CHARACTERISTICS OF CRAYFISH CARDIAC ACTIVITY AS BIOMARKERS OF HABITAT QUALITY

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INTRODUCTION

In connection with constantly growing anthropogenous load on the water objects it is more sharply felt the necessity of development and creation the system, including continuous monitoring of the water environment and an operative water quality control. These methods should be non-invasive, i.e. exclude mass hydrobionts mortification. On the whole biological methods of water quality control are considered to be the most informative, because they allow to give an integrated estimation of a complex influence of different components and factors of the water environment on its population and to estimate the biological effect of the reservoirs pollution by the alive organisms' reactions - biomarkers. The best way to see the dynamics of the water quality changes as a habitat of hydrobionts is to arrange the continuous biomonitoring, particularly tracing changes of physiological characteristics of bioindicators. Perspective branch of such ways of the water quality control is the method based on the registration of the hydrobionts' physiological parameters. The most suitable objects for biomonitoring based on non-invasive cardiac activity registration methods are found the Decapoda order representatives (Aagard et al., 1991). The serviceability of these animals is determined by the presence of tough carapace, which allows to set tightly a sensor on its surface for cardiac activity registration. The group of biological methods based on revelation of ecotoxicological biomarkers is of significant interest for continuous monitoring of water systems' state (Depledge et al., 1995). Elaboration of non-invasive methods of hydrobionts' physiological parameters registration in the real time regime led to the development of system for non-invasive cardiac activity registration of such carapace animals as crayfish (Fedotov et al., 2000). Owing to improved model, this system of water quality control was set at the water intake stations of the State Unitary Enterprise "Vodokanal of Saint-Petersburg".

MATERIALS AND METHODS

In the given work was used the non-invasive instrumental bioanalytical method of the water quality estimation, developed in the laboratory of experimental ecology of water systems of the Saint-Petersburg Scientific Research Center for Ecological Safety of the Russian Academy of Sciences. In a basis of the method lays crayfish heart activity registration with the help of fixed on their carapace fibre optical sensor. The infra-red light beam initially formed in the laser fiber-optic photoplethysmograph (LFOP) is transmitted to the animal by a thin optical fiber with a small sensor attached to the carapace, thus illuminating the heart area with a scattered light. The optical signal modulated by the heart of an animal contains information on cardiac activity. After appropriate amplification and filtration in the LFOP, the analog signal is then transmitted to the analogous digital converter (ADC) and then it is sent to the personal computer (PC) via USB port. As a result one obtains a photoplethysmogram, which can be further analyzed by various mathematical and statistical methods. The method allows to estimate the functional state not only of adults, but also of animals during their early ontogenesis (Fedotov et al., 2002 a). Various crayfish functional states are determined by the features of the cardiac activity, in particular, by the changes of time and amplitude characteristics of the cardiac cycles (Fedotov et al., 2002 b).

EXPERIMENT

<u>Sewage water quality control</u>

Experimental data were obtained during the period from April, 2002 to July, 2005. Cardiac activity registration and decoding of adult animals was carried out on two compared groups of crayfish species *Procambarus clarkii (Girard)*, consisting of ten mature animals each. Exactly crayfish *Procambarus clarkii (Girard)* were chosen as bioindicators as they are well adapted to the maintenance and cultivation in the artificial conditions, that does not render any negative influence on their functional state and fruitfulness, they are capable to maintain fluctuations of the water temperature in a wide range (from 10 up to 28°C) without a change of the functional state at any stage of ontogenesis, they easily live in water with low oxygen content (4-6 mg O_2/l) and pH about 5,8-8,2 and also they are well investigated objects from the point of view of physiology and behavior (Baldridge, Huffman, 1993).

One group was located constantly in aquariums with a stream of biologically purified sewage water at the Northern Station of Aeration of the State Unitary Enterprise "Vodokanal of Saint-Petersburg", another one - in aquariums with conditionally clean water in the laboratory. For animals' maintenece was developed and created a special system with experimental aquariums, providing as the individual keeping, so the maintenance of crayfish family in the stream of biologically purified sewage water.

Heart rate of animals in both groups was registered in the rest and stress functional states. Cardiac activity of each animal was registered with 3 reiterations. Crayfish stress functional state was achieved with the help of their hanger on optical fibres above the bottom in water for 1 hour, that brought animals to the stable stress state. The record of crayfish cardiac activity was carried out three times within one hour during 10 minutes. Further these data were averaged, on the graph they are called the first, the second and the third points of stress accordingly. Besides the heart rate such statistical parameters for quantitative cardiogram estimation as standard deviation and stress-index were counted.

Cardiac activity registration and processing of juvenile crayfish were carried out on descendants of 3 crayfish generations, which were maintained under the same conditions as the adults. From every generation were taken 10 juvenile crayfish in the age of 60 days from each testing group.

Besides the water quality bioindication made by physiological parameters, changes of total body lenght of juvenile crayfish from 3 generations was traced. Metering were made on the 15-th, 30-th and 60-th day after the juveniles hatch with a help of plotting paper and trammel.

Statistical processing of received data was carried out using standard algorithms. Calculated average values were compared with a help of Student criterion. During the statistical processing the standard significance level was used (α = 0,05).

Data from physical-chemical water analysis also were analyzed.

RESULTS

Results received after processing the cardiograms are the following: average values of the heart rate of adult crayfish in the rest functional state, taking place in conditionally clean water, have come to 63,2 ± 2,8 beats/minute. Similar values of the heart rate of crayfish are considered to be normal for the given functional state. At the same time the average values of the heart rate of crayfish in the rest functional state, taking place in the biologically purified sewage water, have come to 82,5 ± 1,9 beats/minute at other equal status. Such values of the heart rate are increased in comparison with norm and rather characterize the stress functional state.

Average values of the heart rate of juvenile crayfish at the rest functional state, taking place in conditionally clean water and in the biologically purified sewage water at other equal status, have come to 181 ± 17 beats/minute and 172 ± 12 beats/minute, respectively. At the stress functional state average heart rate values of selfsame juvenile crayfish have come to 200 ± 21 beats/minute and 204 ± 6 beats/minute being in conditionally clean water and in the biologically purified sewage water, respectively.

Total body length measuring after 15 and 30 days of juvenile crayfish hatch doesn't show significant differences as between different generations of one group, so between two compared groups. Measuring total body length at the 60-th day after hatch average values of this magnitude differed significantly and have come to 34,5 ± 1,5 mm for laboratory group and 30,0 ± 0,9 mm for the group maintained in bioloogically purified sewage water.

Physical-chemical analysis of biologically purified sewage water showed that values of the parameters of sewage water exceed established for reservoirs of the cultural and community water use maximum permissible concentrations of these substances for transparency, suspended substances and in some cases for oil products, BOD_5 and COD.

In special experiments we studied crayfish reactions on the oxygen decrease in water (Sladkova et al., 2006), on the pH decrease (Udalova et al., 2009) and on some other chemical agents (Kholodkevich et al., 2007, 2008). These parameters are solitary, but crayfish gives a synergistic reaction on all water parameters together. At August, 11 2004 we discovered that all animals in aquariums with biologically purified sewage water perished. Data of the water physical-chemical analysis prepared by the officials of the Northern Station of Aeration showed that on August, 11-12 there were significant deviations of the following values: excess of BOD was 2.4 times against norm; excess of ammonia nitrogen - 18 times against norm; excess of phosphates - 10 times against norm; COD - 46 mg/l. Common excess of this parameters led to the death of animals.

DISCUSSION

Crayfish species *Procambarus clarkii (Girard)* are convenient objects for biomonitoring of the biologically purified sewage water quality based on crayfish cardiac activity registration due to the animals' adaptation to maintenance and cultivation in vitro, high repeatability of experiment results and because of rapid and non-invasive test method.

Heart rate of adult crayfish species *Procambarus clarkii (Girard),* as distinct from juvenile crayfish younger than 60 days, could be used as biomarker for biomonitoring of the biologically purified sewage, because the results of the experiments made in 2002-2005 showed significant differences of heart rate values between groups of animals, maintained in biologically purified sewage water and conditionally clean water in laboratory.

By comparison of two juvenile crayfish groups in the rest and stress functional states the absence of significant differences between these groups was discovered in both cases. Nevertheless, there are small but significant differences between heart rate values of crayfish maintained in the biologically purified sewage water in the rest and stress states as distinct from the laboratory group, where no such differences were observed. Consequently, the heart rate values of juvenile crayfish as distinct from adult ones are 2-3 times higher as at the rest, so at the stress functional states. Herewith the differences of heart rate values of juvenile crayfish maintained in the conditionally clean water and biologically purified sewage water are absent. Also there are significant differences of stress-index values between two groups of crayfish in the stress functional state.

Crayfish species *Procambarus clarkii (Girard)* showed adequate biological reproduction cycles during two years, what proves their adaptation to cultivation conditions at the Northern Station of Aeration as biosensors. At the same time chosen biomarkers of crayfish cardiac activity and morphological parameters of growth of juvenile crayfish at the age of 60 days showed obvious differences between animals lived in different conditions. That testifies correctness of the biosensor species choice for estimation the biologically purified sewage water quality.

In biologically purified sewage water excess of maximum permissible concentrations of some substances (transparency, content of suspended substances, oil products, BOD_5 and COD), established for the water objects of cultural and community water use, determines differences of heart rate values of adult crayfish groups, maintained in the conditionally clean water and biologically purified sewage water.

Analysis of the case where crayfish died showed that excess of one parameter didn't lead to the animals‘ death despite of it occured from time to time. Only simultaneous excess of few parameters led to the death of animals. This incident we can regard as practical example of how important is to take into account the synergetic actions of physicochemical agents to the environment. It is necessary to admit that at the present time the synergetic effects on the human and biota health can be found out only with a help of biological methods of water testing.

Creation of the basic network of automatic stations for continuous ecological monitoring will provide the irreplaceable source of the information for systems of preparation the acceptance of the administrative decisions in the field of the water resources integrated management. Developed from the eco-physiological method it permits to discover early reactions of animals on different alarm stressors. And it reveals objective reactions of animals such as crayfish to chemical and physical stress factors. The system of industrial biological water quality monitoring with crayfish usage can be applied at industrial enterprises as an early-warning system to monitor environmental components in potentially dangerous areas (for example in the regions of oil and gas exploration, production and transportation).

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