invertebrate's physiological state can be used in ecosystem health assessment in the Baltic Sea Region.

Key words: Baltic Sea Sub-regions, chronic pollution, bioelectronic systems, heart rate recovery, bivalve mollusks, crabs, crayfish.

Introduction. In modern conditions of escalating anthropogenic impact on the environment, and especially on water ecosystems, there was a need of combination of efforts of a number of the European countries to definition of clear general policy in the field of the strategy of assessment of a condition of the seas and oceans and the strategy of sea researches [1] of the European Parliament and Committee of June 17, 2008 within the Directive of Sea Frame Strategy (Marine Strategy Framework Directive — MSFD). MSFD aims to achieve Good Environmental Status (GES) of the EU's marine waters by 2020 and to protect the resource base upon which marine- related economic and social activities depend. Baltic Sea Action Plan (2007) [2, 3] identifies pollution by hazardous substances as one of the four main issues to improve the health of the Baltic Sea. The Action Plan set a strategic goal related to hazardous substances which is "Baltic Sea with life undisturbed by hazardous substances", and identified a set of Ecological Objectives which corresponds to good environmental status" [4].

Staying on this, the criteria for the achievement of good environmental status are the starting point for the development of coherent approaches in the preparatory stages of marine strategies, including the determination of characteristics of a good environmental status. The modern methods of assessment of anthropogenic impacts on the environment entered into practice need complex efforts on their improvement and development of new technologies and methodological approaches. To achieve this, the development and implementation of "on-line on-site" methods for the assessment of the ecological status of marine areas is a feasible strategy. One approach to identify changes in the aquatic environment potentially leading to threats to the whole ecosystem is the assessment of the physiological status of native for study sites organisms.

However, till now, the fundamental problem in biomonitoring of the Baltic Sea Region is the absence of uniform approaches and evaluation methods of an ecological status (the ecological status is quantitative assessment of a deviation of a controlled water ecosystem from her natural "undisturbed" state) assessment of its water areas significantly differing on ecological conditions, salinity, hypoxia, hydrology, etc. Eutrophication and chemical pollution are typical threats to the ecosystem of the Baltic Sea Regions [5, 6] that can reduce habitat quality, especially for sensitive target species as representatives of indigenous macrozoobenthic communities.

The Baltic Sea is characterized by a significant gradient of water salinity with the majority of its areas being in the so-called critical salinity zone that defines non-uniformity in the distribution of specific fauna and the quantitative indices living in here aquatic organisms. In these cases, effects of environmental pollution on biota health can become stronger and have reflection in biomarker responses to various stress actions.

Regardless of the level of anthropogenic loading, the gradient of salinity can influence essentially, in particular, on physiological condition of a local macrozoobenthos and structure of its communities that causes a certain difficulty in application for this region of the majority of bioindication methods for assessing ecosystem health, especially, in coastal areas of the Gulf of Finland (GoF) from the Neva Bay to a traverse of Helsinki-Tallinn [5—7].

The main features of the approach we are going to discuss here are:

— selection of relevant species for applying developed cardiac monitoring system taking into account also relevant species for caging exposure;

— identify regions as well as specific sites in the Baltic Sea Region, where selected representatives of indigenous biota are significantly stressed by such environmental factor as salinity gradient as well as environmental pollution;

— how to determine the threshold for the assessment of effects of toxic exposure, quantify the degree of sub-lethal stress and

— provide an approach for ranging of aquatic ecosystem states based on cardiac responses to standard stress stimuli and recovery time in selected invertebrates as an addition to much of the contaminant data.

Biological Early warning systems (BEWSs). Biological responses of organisms are commonly accepted to be useful ecological quality indicators. The most effective for the solution of these problems could be the application of biosensor systems of the automated monitoring of surface water quality. The animals in such a systems are used as biosensors as they are directly included in structure of electronic system of registration of these or those behavioral (valve movements in bivalves) biomarkers (e.g., [8—10]), physiological markers indicating chemical stress in target species [11—13]. These complexes, unlike physical and chemical methods, allow receiving in real time the integrated toxicological characteristic of the environment irrespective of the nature and composition of pollutants, and to estimate quality of water as habitat of aquatic organism [12—14].

The systems of valve gape monitoring in mussels and clams are widely and successfully used for control of water quality in sites of concern, in cities and industrial enterprises, on water intakes, in zones of an intensive recreation (e.g., [5, 6, 11]). Such complexes form a basis of systems of early warning (or detection) — Biological Early Warning Systems (BEWSs), which are intensively developed in the leading countries round the world and are a necessary component of obligatory complex monitoring of the water environment. The systems developed in the Europe are based mainly on the defense behavioral reaction of mussels (sea and freshwater) — Musselmonitor and Dreissena-Monitor, respectively (http://www.mosselmonitor.nl; http://www.mermayde.nl; [8, 9, 11]) to close their valves during exposure to polluting agents or their ability to change rhythmic of valve movements as a response to sub-lethal exposure to factors of various modalities, including chemical toxic substances.

However, in the territory of the countries of the former Soviet Union data on use of biosensor systems of monitoring of the water environment of data isn't available. The only exception is the system of monitoring of the incoming water toxicity installed and operating in real time since December 2005 until now at water supply stations of St. Petersburg, whereas bioelectronic system for cardiac activity monitoring in crayfish has been used [14]. The latter system is shown in fig. 1, which presents a block-scheme for cardiac activity registration, signal transformation and automatic data processing in real time.

Systems (a few modifications) for cardiac activity monitoring in selected invertebrates have been used also in environmental studies, both in laboratory and field investigations, for more than 20 years [10—12, 15]. Modified versions of the system are in the development till nowadays [16—19].

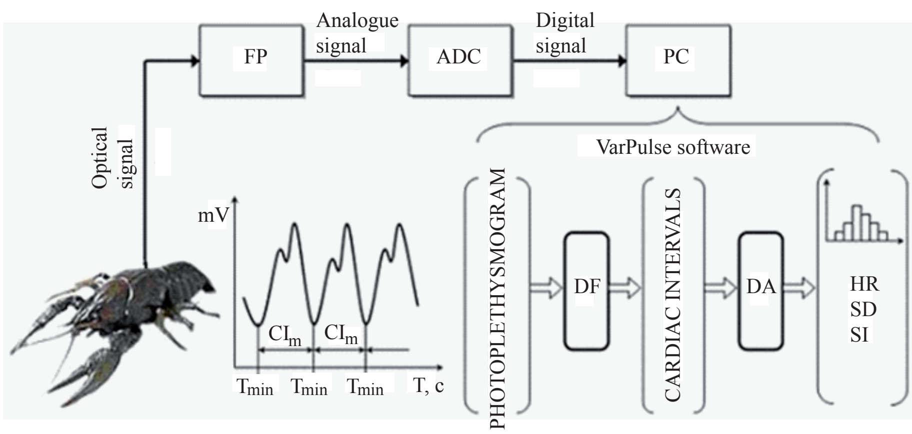

Fig. 1. Experimental set-up and stages of data processing for a sample of cardiac intervals to obtain cardiac activity characteristics of test organisms.
Abbreviations. FP — laser fiber-optic plethysmograph; ADC — analog-to-digital converter; PC — personal computer; DF — digital filter, DA — digital analysis; CI_m — mean interpulse cardiac interval; HR — heart rate; SD — standard deviation of the HR, SI — stress index.

Рис. 1. Экспериментальная установка и основные этапы обработки данных для оценки характеристик кардиоактивности тест-организмов.
Обозначения: FP — лазерный волоконно-оптический плетизмограф; ADC — аналого-цифровой преобразователь; PC – персональный компьютер; DF — цифровой фильтр; DA — цифровой анализ; CI_m — средний междударный кардиоинтервал; HR — ЧСС; SD — стандартное отклонение; HR — ЧСС; SI — стресс-индекс.

Cisar and co-workers (2018) [19] introduced an original system for crayfish heart beat monitoring based on completely fully contactless hardware. The system can determine crayfish heart beat frequency using only the combination of near infra-red illuminator and sensitive camera. But the system has also some disadvantages in use because of the disturbances which could be occurred in water-flow and some other limitations.

Thus, the approach using cardiac monitoring systems in freshwater, brackish waters or in marine, especially, coastal areas is widely used in Europe in the assessment of acute toxicity of surface waters. It was stressed by Gunatilaka and Diehl (2000) that: "The effectiveness and successes of protection measures resulted from among others, a continuous water quality monitoring net built along the Rhine and its tributaries where trans-boundary monitoring, vigilance and peer-pressure has played an important role" [20]. The combined measurement of cardiac activity and body burden has been successfully extended and applied over the coastline and estuarine zones of the British Sea in the UK, in different sub-regions of the Baltic Sea [13, 21].

Selection of relevant species for the assessment of the ecological state of studied areas in the Baltic Sea. Benthic invertebrates are often used as indicators to detect and monitor environmental changes along pollution gradients, because of their integrated responses to long-term natural and/or anthropogenic stresses (e.g., [7, 21, 22]). For the development of automated biomonitoring systems on the basis of behavioral reactions of mussels and/or clams, data on the biology, behavior in the native habitat are defining. Biomarkers of chronic exposure to a mixture of polluting agents (the situation usually we have in environmental studies) in mussels are fast rise of duration of the periods of staying with closed valves, reduction of amplitude of opening state, change in number and frequency of adductions, fast disruption of circadian rhythmicity in valve activity or cardiac rhythm in mussels [21—24].

At the same time, it's essentially important for environmental assessment to use native species as live biomonitors of natural (or surficial) water quality or other environmental challenges in ecosystem they inhabit.

It is known that chemical and physical stresses in aquatic ecosystems can modify ecosystem causing a reduction of sensitive species and an increase in the number of tolerant species [6, 25]. Another specific point at that issue is than using native biota representatives we must choose not high sensitive species, but they also ought to be no high tolerance to the environmental quality changes. At the case with high sensitivity of bioindicator species the system can indicate stress in the situation, when the real pollution level at the site could not cause the drift of the community. It is known that *Mytilus edulis* (as well as *Mytilus trossulus*) is probably relatively tolerant to a range of heavy metal contamination, e.g., Cu, Zn, Mn [13]. Widdows and Donkin (1992) [26] noted that lethal responses give a false impression of high tolerance of *Mytilus*, since adults can close their valves and isolate themselves from adverse environment for days. Analogous behavior bivalves demonstrate in the presence of high concentrations of poly-aromatic hydrocarbons up to 125±28 μg/l of water accommodated diesel oil [27].

The majority of recommended bioindicator species belong to taxonomical types Mollusca and Crustacea, which sensitivity (and more or less tolerance, which provide their survival) to environmental pollution have been well-known. It is established, for example, that mussels and clams are subjected to influence of ions of copper, cadmium, mercury, lead, zinc, surfactants, also some other polluting agents, which cause their inactivation, by closing of their valves, i.e. isolation from the adverse environment [23, 24, 27—29].

The experience of applying the bioelectronics system in fresh water areas and in sub-regions of the Baltic Sea. In the end of 2005 the bioelectronics system [14] has been developed and installed at 11 water supply stations in Saint-Petersburg for on-line water toxicity continuous monitoring in flow-through systems. The freshwater crayfish at such a system has been served as a primary sensing element - live monitors, of water quality.

Since 2010 [30] crayfish have been also used for the control of toxicity of biologically treated effluent (BTE) dumped by South-West Waste Treatment Plant SUE Vodokanal of St.-Petersburg into the Neva Bay, which refers to commercial fishing waters of I category. The practical significance of this biomonitoring system based on BioArgus-WW is as follows:

— as the information basis of an automated system for continuous displaying objective data on safety of BTE discharged into the Neva Bay to interested state authorities and the public;

— as an efficient information and measuring system for preparing decision-making on timely adjustments of wastewater process modes;

— as a tool for the accumulation and storage of objective data showing how to improve the existing regulatory requirements to BTE quality both in terms of the nomenclature and contaminant level.

To develop our approach (in the frame of the “GoF Year 2014”), the research with crayfish cardiac monitoring system was conducted in summer 2014 in the Tallinn Bay (on the base of MSI TTU, Tallinn, Estonia). The aim was to clarify the principle possibility of realization of the system for real time biomonitoring of the GoF brackish waters in combined use with FerryBox physico-chemical measuring system of water [5]. One of the main objective of the study was to investigate whether freshwater crayfish, *Astacus leptodactylus* Esch. could be useful as a test organisms for assessing biologically dangerous changes in the brackish water quality. The changes in the water quality may be caused by pollution of technogenic origin and/or of natural origin, for example, by toxic metabolites during algal blooming in the summer period. Experiment directed on studying of chronic effects of increased salinity (6.5 ‰) on crayfish, *Astacus leptodactylus* Esch., (survival and evaluation of physiological condition) was carried out on previously selected group of healthy crayfish with similar morphometric characteristics and well-expressed circadian rhythm of cardiac activity (as a sign of healthy crayfish). It was revealed that, within a 40-days of exposure in salted water, crayfish (n = 7) didn't show any disturbances in circadian rhythm in HR. Results lead to conclusion of the possibility to use crayfish *Astacus leptodactylus* as test organisms in the system of brackish water biomonitoring The results of preliminary researches confirmed technical capability of creation of such system [31]. In addition, results lead to conclusion of the possibility to use freshwater crayfish *Astacus leptodactylus* as a test organism in monitoring system for assessing brackish waters quality of the Gulf of Finland.

The applicability of the bioelectronic system of heart rate monitoring and above mentioned methodology for the assessing species health status were tested in different sub-regions of the Baltic Sea: in the Belt Sea (western Baltic Sea Region), in the Bothnian Sea, the Riga Bay, the Tallinn Bay, in the Gulf of Finland.

The data on T_{rec} and CV_{HR}, as biomarkers of environmental contamination [32], were examined **in the Belt Sea** (western Baltic Sea) as a part of the BONUS-BEAST project studies in two key species of marine invertebrates - blue mussel (*Mytilus edulis* L.) and shore crab (*Carcinus maenas* L.). Animals were collected from 2 different in anthropogenic pressure locations. Heart rate was recorded by automated system for non-invasive laser fiber-optic technique. The loading was done to evaluate animal’s compensatory response under standardized test stimulus [33] and to assess adaptive capacities of organism to restore undisturbed (rest) state. In laboratory testing of mussels HR was monitored after standardized water salinity change [33].

In ***crabs*** a 1-h anti-ortostatic suspension test was suggested as a standardized stimulus for testing cardiac response to load [34]. The crabs collected at a clean site demonstrated better adaptive capacities, indicated by maintenance an enhanced HR level during a 1h-exposure to suspended state as an added disturbance (test stimulus). During the testing they expressed a high and stable HR with low variability between the individuals tested. In contrast, crabs collected from polluted sites could not maintain enhanced HR values during the disturbance period. Thus, it was shown that organisms inhabiting polluted sites are more vulnerable to energetic loss for their maintenance and survival in severe conditions of chemical environmental stress [35]. Subsequently, their adaptive capacities are reduced, including resistance to additional stressors. In the present study, this was manifested as a prolonged HR recovery time in mussels collected from a polluted site compared to a far less polluted site. HR recovery time (T_{rec}) after experimental load clearly discriminated between the polluted and the local reference sites [34]. Animals from more polluted sites showed significantly increased T_{rec} values in mussels (216±15 mins) and 81±10 mins in crabs (fig. 2) in comparison with those collected from the local reference site (71±12 mins and 33±7 mins, respectively).

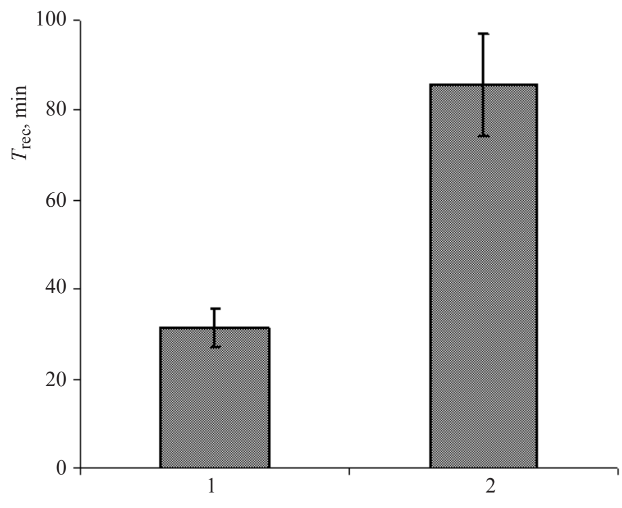

Fig. 2. Recovery time (min) in crabs (n = 7), collected at study stations 1 (2A) and 2 (2F) in the Belt Sea. Mean±SD.

Рис. 2. Время восстановления ЧСС (мин) крабов (n = 7), собранных на разных станциях наблюдения — 1 (2A) и 2 (2F) в Датских проливах Балтийского моря.

For CV_{HR} the values obtained were 7 % in reference site for both tested species, and 24 and 29 %, respectively, for mussels and crabs from moderate sites. Our explanation is that in such case effect of functional loading summarized with chronic chemical stress mediated by contamination level in the area of their habitation. We have seen a prolonged recovery time in mussels collect at a polluted site compared to a fairly pristine site.

In an accompanying study focusing on the health assessment of mussels from the same area using immunological biomarkers [37] the higher phagocytic activity of haemocytes, a measure for the predominant immune defense mechanism was determined at Roskilde Fjord coincided with a reduced T_{rec} (unpublished data). Hence, these observations may provide information on environmental stress. It is feasible to assume that healthy mussels are more likely to recover faster from salinity stress and are more likely to be able to respond to pathogen thread (by phagocytosis).

Similar results [32] were obtained in a caging study conducted **in the northern Baltic Sea**. Mussels were transplanted from a reference site (Hanko, Southwestern Finland) to four differently contaminated sites (S1, S2, G1, G2) near cities Südval and Gävle (Bothnian Sea of the Baltic Sea), with S2 and G2 considered to be local reference sites. It was shown phagocytic activity and T_{rec} slightly correlated as well when omitting the peculiar results of caging site S1. Lowered phagocytic activity was determined in mussels with the highest PCB body burden. Canesi et al. (2003) [38] found that certain PCB congeners indeed have an impact on immune responses and cell signalling pathways, affecting lysozyme release and microbiocidal activity, as well as gene expression.

The applicability of the functional loading method using the bioelectronic system was also approbated in a range of aquatic organisms from different of sites of **the Eastern GoF** with varying salinity from freshwater (0 ‰) in the Neva River estuary to brackish water (3—4 ‰) in the Tallinn Bay (5—6 ‰), using benthic key invertebrates: duck mussel (*Anodonta anatina*) [22] was chosen for the extremely low salinity (0-2, Practical Salinity Scale 1978) in the Eastern GoF, the Baltic mussel *Mytilus trossulus* and the Baltic clam - *Limecola balthica* for the more saline waters (5—6 ‰) found in the Tallinn Bay (the Western GoF) [37].

For freshwater organisms of the GoF, the salted water (as functional loading) was used as dilution medium to adjust required salinity level (6—7 ‰), followed by addition to the tank of a necessary volume of natural water from the site of mollusk's collection, with regard to establish the salinity measured at the sampling site.

Studies in the Eastern GoF showed that the method we used allow to discriminate polluted sites from the reference sites [22]. Testing *A. anatina* showed prolonged T_{rec} after hyper-osmotic test-treatment in mollusks from Petergof and Repino (357±33 mins and 110±15 mins, respectively), instead of those ones collected in Dubki station (reference area [6]) — 45±15 min (ANOVA, $p<0.05$).

The applied for marine species approach and indicative parameter T_{rec} can be used for the assessment of the physiological state of freshwater mollusks from genus Unionidae, namely, — *Anodonta anatina*.

The experience in caged mollusks exposure. Mussel caging has been successfully used to assess environmental stress in coastal environments in many seas areas (e.g., [38—41]. So far, very few studies dealing with the biological effects of WWTP effluents on macroinvertebrates can be found. In the GoF [43] carried out a one-month caging study on *M. trossulus* at sites 0.8 and 1.1 km from the Helsinki WWTP discharge site with a reference site 4 km away. Significant antioxidant, genotoxic and lysosomal responses were observed closer to the WWTP discharge area, coinciding with elevated levels of some contaminants (e.g., pharmaceuticals). Similar to the present study, it was difficult to assess if the negative effects observed were due to the WWTP efflux or other pollution present in the study area under various anthropogenic pressures.

Limecola balthica (former name *Macoma balthica*) has been used as an indicator organism in the assessment of marine pollution and in biomarker responses studies in the Baltic Sea [42—45]. However, no studies are available on the monitoring of cardiac activity in this species. The small size of *L. balthica* complicates cardiac monitoring - it's difficult to fix sensors on their shells. Compared to mussels, physiological responses of *L. balthica* to environmental stressors have been investigated to a lesser extent; therefore, the reasons for the observed differences remain a target for further studies. For example, information is needed on the possible links of cardiac activity with filtration activity and general metabolism in this species.

Using method proposed by the authors of the present paper, the study of biological effects of pollution was performed in Tallinn Bay in clam — *L. balthica*. This species is known to be a sensitive indicator of environmental changes. It is reported that pollution induces cellular oxidative stress in vivo and so lead to adverse health outcome in this mollusks [42]. In regard to *L. balthica*, the following experiment was performed.

The mollusks were collected at reference site near Naissaari Island (Tallinn Bay of the Baltic Sea, Estonia) and deployed in cage near the Tallinn wastewater treatment plant (TWWTP) discharge site [39].

Two examples out of 16 measurements of HR responses after a 10-week exposure near TWWTP were observed. Under the changed salinity some individuals responded by an increased HR, while others showed a decrease in HR. However, after a return to the initial salinity all of them recovered their HR pattern to back to the resting level.

In *L. balthica*, the mean T_{rec} before the caging experiment was 55±7 min while after 2 months of exposure it is increased up to 90±19 min (fig. 3).

Calculation of CV_{HR} failed in this study because of the characteristically for this species irregular fluctuations in HR in this species.

Thus, compared to mussels, physiological responses of *L. balthica* to environmental stressors have been investigated to a lesser extent; therefore, the reasons for the observed differences remain a target for further studies. For example, information is needed on the possible links of cardiac activity with filtration activity and general metabolism in this species.

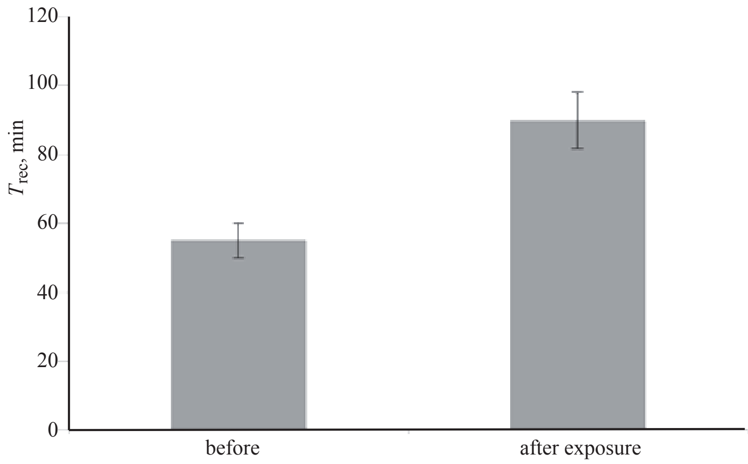

Fig. 3. Heart rate recovery time (min) in *Macoma* (*Limecola*) mollusk before and after 2-months exposure to TWWTP effluents. Mean±SD.

Рис. 3. Время восстановления ЧСС (мин) у моллюска *Macoma* (*Limecola*) до и после 2-х месяцев экспозиции у сброса Таллиннского завода по очистке сточных вод. Среднее±СКО.

In table we summarized the data obtained in our studies in different Sub-regions of the Baltic Sea and the Neva River with the indication of methods applied, species used and references to published data.

Conclusions. Express assessment of ecological state of a water bodies could be obtained by results of integrated assessment of a functional state of benthic invertebrates (on the base of cardiac rhythm monitoring in background conditions (in the absence of an additional stressors) and at the standardized test treatment. However, it is required to add it with more detailed researches accepting in account other biomarker indicators (for example, indicators of an oxidative stress, the index of a state (condition index, Integrated Biological Response), the immunological status of organisms, etc.). All these indicators listed in brackets are obligatory in the system of biological monitoring of water ecosystems of EU countries and are enshrined in the Decision of the Commission of Official Journal of the European Union L 232/14, COMMISSION DECISION of 1 September 2010 on criteria and methodological standards on good environmental status of marine waters (notified under document C(2010) 5956) (Text with EEA relevance) (2010/477/EU), documents of HELCOM (2010, 2012) [1—4].

The method of cardiac activity monitoring detects the effect of a mixture of contaminants presented in the environment and shows impact when the concentration of pollutant do not exceed the allowed limit or are under detection limit. If successfully applied, the method allows seeing the effects of pollution on local biota at a lower level of organismal organization, when survival tests do not reveal any lethal or significant adverse effects.

Sub-regions of the Baltic Sea and the Neva River (including estuary) studied using bioelectronic systems of cardiac monitoring and different methodological approaches

Суб-регионы Балтийского моря и река Нева (включая эстуарий), изучаемые с использованием биоэлектронных систем мониторинга кардиоактивности беспозвоночных с применением различных методологических подходов оценки

Species	Sub-regions of the Baltic Sea	Methods used, biomarkers	References
Astacus leptodactylus	Neva River; Neva Bay	On-line monitoring of water toxicity; HR, ΔHR; ΔHR/Δt	[14]
Astacus leptodactylus	Mesocosm studies; Tallinn Bay	Active bioindication; Trec	[31]
Mytilus trossulus	Tallinn Bay	Caged mussels; Trec	[39]
Macoma (*Limecola*) *balthica*	Tallinn Bay	Caged clams; Trec	[39]
Mytilus edulis	the Belt Sea (western Baltic Sea)	Active bioindication method; Trec ; CV_{HR}	[36]
Carcinus maenas	the Belt Sea (western Baltic Sea)	Active bioindication method; Trec; CV_{HR}	[36]
Mytilus trossulus	the Bothnian Sea	Caged mussels; Trec; CV_{HR}	[21, 32]
Mytilus trossulus	the GoF	Caged mussels; Trec; CV_{HR}	[21]
Limecola balthica	the Gulf of Riga	Active bioindication method; Trec	[46]
Unio pictorum	Rivers and lakes of catchment area of the Gulf of Riga	Active bioindication method; Trec	[46]
Anodonta anatina	Neva Bay; estuary of the Neva River	Active bioindication method; Trec	[22, 39, 47]
Unio pictorum	Neva Bay; estuary of the Neva River	Active bioindication method; Trec	[47]
Dreissena polymorpha	Neva Bay; estuary of the Neva River	Active bioindication method; Trec	[47]

*Active bioindication method — the methodology with the use of functional loading [33, 36].

It has been shown that cardiac monitoring and the assessment of organism's state, by testing adaptive potential using functional load (followed by the recovery), are a sensitive and ecologically meaningful biological response that can provide a powerful, rapid (i.e. results can be obtained within a day of sampling), and cost-effective method for monitoring changes in environmental quality [21, 22, 23]. Therefore, field studies have demonstrated that this approach is able to detect and quantify changes in environmental quality, as well as identify some of the cause(s) of these changes through the use of complex analysis (for example, PCA) (e.g., [7, 32]). In future, it's a real need to search and try to establish a list of cause-effect relationships for a number of hazardous substances and to clarify correlations between contaminant concentrations in mussel tissues and cardiac disturbances caused by chemically induced stress, of course, in that case when it is possible.

Another point we need to outline is the necessity to search a new species, which could be used as indicators of the environmental pollution. Here, Saduria entomon L. (endemic in the Baltic Sea crustacean) could be discussed as a candidate because of it numerous distribution in the Baltic Sea offshore zones. The potential applicability of Saduria entomon L. in cardiac activity monitoring systems was tested in our laboratory (unpublished data).

Cardiac monitoring data provides an instantaneous measure of the energy status of an animal, its general health, which can range from maximum positive values (norm of reaction) under optimal conditions, declining to negative values when an animal is severely stressed and utilize functional reserves. Although direct measurements of total energetic costs under toxic exposure are often difficult, as well as to quantify and interpret impact in relation to pollution [26], cardiac activity monitoring is rapidly determined, providing a sensitive, quantitative, and integrated response of organism to various stress actions [7, 10, 16]. The later approach has been successfully applied in laboratory and mesocosm experiments to assess the toxic effects of hazardous substances on organism status [10, 12, 21, 23].

Field studies conducted in the areas of the Gulf needs taking into account specific features of the ecology of target species from different areas on the basis of biomarkers of pollution, i.e. the responses of local species of the invertebrates, for example, instrumentally measured by testing adaptive potential of the cardiac system of animals in the presence of additional load (a short-term salinity change). In the case of salinity gradient in the sites of concern, one must need to develop zone approach in water areas of transitional type from sea to brackish water and from the brackish to nearly freshwater areas with its specific fauna and characteristics of organism's functioning.

To summarize, taking into account previously discussed, we are ready to propose the common scheme for ecosystem health assessment in the Baltic Sea.

Principles of assessment of ecological trouble (hot spots) in the water bodies and the Gulf of Finland areas could be as follows:

- Assessment based on biological indicators
- Identification of a condition of ecological trouble at emergency and assessment of its degree is proposed to be carried out:

— according to assessment of a functional condition of animals (Mollusca and Crustacean), characterizing changes in ecosystem they live in;

— on biochemical data, in particular, integrated indicators of oxidative stress (antioxidative stress parameters and lipid peroxidation) in these animals;

— according to bio-accumulation of hazardous substances in tissues of animals (especially, in gills and a hepatopancreas);

— according to integrated biological response patterns expressed as Index of Biological Response (IBR), Principle Component Analysis (PCA), Scope of Growth (SG), etc.

Based on the results discussed in the paper, T_{rec} and CV_{HR} in mussels and crabs seemed to be a powerful tool and a useful endpoint for the evaluation of the health of aquatic organisms and, subsequently, the quality of water, where they live. The methodology could be useful also in the assessment of ecosystem health.

Obtained by the authors field and laboratory studies are rather good basis for further work in development and deployment in the Gulf of Finland waters of a net of technologies and automated biomonitoring systems using benthic invertebrates as live monitors of environmental quality, in all spheres of modern water use. The major advantages of the considered system are: high extent of automation of monitoring, easy to create large networks of the continuous, automated control of the water environment, high reliability, simplicity in service, high degree of autonomy, durability and rather low cost.

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Литература

1. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy // Official Journal of the European Communities. 2000. L. 327, 22. 12. 72 p.
2. HELCOM Baltic Sea[2] HELCOM Baltic Sea Action Plan, 2007. HELCOM Ministerial Meeting Krakow, Poland, 15 November 2007, 100 p.
3. HELCOM. 2010. Hazardous substances in the Baltic Sea: An integrated thematic assessment of hazardous substances in the Baltic Sea. HELCOM. Helsinki Commission, 2010.Baltic Sea Environment Proceedings 120B. P. 54—60.
4. HELCOM. 2012. Development of a set of core indicators: Interim report of the HELCOM CORESET project. PART B: Descriptions of the indicators. Baltic Sea Environment Proceedings No. 129 B.
5. *Lips I., Rünk N., Kikas V., Meerits A., Lips U.* High-resolution dynamics of spring bloom in the Gulf of Finland, Baltic Sea // J. Mar. Syst. 2014. V. 129. P. 135—149.
6. *Berezina N. A., Gubelit Y. I., Petukhov V. A., Polyak Y. M., Sharov A. N., Kudryavtseva V. A., Lubimtsev V. A., Shigaeva T. D.* An integrated approach to the assessment of the Eastern Gulf of Finland health: A case study of coastal habitats // J. Mar. Systems. 2017. V. 171. P. 159—171. DOI: 10.1016/j.jmarsys.2016.08.013
7. *Lehtonen K. K., Sundelin B., Lang T., Strand J.* Development of tools for integrated monitoring and assessment of hazardous substances and their biological effects in the Baltic Sea // AMBIO. 2014. V. 43, N1. P. 69—81. DOI: 10.1007/s13280-013-0478-3
8. *Kramer K. J. M., Botterweg J. M.* Aquatic biological early warning systems. Bioindicators and environmental management / D.W. Jeffrey, B. Madden (eds). London: Academic Press, 1991. P. 95—126.
9. *Borcherding J.* Ten years of practical experience with the Dreissena-Monitor, a biological early warning system for continuous water quality monitoring // Hydrobiologia. 2006. V. 556. P. 417—426.
10. *Depledge M. H., Andersen B. B.* A computer-aided physiological monitoring system for continuous, long-term recording of cardiac activity in selected invertebrates // Comp. Biochem. Physiol. A. 1990. V. 96. P. 473—477. http://dx.doi.org/10.1016/0300-9629(90)90664-E.

11. *Kees J. M., Kramer K.J.M., Jenner H. A., de Zwart D.* The valve movement response of mussels: a tool in biological monitoring // Hydrobiologia. December 1989. V. 188, Issue 1. P. 433—443. https://link.springer.com/article/10.1007/BF00027811
12. *Depledge M. H., Aagaard A., Gyorkos P.* Assessment of trace metal toxicity using molecular, physiological and behavioral biomarkers // Mar Poll Bull. 1995. V. 31, N1-3. P. 19—27.
13. *Brown R. J., Galloway T. S., Lowe D., Brown M. A., Dissanayake A., Jones M. B., Depledge M. H.* Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers // Aquat. Toxicol. 2004. V. 66. P. 267—278.
14. *Kholodkevich S. V., Ivanov A. V., Kurakin A. S., Kornienko E. L., Fedotov V. P.* Real time biomonitoring of surface water toxicity level at water supply stations // J. Envir. Bioindicators. 2008. V. 3, N1. P. 23—34.
15. *Baldwin I. G., Kramer K.J.M.* Biological early warning systems (BEWS) / KJM Kramer (ed) // Biomonitoring of Coastal Waters and Estuaries, CRC Press, Boca Raton, 1994. P. 1—28.
16. *Kholodkevich S. V., Kuznetsova T. V., Trusevich V. V., Kurakin A. S., Ivanov A. V.* Peculiarities of valve movements and cardiac activity in bivalve mollusks under different stressors actions // J. Evol. Biochem. Physiol. 2009. V. 45, N 4. P. 524—526.
17. *Burnett N. P., Seabra R., de Pirro M., Wethey D. S.,Woodin S. A., Helmuth B., Zippay M. A., Sarà G., Monaco1 K., Lima F. P.* An improved noninvasive method for measuring heartbeat of intertidal animals // Limnol. Oceanogr.: Methods. 2013. V. 11. P. 91—100. DOI: 10.4319/lom.2013.11.91
18. *Pautsina A., Kuklina I., Štys D., Císař P., Kozák P.* Noninvasive crayfish cardiac activity monitoring system // Limnology and Oceanography: Methods. 2014. V. 12. P. 670—679.
19. *Císař P., Saberioon M., Kozák P., Pautsina A.* Fully contactless system for crayfish heartbeat monitoring: Undisturbed crayfish as bio-indicator // Sensors and Actuators B: Chemical. 2018. V. 255. P. 29—34.
20. *Gunatilaka A., Diehl P.* Brief Review of Chemical and Biological Continuous Monitoring of Rivers in Europe and Asia // Biomonitors and Biomarkers as Indicators of Environmental Change, Volume II' / Eds. Butterworth, Gunatilaka & Gonsebatt. New York: Plenum Press, 2000. P. 9—28.
21. *Kholodkevich S. V., Kuznetsova T. V., Lehtonen K. K., Kurakin A. S.* Experiences on ecological status assessment of the Gulf of Bothnia different sites based on cardiac activity biomarkers of caged mussels (Mytilus edulis) // ICES Annual Science Conference 2011, 19—23 September, Gdansk, Poland. http://www.ices.dk/products/CMdocs/CM-2011/R/R2011.pdf
22. *Холодкевич С. В., Шаров А. Н., Кузнецова Т. В.* Перспективы и проблемы использования биоэлектронных систем в мониторинге экологической безопасности акваторий Финского залива // Региональная экология. 2015. N 2 (37). С. 16—26.
23. *Kuznetsova T. V., Kholodkevich S. V.* Comparative assessment of surface water quality through evaluation of physiological state of bioindicator species: searching a new biomarkers // Proceedings — 2015 4rd Mediterranean Conference on Embedded Computing, MECO. IEEE conference publications ISBN 978-9-9409-4364-6, Budva, Montenegro, 2015. P. 339—344.
24. *Трусевич В. В., Гайский П. В., Кузьмин К. А.* Автоматизированный биомониторинг водной среды с использованием реакций двустворчатых моллюсков // Морской гидрофизический журнал. МГИ НАНУ. Севастополь. 2010. № 3. С. 75—83.
25. *Rosenberg R., Blomqvist M., Nilsson H. C., Cederwall H., Dimming A.* Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within European Union Water Framework Directive // Mar. Pollut. Bull. 2004. V. 49. P. 728—739.
26. *Widdows J., Donkin P.* Mussels and environmental contaminants: bioaccumulation and physiological aspects // The Mussel Mytilus: Ecology, Physiology, Genetics and Culture / ed. E.M. Gosling. Elsevier Science Publishers. B.V., Amsterdam. 1992. P. 383—424.
27. *Abel P. D.* Effect of some pollutants on the filtration rate of Mytilus // Mar. Poll. Bull. 1976. V. 7. P. 228—231.
28. *Davenport J.* A study of the effect of copper applied continuously and dis continuously to specimens of Mytilus edulis (L.) exposed to steady and fluctuating salinity levels // J. Mar.Biol. 1977. Ass. UK 57, 63—74.
29. *Davenport J., Redpath K. J.* Copper and the mussel Mytilus edulis L. // Toxins, Drugs and Pollutants in Marine Animals / Bolis L., Zadunaiy J., Gilles R. (eds). Springer-Verlag, Berlin Heidelberg, 1984, P. 176—189.
30. *Мельник Е. А., Рублевская О. Н., Панкова Г. А., Холодкевич С. В., Иванов А. Б., Корниенко Е. Л., Сладкова С. В., Любимцев В. А., Куракин А. С.* Биоэлектронная система контроля токсикологической безопасности биологически очищенных сточных вод, сбрасываемых Юго-Западными очистными сооружениями ГУП «Водоканал Санкт-Петербурга» в Невскую губу // Водоснабжение и санитарная техника. 2013. № 1. С. 7—12.
31. *Kholodkevich S. V., Kuznetsova T. V., Lips U., Kolesova N., Ljubimtsev V. V., Sladkova S. V.* On the possibility of realization of the system for real time biomonitoring of Gulf of Finland surface water quality in parallel with FerryBox measuring system: preliminary results. Gulf of Finland 2014 Trilateral Seminar, 26-27 November 2014, Helsinki/ gof2014 internet source (http://www.gof2014.fi/en/events/gof-research-forum, p. 17—18).
32. *Turja R., Höher N., Snoeijs P., Baršienė J., Butrimavičienė L., Kuznetsova T., Kholodkevich S. V., Devier M.-H., Budzinski H., Lehtonen K. K.* A multibiomarker approach to the assessment of pollution impacts in two Baltic Sea coastal areas in Sweden using caged mussels (Mytilus trossulus) // Sci. Total Environ. 2014. V. 473—474. P. 398—409.
33. *Kuznetsova T. V.* Change of salinity of medium as a functional loading in estimating physiological state of the crayfish Astacus leptodactylus // J. Evol. Biochem. Physiol. 2013. V. 49, N 5. P. 498—502.
34. *Udalova G. P., Kholodkevich S. V., Fedotov V. P., Kornienko E. L.* Changes in heart rate and circadian cardiac rhythm as physiological biomarkers for estimation of functional state of crayfish Pontastacus leptodactylus Esch. upon acidification of the environment // Inland Water Biology. 2012. V. 5, № 1. P. 119—127.
35. *Kuznetsova T. V., Sladkova S. V., Kholodkevich S. V.* Evaluation of physiological state of crayfish Astacus leptodactylus Esch. in normal and toxic environments // J. Evol. Biochem Physiol. 2010. V. 46, N 3. P. 203—210.
36. *Kholodkevich S. V., Kuznetsova T. V., Lehtonen K. K., Strand J., Kurakin A. S., Kamardin N. N., Kornienko E. L.* Bioelectronic system for monitoring of cardiac activity in mussels and crabs and its implementation to environmental assessment // 8th Baltic Sea Science Congress-2011, 22-26, August, 2011, St. Petersburg, Russia. Book of Abstracts. P. 359.

37. *Höher N., Köhler A., Strand J., Broeg K.* Effects of various pollutant mixtures on immune responses of the blue mussel (Mytilus edulis) collected at a salinity gradient in Danish coastal waters // Mar.Environ.Res. 2012. V. 75. P. 35—44. https://doi.org/10.1016/j.marenvres.2011.11.003
38. *Canesi L., Betti M., Ciacci C., Lorusso L. C., Pruzzo C., Gallo G.* Cell signaling in the immune response of mussel hemocytes // Invertebrate Surviv. Journal. 2006. V. 3. P. 40—49.
39. *Kholodkevich S., Kuznetsova T. V., Sharov A. N., Kurakin A. S., Lips U., Kolesova N., Lehtonen K. K.* Applicability of bioelectronic cardiac monitoring system for the detection of biological effects of pollution in bioindicator species in the Gulf of Finland // J. Mar. Systems. 2017. V. 171. P. 151—158. http://dx.doi.org/10.1016/j.jmarsys.2016.12.005.
40. *Regoli F., Frenzilli G., Bocchett R., Annarumma F., Scarcelli V., Fattorini D., Nigro M.* Time-course variations of oxyradical metabolism, DNA integrity and lysosomal stability in mussels, Mytilus galloprovincialis, during a field translocation experiment // Aquat. Toxicol. 2004. V. 68. P. 167—178.
41. *Serafim A., Lopes B., Company R.* et al. A multi-biomarker approach in cross-transplanted mussels Mytilus galloprovincialis // Ecotoxicol. 2011. V. 20. P. 1959—1974.
42. *Turja R., Soirinsuo A., Budzinski H., Devier M. H., Lehtonen K. K.* Biomarker responses and accumulation of hazardous substances in mussels (Mytilus trossulus) transplanted along a pollution gradient close to an oil terminal in the Gulf of Finland (Baltic Sea) // Comparative Biochemistry and Physiology C. 2013.V. 157. P. 80—92.
43. *Turja R., Lehtonen K. K., Meierjohann A., Brozinski J.-M., Vahtera E., Soirinsuo A., Sokolov A., Snoeijs P., Budzinski H., Devier M.-H., Peluhet L., Pääkkönen J.-P., Viitasalo M., Kronberg L.* The mussel caging approach in assessing biological effects of wastewater treatment plant discharges in the Gulf of Finland (Baltic Sea) // Mar. Pollut. Bull. 2014. V. 473—474. P. 398—409.
44. *Lehtonen K. K., Leiniö S., Schneider R., Leivuori M.* Biomarkers of pollution effects in the bivalves Mytilus edulis and Macoma balthica collected from the southern coast of Finland (Baltic Sea) // Marine Ecology Progress Series. 2006. V. 322. P. 155—168.
45. Putna I., Strode E., Bârda I., Puriòa I., Rimða E., Jansons M., Balode M., Strâíe S. Sediment quality of the eco-region Engure, Gulf of Riga, assessed by using ecotoxicity tests and biomarker responses // Proceedings of the Latvian Academy of Sciences. Section B. 2014. V. 68 (1/2) (688/689). P. 101—111.
46. *Куракин А. С., Холодкевич С. В., Пурвиня С., Барда И., Римша Е., Кулинкович А. В.* Оценка экологического состояния отдельных акваторий Рижского залива и внутренних водоемов Латвии используя метод активной биоиндикации // Журнал «Научно-технические ведомости», серия «Наука и образование». 2012. № 1 (142). С. 267—272.
47. *Шаров А., Холодкевич С.* Некоторые особенности использования мониторинга сердечных сокращений у пресноводных моллюсков с помощью волоконно-оптического метода для экотоксикологических исследований // Принципы экологии. 2015. V. 4, № 2. С. 23—30.