Journal of Marine Systems xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Marine Systems

journal homepage: www.elsevier.com/locate/jmarsys

# Applicability of a bioelectronic cardiac monitoring system for the detection of biological effects of pollution in bioindicator species in the Gulf of Finland

Sergey V. Kholodkevich [a,b], Tatiana V. Kuznetsova [a], Andrey N. Sharov [a,*], Anton S. Kurakin [a], Urmas Lips [c], Natalia Kolesova [c], Kari K. Lehtonen [d]

[a] Saint-Petersburg Scientific Research Center for Ecological Safety, Russian Academy of Sciences, Korpusnaya 18, St Petersburg 197110, Russia
[b] Saint-Petersburg State University, 7-9 Universitetskaya nab., St Petersburg 199034, Russia
[c] Marine Systems Institute, Tallinn University of Technology, Akadeemia tee 15a, 12618 Tallinn, Estonia
[d] Finnish Environment Institute, Hakuninmaantie 6, FI-00430 Helsinki, Finland

A R T I C L E I N F O

*Article history:*
Received 14 April 2016
Received in revised form 15 December 2016
Accepted 19 December 2016
Available online xxxx

*Keywords:*
Gulf of Finland
Biomonitoring
Bioelectronic systems
Heart rate recovery cardiac activity
Bivalve mollusks
Pollution

A B S T R A C T

Field testing of an innovative technology based on a bioelectronic cardiac monitoring system was carried out in the Gulf of Finland (Baltic Sea).
The study shows that the bioelectronic system is suitable for the selected bivalve mollusks *Mytilus trossulus*, *Macoma balthica* and *Anodonta anatina*. Specimens taken from reference sites demonstrated a heart rate recovery time of <60 min after testing with changed salinity load, while those collected from sites characterized by high anthropogenic pressure demonstrated a prolonged recovery time of up to 110–360 min. These results make possible a discrimination of the study sites based on the assessment of physiological adaptive capacities of inhabiting species. In addition, the approach of measuring heart rate characteristics in *M. balthica* transplanted in cages to specific target areas was successfully used to evaluate the decline in the adaptive potential of mollusks exposed at polluted sites.
Application of the novel system is a useful tool for the biomonitoring of freshwater and brackish water areas. Development of methodological basis for the testing of adaptive capacities (health) of key aquatic organisms provides new knowledge of biological effects of anthropogenic chemical stress in aquatic organisms.


## 1. Introduction

Maintenance of a good quality of surface waters to ensure their ecological safety for human use and the living biota is currently a major issue globally. To achieve this, the development of biological “early warning” technologies for monitoring of aquatic systems exposed to anthropogenic pressures is of high priority. These technologies are highly useful in the early detection of emergency situations (chemical accidents, oil spills, unauthorized dumping of untreated waters, illegal emissions from industry, etc.), which are likely to cause serious hazards for the aquatic environment as well as human populations.

In the Gulf of Finland (GoF; northeastern Baltic Sea), high anthropogenic pressures influencing all parts of the marine area impose special requirements for the rapidity of detection and identification of undesirable effects as well as the subsequent decision-making process for the implementation of adequate protection measures to ensure ecological safety of the region. 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 ecosystem is the assessment of the physiological status of native organisms. Various aquatic species have been used for biomonitoring in different freshwater and marine environments, including the Baltic Sea (e.g., Lehtonen et al., 2014), and their biological responses are commonly accepted to be useful ecological quality indicators. By using biological effect methods it is possible to take into account the cumulative effects of all the influencing factors in an integrative way to reveal and, to some extent, predict any negative changes in habitat water quality. In addition, it is essentially important to apply representatives of local biota for biomonitoring, in this way ensuring “ecological compliance”, i.e., the conditions prevailing in the ecosystem being favourable for the inhabiting biota (Handy and Depledge, 1999).

One approach to examine the health of organisms is the use of bioelectronic methods that record physiological parameters. In biosensor systems, the test animals applied are included directly in the system structure as primary converters; thus, they are an integral part of an electronic recording system of certain physiological and behavioural

* Corresponding author.
*E-mail address:* sharov_an@mail.ru (A.N. Sharov).

http://dx.doi.org/10.1016/j.jmarsys.2016.12.005
0924-7963/© 2016 Elsevier B.V. All rights reserved.

parameters reflecting the integrated response of animals to changes in environmental conditions (Depledge et al., 1995; Depledge and Galloway, 2005; Kholodkevich et al., 2008). An essential benefit of biosensor methodologies is the application of rapid, integrated criteria to assess biological effects of contamination and their use in on-line monitoring (Kholodkevich et al., 2011; Kuznetsova and Kholodkevich, 2015).

In the GoF, marked gradients in major environmental factors such as salinity and hypoxia determine to a large extent the spatial distribution of fauna, which is often very heterogeneous in the region. Half of the GoF consists of a so-called "critical salinity" zone (Telesh and Khlebovich, 2010) where aquatic organisms of both freshwater and marine origin are found in a weakened physiological state due to the prevailing suboptimal salinity conditions.

In the present study, different species in different parts of the GoF were used for the testing of a biosensor system. The freshwater duck mussel (*Anodonta anatina*) was chosen for the extremely low salinity (0–2, Practical Salinity Scale 1978) eastern GoF, and the Baltic mussel *Mytilus trossulus* and the Baltic clam *Macoma balthica* for the more saline waters (5–6) found in the Tallinn Bay (western GoF). All three are key species in their respective habitats. By filtering (*M. trossulus*) or filtering and deposit feeding (*A. anatina* and *M. balthica*) they process large amounts of water and take up particles, and therefore also a large variety of soluble and particle-bound contaminants. At the individual level, the measuring of molecular, cellular, physiological and behavioural responses, the so-called biomarkers, to environmental challenges allows for the assessment of biological effects of contaminants in biota and, to some extent, to predict the sustainability of populations (e.g., Lehtonen et al., 2006). In particular, physiological biomarkers (e.g., characteristics of cardiac activity, respiration, physical activity) are effective tools for bioindication of water quality as they reflect the integrated response of an organism to contamination (Depledge et al., 1995; Hagger et al., 2009; Kholodkevich et al., 2011; Kuznetsova et al., 2010), making possible to obtain information on the ecological status of ecosystems ("ecosystem health") at the organism level (Depledge and Andersen, 1990; Depledge and Galloway, 2005).

During the BEAST project of the Baltic Sea BONUS programme (Lehtonen et al., 2014), a battery of biomarkers were applied in the Baltic Sea, some of them for the first time , (Kholodkevich et al., 2011; Turja et al., 2014). Among these, the time of adaptive heart rate recovery to the background pattern after the introduction and subsequent removal of an additional short-term stress factor (Kholodkevich et al., 2011; Kuznetsova and Kholodkevich, 2015) was applied. Later on, results from the studies above were considered to examine the applicability of the method in the GoF. In this region, regular environmental monitoring has been conducted already for a long period (Telesh et al., 2008; Lips and Lips, 2008; Lips et al., 2014), but, as in most of the Baltic Sea, monitoring of biological effects of contaminants has not been a part of the programme, largely due to the lack of suitable and validated methodologies (Lehtonen et al., 2006, 2014).

The present study was carried out in the frame of the trilateral (Estonia, Finland and Russia) "Gulf of Finland Year 2014" research programme, and had the following specific objectives:

- to study the applicability of a bioelectronic system for the bioindication of water quality in different areas of the GoF characterized by varying levels and types of anthropogenic pressures;
- to select and test local macrozoobenthos species to be used as biosensor organisms using the technology developed;
- to study physiological responses of cardiac systems of the selected organisms periodically exposed to additional stress factors.

The results of the project were foreseen to promote the development of technologies for the detection and indication of biological effects of pollution globally but also especially concerning the specific characteristics of the GoF ecosystem.

## 2. Material and methods

### 2.1. Study sites and sampling

The applicability of the method was tested in different areas of the GoF with varying salinity from freshwater in the Neva estuary to brackish water (5–6) in the Tallinn Bay, and using local benthic key species (Fig. 1).

In the eastern GoF study area, the experiments were conducted using *A. anatina*, sampled (n = 16) in the shallow waters of the Kurortny District (Sestroretsk, Dubki Park, Repino, Komarovo) and Peterhof on the eastern shore (Fig. 1B). On the basis of a number of hydrobiological and hydrochemical indicators, the water area around Dubki Park can be considered as a reference site for the Neva Bay estuary whereas the Peterhof area is significantly contaminated by anthropogenic activities (Telesh et al., 2008).

Monthly samples (May to September 2014) of 10–16 individual *A. anatina* of 70–80 mm in length were collected. Within 1–3 h after collecting the mussels were brought to the laboratory in 10 l plastic isothermal containers. In the laboratory, fiber optical sensors of 3 mm in diameter were glued on the shells of the mussels in the area of heart projection. The mussels were then kept in an aquarium with natural water from the sampling site with constant aeration. After a 1–2 h period of stabilization of the heart rate (HR) to the level corresponding to that recorded during active filtering (open shells), the testing of their physiological condition was initiated by a so-called functional load method; in the case of *A. anatina* this signified the increase of water salinity up to 6 with a subsequent return to the ambient one (Kholodkevich et al., 2015). All the individuals survived the test procedure and were then returned to their natural habitats.

*M. trossulus* and *M. balthica* were collected from their respective reference sites, the Lahepere Bay and the Naissaar Island (Fig. 1A). One hundred and twenty individuals of *M. trossulus* were collected by diving from the depth of 3–4 m from a stony substrate. *M. balthica* (130 individuals) were collected by dredging from the depth of 43 m. Only undamaged individuals were selected for the testing with at least 48 individuals of each species, and the fiber optical sensors were glued on their shells. The collected specimens were transported to the laboratory in isothermal boxes with ambient water. In the laboratory they were kept in tanks in a climate room at 7–10 °C in aerated water collected from their habitats and under a 12 L:12D (light:dark) illumination regime.

After testing the cardiac activity baselines for each individual (see below), the bivalves (a total of 48 individuals of each species) were divided into two groups and deployed in cages for 10 weeks at a reference and a contaminated site in the Tallinn Bay region (Fig. 1A). The infaunal species *M. balthica* were deployed in cages placed in plastic boxes with sediment from the collection site.

### 2.2. The bioelectronic system for the measuring of cardiac activity in mollusks

In 1999, the laser fiber optical photoplethysmograph (Fedotov et al., 2000; Kholodkevich et al., 2008) was developed in the Laboratory of Experimental Ecology of Aquatic Systems of the St. Petersburg Research Center for Ecological Safety of the Russian Academy of Sciences. In 2003–2004, a software was designed to enable real-time estimation of the physiological stress level in benthic invertebrates in the presence of chemical stressors. A limitation to the method is the requirement to use only animals with an external skeleton (crayfish, crabs and shelled mollusks) to which a small sensor (<2 g of weight) can be attached for the registration of cardiac activity (Fedotov et al., 2000; Kholodkevich et al., 2007, 2008). In the papers above it has been demonstrated that the attached sensor does not exert any impacts on normal activities on any of the groups of species tested; animals with a fixed sensor remained in a normal physiological state for several

Please cite this article as: Kholodkevich, S.V., et al., Applicability of a bioelectronic cardiac monitoring system for the detection of biological effects of pollution in bioindicator sp..., J. Mar. Syst. (2016), http://dx.doi.org/10.1016/j.jmarsys.2016.12.005