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Noninvasive crayfish cardiac and behavioral activities monitoring system

Ph.D. THESIS

Aliaksandr Pautsina, M.Sc.

Supervisor: Prof. RNDr. Dalibor Štys, CSc.
University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters

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Annotation

Crayfish provide a model which is simple, has an easily-accessible cardiovascular system and can be maintained in the laboratory conditions; the model has good utility for water quality assessment and ethophysiological studies. A noninvasive crayfish cardiac and behavioral activities monitoring (NICCBAM) system is discussed in the thesis. The system is inexpensive, has relatively few components and permits long-term continuous simultaneous monitoring of cardiac and behavioral activities of several crayfish. Moreover, compared to other available systems, it provides a novel approach of cardiac activity shape analysis which allows improving monitoring accuracy as well as obtaining additional information on crayfish functional state.

The NICCBAM system was evaluated by comparing with the well-known electrocardiography system which demonstrated that cardiac contractions with both approaches were synchronous and that both signal shapes were similar. Experiments on crayfish cardiac activity relative to selected odors and chemicals demonstrated the promising potential of cardiac signal shape analysis, not only for detecting changes in the aquatic environment, but also for their classification.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

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České Budějovice, 02.11.2015
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   AP participated in the development of the system, developed the data processing method, analyzed the experimental data and wrote the manuscript.

2. **A. Pautsina**, P. Císař, I. Kuklina, A. Kouba, and P. Kozák. 2014. Non-invasive sensing element. Registered national utility model number: 27114. Application number: 2014-29595.
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4. **A. Pautsina**, P. Císař, D. Štys, B. F. Terjesen, and Å. M. O. Espmark. 2015. Infrared reflection system for indoor 3D tracking of fish. Aquacult. Eng. 69:7-17 [doi:10.1016/j.aquaeng.2015.09.002]. (IF 2014 = 1.181)

Dedicated to my mother

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CONTENTS

LIST OF ABBREVIATIONS

ADC	Analog-to-digital converter
BA	Behavioral activity
BCA	Biochemical analyses
BEWS	Biological early warning system
CAPMON	Computer-aided physiological monitoring
CCD	Charge-coupled device
ECG	Electrocardiography
FPS	Frames per second
HR	Heart rate
HW	Hardware
IPG	Impedance pneumography
LED	Light-emitting diode
LFOP	Laser fiber-optic photoplethysmograph
NICCBAM	Noninvasive crayfish cardiac and behavioral activities monitoring
NIR	Near infrared
PC	Personal computer
PPG	Photoplethysmograph
SD	Standard deviation
SIBWQM	System for Industrial Biological Water Quality Monitoring
SW	Software
VR	Ventilatory rate

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1. Introduction

1.1. Biomonitoring of water quality

Biological monitoring (biomonitoring) implies monitoring of some physiological and/or behavioral functions in a test organism which respond when exposed to sufficient concentration of toxic substances or atypical physical conditions (Gunatilaka and Diehl 2001). It provides the integrated response to the combination of physical/chemical environmental stress factors and indicates the overall effect in the water body (Chapman and Jackson 1996). In comparison to physico-chemical monitoring, that provide a result valid only for moment in time when the sample was collected, biological methods reflect the consequences of physical and chemical conditions to which the organisms were exposed relative to a time period. Examples of biomonitoring methods are monitoring of community structure and presence/absence of species, community metabolism (e.g. oxygen production/consumption), biochemical effects (e.g. enzyme inhibition) in communities/individuals, measuring the effects of specific environment conditions on organism's growth rate, death, reproduction capacity, physiological functions, behavior, bioaccumulation of specific contaminants in tissues, and cellular and morphological (e.g. gill damage and fish skin lesions) changes (Chapman and Jackson 1996).

The advantage of some biomonitoring methods is the capacity to rapidly detect acutely harmful conditions in water, whereas laboratory chemical screening of a large number of parameters including organic micropollutants (e.g. halogenated hydrocarbons, herbicides and pesticides, nitro aromatics and phosphoric esters) requires several hours, and still cannot detect all of them (Gunatilaka and Diehl 2001). Rapid water quality assessment can be especially critical in case of short and sudden water contamination, e.g. accidental waste water treatment plant malfunction.

Despite all the advantages of biomonitoring, it should not be considered as a replacement for physico-chemical monitoring, but rather as a useful complementary approach. Usually, biological methods cannot precisely identify the contaminant unless additional information from chemical analysis is available. Moreover, without some basic physico-chemical measurements, they can fail to relate the observed effects to a specific environment disturbance, such as contamination, natural changes in the environment or natural organism cycles (Chapman and Jackson 1996). Therefore, relatively inexpensive and rapid biomonitoring methods should be used to determine the presence and severity of an environmental effect and to trigger the need for more advanced methods.

Applications of automated, continuous biomonitoring methods are usually called biological early warning system (BEWS). The biological organism or material in them is employed as a primary sensing element. The operating principle of BEWS is usually based on continuous diversion of water from the water body of interest through special tanks which contain the test organism (Chapman and Jackson 1996). The response of the organism is continuously measured by an appropriate sensing device and recorded by, e.g. personal computer (PC) or pen recorder, for further automatic and/or manual analysis. If BEWS is used close to important drinking water intakes, the biological response can trigger a temporary intake shutdown until further advanced analyses are done. When used close to effluent discharges, the response can signal a sudden change in discharge nature, e.g. caused by treatment plant malfunction, and rapid response actions can be taken, thereby reducing harmful environmental effects without waiting for the chemical analysis results (Chapman and Jackson 1996).

1.2. Crayfish as a promising model for biomonitoring

Efficient environment biomonitoring requires selecting an organism which is sensitive enough to detect and signal harmful conditions, yet being able to tolerate and survive them. Also, the ease of maintenance and experimentation in the labs, early maturation, high reproduction rate and long life-span are important requisites for the test animal. Crayfish are considered to be pivotal organisms in the freshwater ecosystems and have promise for biomonitoring of water quality; they are highly sensitive to changes in the aquatic environment and they are adaptable for experimentation (Kholodkevich et al. 2008; Kuklina et al. 2013; Kuklina 2014). Further, their nervous system is relatively simple compared with vertebrates, thus a crayfish model is useful for studying the mechanisms underlying behavior (Li et al. 2000).

Assessing the state of the organism can be done by evaluation of its internal functions. Crayfish cardiac and respiratory activities can be relatively easy and rapid to evaluate (Villarreal 1990; Bini and Chelazzi 2006; Bierbower and Cooper 2009). Cardiac activity provides a general indication of crayfish metabolic status (Depledge and Galloway 2005). It can signal the integrated impact of natural and anthropogenic stressors and may reflect the availability of energy that is required for crayfish normal life, growth, and reproduction. Heart rate (HR) and ventilatory rate (VR) being within the normal ranges indicate the undisturbed state or base level of the organism (Depledge and Galloway 2005). Despite the relatively simple organization of the crayfish cardiovascular system, it is capable of reflecting the environmental effects via cardiac activity alterations (Reiber and McMahon 1998; Bierbower and Cooper 2010).

Introducing a stress stimulus can result in a chain of organism reactions, starting with the reception of the external impact, then an increase of cardiac and ventilatory rates and oxygen consumption and finally a change in locomotor activity. Many studies have been devoted to ethophysiological aspects of a crayfish model; e.g. it has been shown that cardiac and locomotor activities in crayfish are circadian with a significant increase at night time (Styrishave et al. 2007), that they are closely related to each other, as well as to ambient temperature and light intensity (Bojsen et al. 1998), and hypoxic conditions (Bierbower and Cooper 2010). Therefore, combined monitoring of crayfish physiological parameters and behavioral activity (BA) presents a more accurate and detailed assessment of crayfish functional state.

1.3. Crayfish HR and BA monitoring: state of the art

The methods and systems described below allow HR and BA monitoring of crayfish as well as other crustaceans (shrimps, lobsters, crabs or amphipods) and mollusks (bivalves or gastropods). Despite the fact that some of the approaches were originally developed for other species, they have been successfully tested with crayfish or can be potentially applied to crayfish studies. The existing methods/systems described below are arranged in chronological order as they have appeared in the literature.

It also should be noted that the invasive and noninvasive HR monitoring methods described below can be applied to VR monitoring in crayfish (Schapker et al. 2002; Shuranova and Burmistrov 2002; Shuranova et al. 2003; Bini and Chelazzi 2006; Bierbower and Cooper 2010; Shuranova and Burmistrov 2010). The modification is in the sensor placement: the sensor electrodes should be inserted under the cuticle in the rostral area of the gill chamber, or it should be placed latero-ventrally on the gill chamber for invasive and noninvasive VR measurements, respectively.

1.3.1. Invasive measuring of HR

Invasive HR measurements imply direct insertion or implantation of two electrodes into the animal. Because of the stress invoked by handling and surgery the animal should be left undisturbed for a period of several hours or even days before the start of the experiment. During measurements instantly emerged voltage or changes in impedance between the electrodes caused by cardiac activity is processed and recorded.

Impedance pneumography (IPG)

An IPG method is used for detection of pulsatile changes in heart volume (Helm and Trueman 1967; Hoggarth and Trueman 1967). Before starting the measurements, two fine wire electrodes are inserted into the pericardial cavity of the animal through small holes drilled through the carapace and then sealed with wax. During the function a small oscillatory current passes between the electrodes and the volume change caused by heartbeat affects the impedance between them. The output voltage signal proportional to changes of the impedance is then fed to a recorder by A.C. coupling. The IPG method was successfully applied to HR recording of the blue mussel (*Mytilus edulis*) during its exposure to the air (Helm and Trueman 1967) and at different temperature and salinity conditions (Braby and Somero 2006). Also it was applied to HR recording of three species of portunid crabs concomitant with oxygen level changes (Uglow 1973) and brown crab (*Cancer Pagurus*) during starvation (Ansell 1973).

This method was later modified for HR measurements in small 0.3 – 0.4 g crustaceans (Dyer and Uglow 1977). A modification from the original IPG is the use of two unequally-sized electrodes: the smaller one is implanted to the animal while the larger one is sited in the water of the aquarium. The modified IPG was successfully applied to the HR recording of brown shrimp (*Crangon crangon*) during a variety of its normal activities (Dyer and Uglow 1977), shore crab (*Carcinus maenas*) during handling, tactile and optical stimulation (Cumberlidge and Uglow 1977) and intertidal prawn (Morris and Taylor 1985) under hypoxic and hyperoxic conditions.

The IPG has not been tested with crayfish, but because of its simplicity and confirmed compatibility with many crustacean species, I believe it can be used for HR measurements in crayfish.

Electrocardiography (ECG)

ECG records the electrical activity of the heart. To obtain ECG recordings from crayfish, 1-3 days prior to experimentation, two fine stainless steel fine wires are placed under the dorsal carapace over the heart (Florey and Kriebel 1974; Listerman et al. 2000). The wires are inserted through holes drilled in the carapace, then cemented in place with instant adhesive. During recording, the instantaneous small amplitude voltage emerged between electrodes is either directly amplified with high gain AC amplifier (Florey and Kriebel 1974; Pollard and Larimer 1977; Goudkamp et al. 2004) or the correlated impedance changes are estimated and converted to voltage using an impedance detector (Listerman et al. 2000; Chabot and Webb 2008; Bierbower et al. 2013) prior to being fed to a recorder. The ECG method was successfully applied to HR recording in red swamp crayfish (*Procambarus clarkia*) during social interactions (Listerman et al. 2000), copulation (Cooper et al. 2011) and under acute and chronic cold exposure (Chung et al. 2012), blind cave crayfish (*Orconectes australis packardi*) during environmental disturbances and social interactions (Li et al. 2000) and freshwater crayfish (*Cherax destructor*) during heating and cooling (Goudkamp et al. 2004).

1.3.2. Noninvasive measuring of HR

Noninvasive monitoring methods theoretically allow measuring the most relevant physiological data, while minimizing manipulation time and disturbance to the animals.

The noninvasive HR measuring systems discussed in the literature are all based on estimating the amount of scattered near infrared (NIR) light from the animal’s heart modulated by changes of the beating heart volume. They consist of a reflective optocoupler in a small package having at least one light emitter and one photoreceiver and a signal conditioning/processing module. The scattered light from the optocoupler’s source illuminates the animal’s heart through the carapace and partially reflects to a photoreceiver. Changes in shape and/or volume of the heart during contractions cause changes in the amount of reflected light. The varying incident light at the photoreceiver is converted into an electrical signal which is then amplified, filtered and digitized in the conditioning/processing module.

The majority of the systems discussed below were initially tested on the crab model, but later studies showed that they could be successfully used with selected crayfish, as well as with prawns, shrimps, lobsters, thin-shelled bivalves, polychaete worms, mussels and terrestrial snails.

Photoplethysmograph (PPG)

The first noninvasive HR measurement system for decapod crustaceans described in literature was called PPG (Depledge 1984). It consisted of two small low intensity light bulbs and a photosensor embedded in transparent resin (see Fig. 1.1). During operation, the light from the bulbs passed through the dorsal carapace into the pericardium and part of the light was reflected back to the photosensor being modulated by the ventricle contractions. The incident on the photosensor light was then converted to an electrical signal and transmitted via the wires to an oscillograph for visualization of cardiac activity. The dimensions of the bulbs/photosensor package were relatively small and could be attached to animal's carapace with minimum width of 2 cm using water-resistant, instant adhesive. The combined experiment with the IPG method showed no significant changes in the crab's (*Carcinus maenas*) heart and ventilatory (scaphognathite) rates in accordance with the switched on/off state of the PPG. The recordings using the PPG were successfully obtained from crabs (*Carcinus*, *Macropipus*, *Sesarma*, *Uca*, *Ocypode*), from large prawns and shrimps and from the Norwegian lobster (*Nephthrops norvegicus*).

Computer-aided physiological monitoring (CAPMON) system

The CAPMON system for continuous, long-term recording of cardiac activity in selected invertebrates (Depledge and Andersen 1990) represents a modification of the PPG system for monitoring of up to eight animals simultaneously. The light bulbs and special photosensor used in the old system were replaced in the new design by NIR light-emitting diode (LED) and phototransistor mounted parallel to each other and facing in the same direction. The new components allowed the transducer to be smaller and lighter. Additionally, the special electronic circuit was developed for processing the electric signal from the phototransistor, so as to count the cardiac activity peaks and transfer the data in a digital form to the PC for visualization and storing (see Fig. 1.2). The software (SW) of the CAPMON system allows displaying the HR of up to eight animals simultaneously and, optionally, visualizing the raw cardiac activity signal for performing the hardware (HW) calibration. The evaluation of the system realized by direct observation of the heart contractions via a small hole drilled in the carapace and the peaks displayed by the SW showed their agreement. The CAPMON system was originally developed for reptant decapods but was also successfully used with thin-shelled bivalves and polychaete worms. The system was applied to study the circadian rhythm of HR in noble crayfish (*Astacus astacus*) under influence of selected bulk and trace metal (Styrishave et al. 1995) and HR response in signal crayfish (*Pacifastacus leniusculus*) to fluctuations of ammonia concentration (Bloxham et al. 1999). Combined physiological and BA study (Aagaard et al. 1991) using CAPMON system was performed for the shore crab (*Carcinus maenas*).

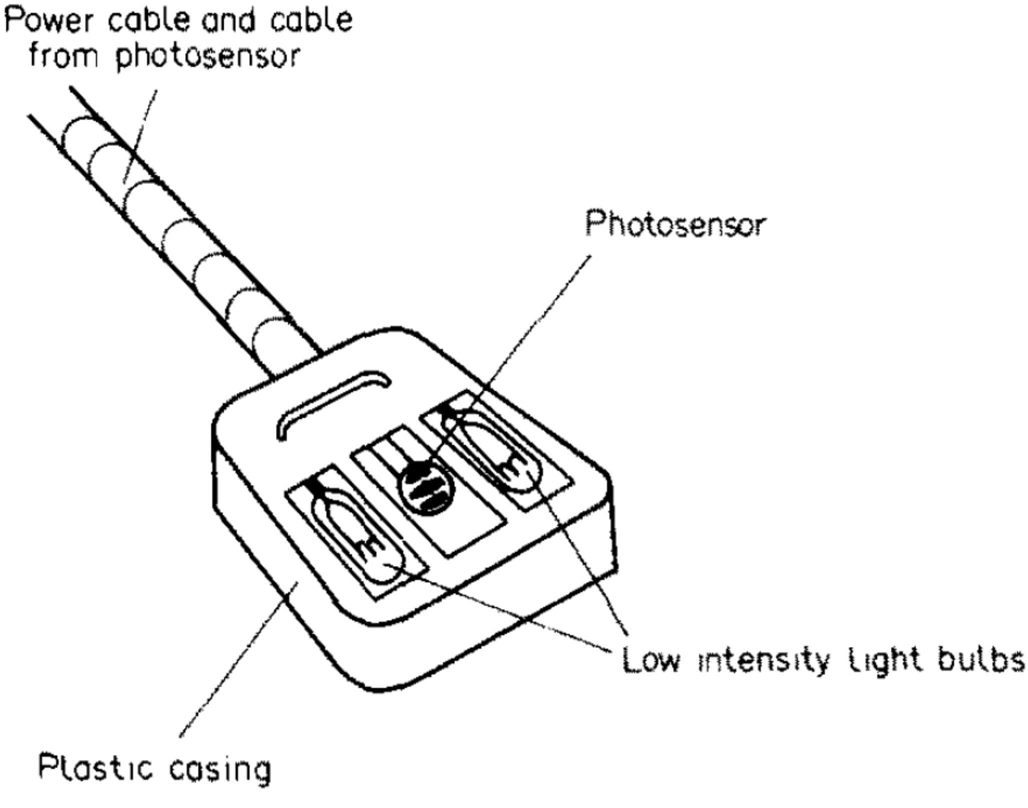


Fig. 1.1. The PPG (Depledge 1984).

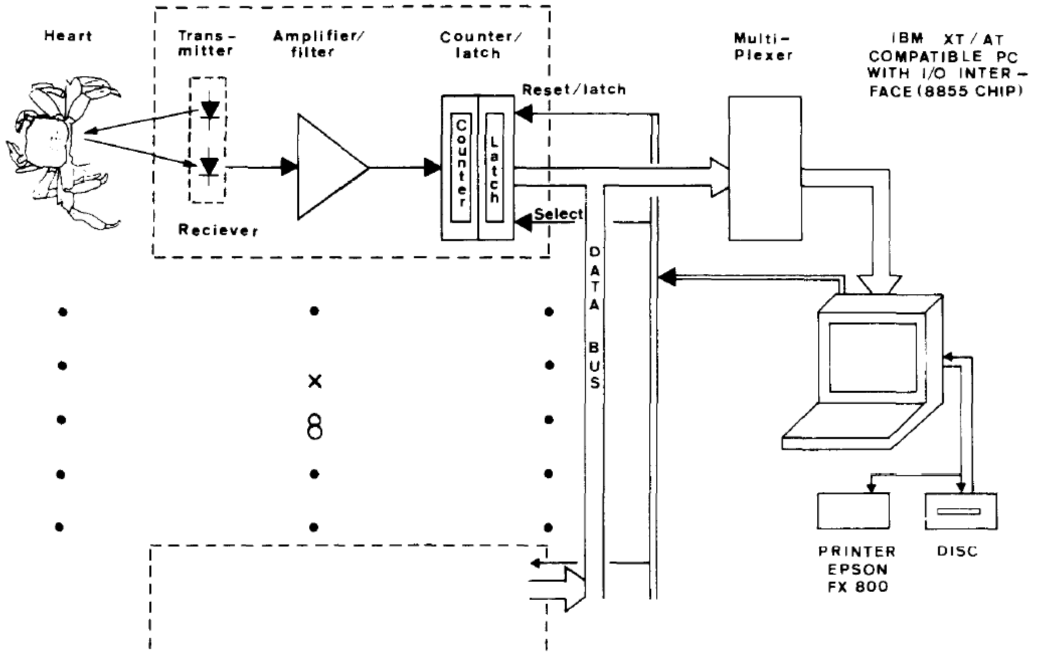


Fig. 1.2. Overview of the CAPMON system (Depledge and Andersen 1990).

The original data processing algorithms of the CAPMON system were later improved to allow distinguishing between different HR patterns (Depledge et al. 1996). The technique got the name Automated Interpulse Duration Assessment (AIDA) and was capable of detecting HR irregularities which were not evident when measuring the mean HR alone. It allowed successful detection of the handling stress in mussels (*Mutilus Edulis*) and changes of the frequency distribution of interpulse duration in crabs (*Carcinus maenas*) according to the nutritional states.

System for Industrial Biological Water Quality Monitoring (SIBWQM)

The SIBWQM system (Kholodkevich et al. 2008) and its earlier prototype (Fedotov et al. 2000) are based on the same cardiac monitoring approach as the PPG and CAPMON systems but have considerably different HW. The laser NIR light source and photoreceiver forming the laser fiber-optic photoplethysmograph's (LFOP) are placed separately from the animal and connected to its carapace via two thin optical fibers. One optical fiber line is used for transmitting light from a laser to the animal's cardiac sac, while the second one is used for receiving the reflected part of the light modulated by the heart contractions. The system prototype (Fedotov et al. 2000) had only one LFOP (channel) that was connected to the oscillograph and low-inertia recorder for cardiac signal visualization and storing. The final version of the system (Kholodkevich et al. 2008) was multichannel, contained an analog-to-digital converter (ADC) and had an interface to the PC (see Fig. 1.3). The specially developed SW running on the PC analyzed the cardiac activity signal, determined the duration of cardiac intervals and calculated a set of HR statistical characteristics. The SIBWQM system was successfully tested with four species of freshwater crayfish, including noble crayfish (*Astacus astacus*) and narrow-clawed crayfish (*Pontastacus leptodactylus*), one species of crabs, four species of freshwater bivalves, two species of gastropods, three species of marine bivalves and two species of terrestrial snails.

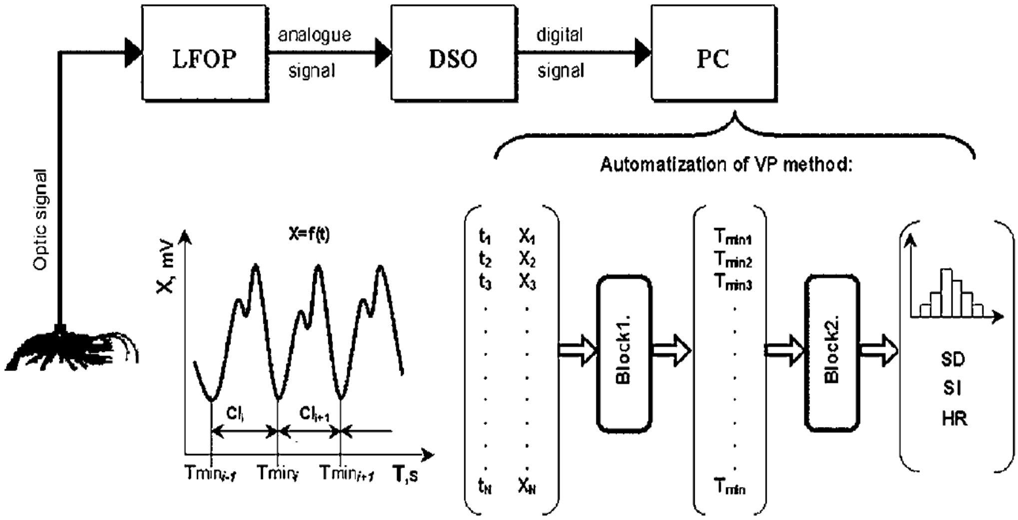


Fig. 1.3. SIBWQM system: signal processing stages (Kholodkevich et al. 2008).

It should be also noted that the discussed system is mainly focused on the astacological research and was used in many cardiac activity studies in crayfish, e.g. in different functional states (Fedotov et al. 2002; Fedotov et al. 2006), in varying natural conditions (Sladkova et al. 2006; Udalova et al. 2009; Udalova et al. 2012) and under the selected chemical impacts (Kozák et al. 2009; Kuznetsova et al. 2010; Kozák et al. 2011).

Improved CAPMON

The ideas behind the development of the improved CAPMON system (Burnett et al. 2013) were modifications of the original HW design using the electronic parts currently available from distributors and its dissemination together with the uniform method of data collection among a wide range of scientists. In contrast to the original CAPMON, the improved system's conditioning circuit only amplifies and filters the signal but doesn't transform the analog signal into square waves for automatic HR calculation (see Fig. 1.4). Because each animal can exhibit a unique cardiac activity pattern, processing the raw, non-square signal allows for more flexible tuning of SW data processing algorithms and thus reducing the number of data misinterpretation errors in comparison to automatic HW counting circuit.

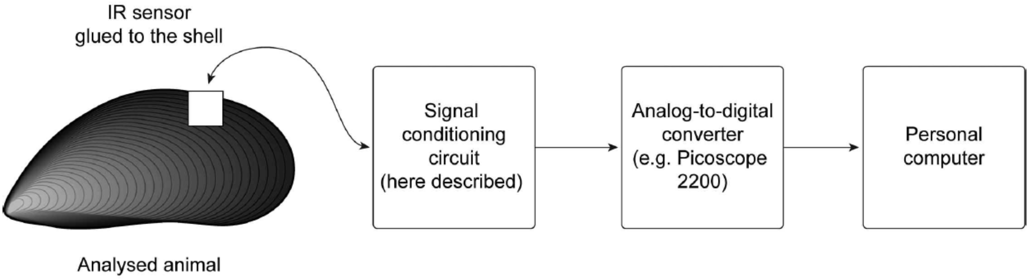


Fig. 1.4. Improved CAPMON system: cardiac activity signal flowchart (Burnett et al. 2013).

The system was evaluated by comparing the cardiac activity patterns in the Atlantic blue crab (*Callinectes sapidus*) simultaneously recorded using the discussed method and the invasive impedance method. Both methods showed the same heartbeat patterns and frequency. Like the original CAPMON the system can be used with many intertidal and marine invertebrates.

1.3.3. Camera-based BA monitoring

Systems based on video signal analysis, in contrast to earlier used methods, e.g. based on LED/photodetector pairs (Bolt and Naylor 1985; Aagaard et al. 1991), are currently the most versatile systems for automated analysis of behavior (Kruk 1997). The recent advances in computer engineering and machine vision technology allows performing the BA recording and analysis fully automated (Celi et al. 2013). Also it allows overcoming the inherent fundamental limitations of the direct behavioral observation: efficient estimation of only qualitative properties of behavior, while the quantitative features, such as intensity, acceleration or trajectories cannot be easily measured (Kruk 1997).

Behavior of two crayfish species (*Orconectes rusticus* and *Orconectes virilis*) in two lakes was recorded using the system containing the charge-coupled device (CCD) camera in the underwater housing and white light (Bergman and Moore 2003). The recorded videos were later manually analyzed to draw the crayfish ethograms and estimate the effects of extrinsic and intrinsic factors on the crayfish intraspecific agonistic behavior. Similar approaches based on manual processing of the recorded video data were used during the crayfish (*Astacus astacus*) behavior studies on the formation and maintenance of hierarchies (Goessmann et al. 2000) and on the effect of serotonin on agonistic interactions (Huber et al. 1997; Huber and Delago 1998).

The BA monitoring system used for studying the effects of psychostimulants on rusty crayfish (*Orconectes rusticus*) contained a video camera connected to a PC and a fluorescent diffused lighting module placed below the aquarium in order to enhance the resolution of video tracking (Panksepp and Huber 2004). The system's SW based on freeware JavaGrinders library was capable of automatically obtaining the crayfish spatial coordinates at a temporal resolution 1/3 Hz.

The system for tracking of two dimensional position and orientation of the Caribbean spiny lobster (*Panulirus argus*), except for the video camera, also contained a backpack with two red LEDs glued to the animal's carapace to facilitate tracking (Horner et al. 2004). The experiments were conducted during the day under low ambient illumination. The presence of the backpack had no apparent effect on the behavior of the animal.

Many crayfish species are nocturnal animals (Patullo et al. 2007). To solve the problem of reduced resolution and clarity of the images obtained during night time recording the tracking system with NIR LED illumination and NIR sensitive CCD camera was proposed (Patullo et al. 2007). The NIR lamps in the setup were fixed above the monitored tank so as not to point directly perpendicular to the tank bottom to minimize reflection. The system was successfully tested with freshwater crayfish (*Cherax destructor*). The SW- and observer-acquired behaviour data were not significantly different.

1.3.4. Combined HR and BA monitoring

The first systems for combined HR and behaviour monitoring were based on the sensors from the CAPMON system for HR recording and the same or similar NIR optocoupler based sensors for locomotor activity recording (Aagaard et al. 1991; Bojsen et al. 1998; Styrishave et al. 2007). One or two activity sensors were fixed under the transparent floor of each of the experimental chambers. Movements were detected when the NIR light was reflected back from the animal. The sensor was positioned so that even a few centimeter movement of the animal was detected. The HR and locomotor activity data from both types of the sensors were continuously recorded and stored on the PC. The above described monitoring approach was successfully applied to combined HR and locomotor activity monitoring in shore crab (*Carcinus maenas*), noble crayfish (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*).

In later combined studies, the BAs were monitored using video cameras. However, the records were analyzed manually, e.g. the activity of red swamp crayfish (*Procambarus clarkia*) during copulation (Cooper et al. 2011) and blind cave crayfish (*Orconectes australis packardi*) during social interactions (Bierbower et al. 2013).

1.4. Crayfish model based studies

The key published studies based on crayfish model for estimating the effects of different physical and chemical stimuli are summarized in Table 1.1. It contains the information on the types of stimuli, crayfish species, measured ethophysiological parameters (HR, VR and BA), the name of the methods/systems used during the research as well as main outcomes. If biochemical analyses (BCA) were used in combination with HR, VR or BA monitoring, the methods and the main outcomes are also briefly described in the Table 1.1.

Table 1.1. Brief description of key crayfish model based studies

Stimulus	**Species**	**Parameters**	**Method/ System**	**Main outcomes**	**References**
Chemical					
Ammonia	*Pacifastacus leniusculus*	HR	CAPMON	The significant stimulatory effect on HR was observed at chemical concentrations starting from 5 mg/L.	Bloxham et al. (1999)
Amphetamine	*Orconectes rusticus*	BA	Camera	Injection of D-amphetamine stimulated active explorative crayfish behaviors in a dose-dependent fashion.	Alcaro et al. (2011)
Carbon dioxide	*Procambarus clarkia*	HR + VR BA	ECG Camera	HR and VR considerably decreased during acute exposure to 50% of CO_2 and showed a complete cessation at 100%. Behavioral responses demonstrated avoidance and repelling effects at 50% and 100% of CO_2, respectively.	Bierbower and Cooper (2010)
Chloramine	*Astacus leptodactylus*	HR	SIBWQM	During exposure to chloramine-T no significant changes were observed in crayfish HR for concentrations 2 and 5 mg/L, while 10, 20 and 50 mg/L	Kuklina et al. (2014)

				concentrations caused a significant HR increase. Repetitive exposures to concentrations 10 and 50 mg/L resulted in less expressed increase in HR in comparison to the first trial and showed significant differences in HR only for the 50 mg/L concentration.	
Food odor	*Orconectes rusticus*	BA	Camera	In the presence of chemical food cues, crayfish initiated encounters with increased intensity and fighting lasted longer compared to controls. The satiated crayfish escalated fights less rapidly than hungry individuals.	Stocker and Huber (2001)
Hydroquinone	*Pontastacus leptodactylus*	HR BCA	SIBWQM Lowry method	Short-term (1 h) exposure to hydroquinone in concentration 1 g/L resulted only in HR increase while long-term (1 d) exposure caused tachycardia and considerable decrease of the hemolymph total protein.	Kuznetsova et al. (2010); Sladkova and Kholodkevich (2011)
Metals	*Procambarus clarkia*	HR + VR	CAPMON	Exposure to copper in concentrations up to 10 mg/L caused HR and VR reduction which fully reversed after 3 h of crayfish	Bini and Chelazzi (2006)

				residence in clean water. Pre-exposure to low copper concentration significantly decreased HR and VR reduction during the following exposure to 10 mg/L copper concentration. Higher copper concentration starting from 50 mg/L resulted in increasing crayfish mortality.	
	Astacus astacus	HR	CAPMON	The circadian rhythmicity in crayfish HR was disrupted by mercury and copper in concentrations 0.1 and 8 mg/L, respectively. Moreover, the exposure to mercury resulted in a later crayfish death.	Styrishave et al. (1995); Styrishave and Depledge (1996)
Morphine	*Orconectes rusticus*	BA	Camera	Single or repeated intra-circulatory injections of morphine (2.5, 5 and 10 mg/g) resulted in persistent locomotor sensitization and morphine-induced conditioned place preference behavior even five days following the infusion. It was shown that long-lasting behavioral sensitization can be induced by a single exposure to morphine and the effect can be	Nathaniel et al. (2009); Nathaniel et al. (2010)

				comparable to repeated drug regimes.	
Oxygen	*Astacus astacus, Pontastacus leptodactylus, Procambarus clarkia*	HR	SIBWQM	Under hypoxic conditions the crayfish HR and amplitude of the cardiac activity showed tendencies to decrease and increase, respectively. Under anoxic conditions the crayfish demonstrated heart contractile activity for almost 10 h while desynchronization of regulatory processes in the central and peripheral chains of cardiovascular system control was observed.	Sladkova et al. (2006)
Own, conspecific odors	*Orconectes australis packardi*	HR BA	ECG Camera	Exposure to their own and conspecific odors can result in significantly elevated HR while the BA changes estimated by the body movements may not be manifested.	Li et al. (2000)
pH	*Pontastacus leptodactylus*	HR	SIBWQM	At low pH (3.4±0.1) elevated HR and disturbance in the circadian cardiac rhythm were observed.	Udalova et al. (2012)

Serotonin, social interactions	*Procambarus clarkia*	HR BA	ECG Camera	During social status establishment interactions HR in crayfish can increase. Nevertheless, it can be within seconds dampened during the pauses in interaction even if the crayfish is still posturing in an aggressive or submissive state. Injection of 100 nM – 10 µM of serotonin to crayfish can substantially increase the HR for hours.	Listerman et al. (2000)
Sodium chloride	*Astacus leptodactylus*	HR	SIBWQM	The evident increase in HR was obtained for sodium chloride concentrations staring from 3200 mg/L. Application of chlorides prior to high concentration nitrite exposure helps to withstand stress and has a positive effect on cardiac activity.	Kozák et al. (2009); Kozák et al. (2011)
	Astacus astacus	HR	CAPMON	The circadian rhythmicity in crayfish HR was disturbed by sodium chloride in concentration 1400 mg/L.	Styrishave et al. (1995)

Physical					
Acoustic stimulus	*Procambarus clarkia*	BA BCA	Camera Hemolymph analysis	Exposure to acoustic stimulus resulted in significant variations in hemato-immunological parameters and reduction of agonistic behavior.	Celi et al. (2013)
Conspecific social interactions	*Procambarus clarkia*	HR + VR BA	ECG Camera	During agonistic interactions HR and VR in both crayfish can show a dramatic increase even without detectable difference in BA. The observed changes in HR and VR were not always synchronized and could be rapidly altered independently.	Schapker et al. (2002)
Illumination	*Orconectes australis packardi*	HR BA	ECG Camera	Exposure to white light resulted in HR increase both during the walking phases and between them. Elevated HR was not observed during the exposure to infrared and dim red lights.	Li et al. (2000)
Physical load	*Pontastacus leptodactylus*	HR	SIBWQM	Short-term (less than 1 hour) and weakly expressed HR increase during physical loads reflects instability of excitation and	Udalova et al. (2012)

				inhibition processes in nervous regulation of cardiac activity and indicates stressed functional state in crayfish.	
Temperature	*Astacus astacus*	HR BA	CAPMON	Positive correlation was observed between HR and locomotor activity and both parameters showed increase at higher temperature.	Bojsen et al. (1998)
	Procambarus clarkia	HR	ECG	The acute and slow temperature changes from 21 to 5 °C resulted in instantaneous substantial and gradual reduction of HR, respectively. The crayfish survived and remained responsive to sensory stimuli during both exposure modes.	Chung et al. (2012)
	Cherax destructor	HR	ECG	The experiments with convective heating and cooling of crayfish showed that HR during heating (from 20 to 30 °C) was significantly faster than during cooling for any given body temperature (HR hysteresis). The observed HR hysteresis pattern was similar to the patterns reported for many reptiles.	Goudkamp et al. (2004)

Waterborne vibrations	*Orconectes australis packardi*	HR BA	ECG Camera	The fallen drops of water resulted in a brief up to several minutes HR increase while a dropped pebble caused a larger increase both in HR and duration of the effect. In both experiments the behavioral responses to the stimuli were not observed.	Li et al. (2000)
	Procambarus clarkia	HR + VR BA	ECG Camera	The environmental disturbances such as drops of water and pebbles can cause a rapid decrease for a few beats followed by significant increase over basal conditions of both HR and VR. BA changes were not observed after drops of water stimulus while after a dropped pebbles crayfish reacted with tail flipping, walking movements or antennae sweeping.	Schapker et al. (2002)

1.5. Motivation

As was shown above, crayfish are keystone species in freshwater ecosystems and are promising organisms for biomonitoring. Due to relative simplicity of the cardiovascular system measurements in comparison to vertebrates, experimentally accessible and sensitive nervous system, straightforward growing, reproduction and maintenance under the laboratory conditions, the crayfish model has a powerful potential for water quality assessment applications, e.g. as a basis of BEWS. Moreover, the model can be used in a variety of ethophysiological studies, e.g. being sensitive to human drugs of abuse it can offer a comparative and complementary approach in studying basic biological mechanisms of drug addiction (Huber 2005; Huber et al. 2011) and having a highly structured and easily evoked behavioral system, it can be used to quantitatively study formation and maintenance of dominance relations and aggression (Kravitz and Huber 2003).

Currently available invasive HR measurement methods based on IPG or ECG recording require drilling through the crayfish dorsal carapace directly over the heart and implanting two metal electrodes (see Section 1.3.1). However, implanting electrodes can result in injuring the crayfish and alter its behavior, and connecting the sensory device to crayfish requires complex and precise surgery.

One disadvantage of the noninvasive systems described in Section 1.3.2 is the complexity of HW: both the original (Depledge and Andersen 1990) and the improved CAPMON (Burnett et al. 2013) systems require a conditioning circuit for filtering and amplification of the signal, whereas a SIBWQM (Fedotov et al. 2000; Kholodkevich et al. 2008) additionally requires precise optic components. Recent advances in electronics and optoelectronics eliminate the need for a HW amplification circuit, whereas the increased computational power of modern PCs allows real-time digital filtering of the signal in SW. Processing the signal in SW permits easier and more flexible control over the data processing parameters and allows excluding HW signal conditioning circuit, thus making the whole system cheaper and easier to manufacture.

Another drawback of the noninvasive systems is that they are focused on measuring and analyzing HR, whereas other chronotropic and inotropic parameters describing the shape of the cardiac signal are not considered.

Previous studies (Depledge and Andersen 1990; Fedotov et al. 2000; Burnett et al. 2013) describe that crayfish cardiac activity generally has a double peak shape: the primary peak that defines the HR and a periodic smaller secondary peak that is seen (more often at low HR) in the diastolic phase between primary peaks. Occasionally, the amplitude of the secondary peak can increase considerably and become comparable with the amplitude of the primary peak. Previous experimental results showed that it can reach 54.5% and 68.7% of primary peak amplitude in water and in air, respectively (Fedotov et al. 2000).

The origin of the secondary peaks can be related to the nonsynchronous work of two pacemaker groups of cardioneurons at crayfish cardiac ganglion that provide functioning of the crayfish heart (Fedotov et al. 2009). Particularly, when activity of small neurons (intrinsic cardiac pacemakers) dominates over the work rhythm of large cells (anterior group connected with cardiac muscle).

An individual crayfish can have a unique heartbeat pattern (Burnett et al. 2013) depending on its size, age, physiology, and species, as well as modifications that can be influenced by different stimuli. Therefore, analysis of the shape of the cardiac signal can provide additional information on the crayfish functional state that is not included in HR.

Monitoring of a combination of cardiac and behavioral activities can provide more accurate and detailed information on the functional state of crayfish. Several approaches for combined monitoring of HR and BA were earlier reported in the literature. The BA monitoring approaches based on optocouplers and CAPMON system (Aagaard et al. 1991; Bojsen et al. 1998; Styrishave et al. 2007) require at least one sensor per individual crayfish. Another drawback is that the aquarium need to be relatively small and should not contain obstacles (e.g. crayfish shelters) in order to guarantee accurate monitoring without optical dead zones. The later reported video camera based approaches (Cooper et al. 2011; Bierbower et al. 2013) used manual data processing methods which are time consuming and limited in providing quantitative BA parameters (trajectories, velocities, accelerations, etc.). Recent advances in computer engineering and image processing algorithms (e.g. OpenCV SW library) allow development of camera-based systems for real-time automatic extraction of BA parameters from the video footage. Moreover, one single system can be used for simultaneous BA monitor of several crayfish.

Some of the previously developed biomonitoring systems were designed to be multichannel, e.g. CAPMON (Depledge and Andersen 1990) and SIBWQM (Kholodkevich et al. 2008). Simultaneous monitoring of several crayfish during one experiment increase the reliability of data by reducing the effects of natural peculiarities among individual crayfish, their instantaneous health status, functional state, etc.

Therefore, there is a need for a system which is inexpensive, straightforward, and has effective HW and SW tools so as to simultaneously record and analyze ethophysiological parameters in multiple crayfish. The tools should be capable of analyzing the complex shape of the crayfish cardiac activity signal, automated real-time monitoring of BA and provide additional (compared to alone HR measurements) information about the crayfish functional state.

1.6. Aims of the thesis

The aims of the thesis were to:

- study various methods and systems described in the literature for monitoring crayfish cardiac and behavioral activities.
- develop an inexpensive and easy-to-use noninvasive biomonitoring system for simultaneous recording of ethophysiological parameters in several crayfish,
- develop a cardiac activity data processing method based on signal shape analysis,
- assess the biomonitoring system and data processing methods in the experiments with selected natural odors and harmful chemical agents that can be found in aquatic environment,
- establish a foundation for possible future applications of the system as a basis of BEWSs and in crayfish model ethophysiological studies.

2. Noninvasive crayfish cardiac and behavioral activities monitoring (NICCBAM) system

2.1. Overview

The NICCBAM system is comprised of a set of 16 NIR optical sensors for crayfish, a multichannel 14 bit ADC with USB interface, crayfish motion detection module and a PC with SW for data processing. It allows:

- obtaining raw cardiac activity data of up to 16 crayfish simultaneously with a sampling rate of up to 500 samples/second,
- processing the data to obtain *HR* and other 15 cardiac signal inotropic/chronotropic parameters,
- detecting the movement of each monitored crayfish with a sampling rate of 1 sample/s,
- storing the calculated cardiac parameters and motion data to local hard drive.

A schematic representation of the system is shown in Fig. 2.1.

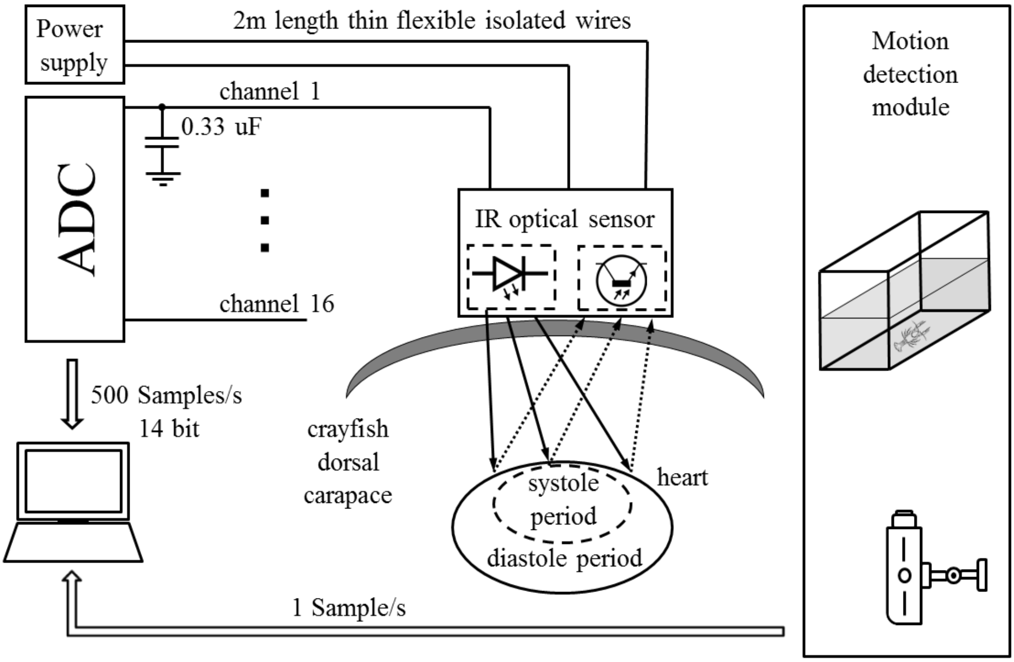


Fig. 2.1. Overview of the NICCBAM system.

The SW's graphical user interface is capable of displaying raw cardiac activity signals of all monitored crayfish and their main parameters in real-time. Additionally, motion detection module allows visualizing the aquaria and indicating to the operator which crayfish are moving. The user interface allows choosing which cardiac activity parameters from the set will be calculated and recorded. Optionally, raw cardiac activity can be recorded for further manual or semiautomatic analysis.

2.2. NIR optical sensors

The NIR optical sensor is comprised of an NIR LED axially coupled with a phototransistor (Fig. 2.2). It is placed in the water-proof package and can be fixed for several weeks on the dorsal side of crayfish carapace above the heart with non-toxic epoxy glue. The connection of sensors to the ADC is done with thin flexible wires of 2 m length.

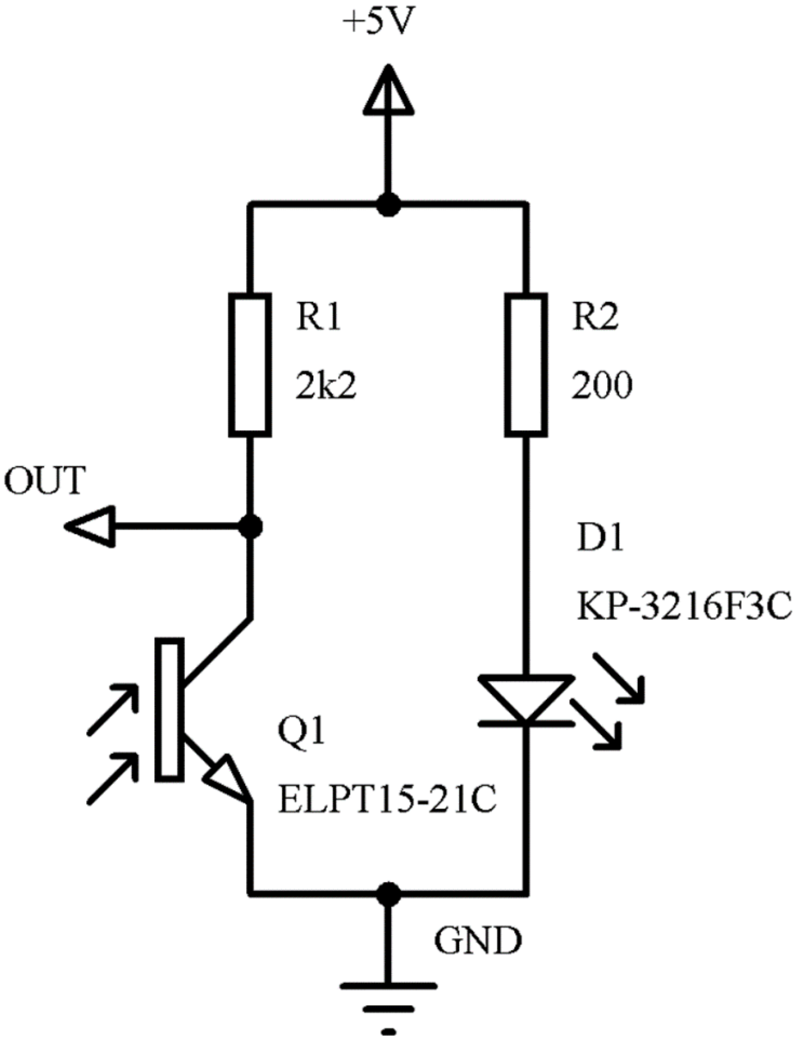


Fig. 2.2. Circuit diagram of NIR optical sensor.

The operation of the sensor is based on the photoplethysmography principle and the sensor is a reflective optocoupler. The electrical signal on the phototransistor output depends on the ambient illumination level and the amount of light emitted by the LED and then reflected by the objects in the phototransistor's field of view. When the sensor is placed on the crayfish carapace, the output signal is modulated by the amount of hemolymph filling the heart that effectively scatters the incident light from the LED. The described approach allows noninvasive monitoring of cardiac activity as a function of size and shape of the crayfish heart.

Except for a capacitor which suppresses high frequency noise (cutoff frequency is ~200 Hz) at the ADC's input, the sensor's output signal does not require any specific amplification and/or filtering circuits, unlike the CAPMON system (Depledge and Andersen 1990) or the improved CAPMON system (Burnett et al. 2013). The increased efficiency of LEDs that have been developed in the last decade, provides considerably more optical power from a single device at the same electrical power consumption. Currently, a typical commercially available NIR LED has a total optical output power of ~15 mW, assuming a driving current of 20 mA and an efficiency of 60%. The increase in optical output power leads to an increase of the optical power that is reflected towards the phototransistor, thus, amplifying its electrical output signal and eliminating the need for an external amplification circuit. Installing several small size surface mounted LEDs (e.g. in 0402 packages) in one sensor can amplify the electrical output signal even more without too much increase in overall sensor size.

It should be noted that only a small part of the total unfocused optical irradiation that is emitted by the LED reaches the crayfish heart, and it neither harms the animal nor affects its behavior. Most of the light is radiated elsewhere because of the wide 120° viewing angle of the LED, and is scattered on boundaries among several optically different layers, e.g. the water-proof sensor's covering, the thin water layer between crayfish and sensor, and the crayfish carapace.

The benefits of not using external HW amplification and filtering circuits include the preservation of the original signal shape for further SW processing, minimizing signal distortion caused by improper HW adjustments, eliminating of clipping problems (Burnett et al. 2013), and the greater simplicity and lower cost of HW.

With the current version of the sensor HW, typical heartbeat and noise amplitudes are approximately 50 mV and 2 mV, respectively. The noise level can be reduced later in SW. The signal's fluctuations caused by changes in ambient illumination fall in the range from 2 to 3 V.

2.3. Motion detection module

Crayfish motion detection module is comprised of two wide field of view NIR cameras with ethernet interfaces and two external NIR illuminators. One camera together with one illuminator can be used for BA monitoring of up to eight crayfish simultaneously. Monitoring of more than eight crayfish by a single camera is also possible but it can be technically complicated to arrange and install all the aquaria so that they all appear in the camera's field of view. Also the motion detection accuracy can decrease as the result of lower resolution obtained for each individual aquarium on the image.

Each illuminator has a peak wavelength of 850 nm and contains an internal photo sensor for switching ON and smoothly increasing the light output in the case of low ambient illumination level. The use of the external illuminator together with a camera allows monitoring of the crayfish motion during night time. Because the visual pigments of crustaceans are not sensitive to the wavelengths longer than 650 – 700 nm (Goldsmith 1972; Tsuchida et al. 2004), the selected NIR wavelength will not affect their circadian rhythm.

The cameras and the illuminators are placed under the aquaria at a distance which allows capturing all of them in the camera's field of view. Monitoring of the aquaria from the bottom side eliminates the effects of image distortion caused by water surface ripples and thus, allows obtaining better quality images for later processing.

2.4. Data processing

2.4.1. Cardiac activity

Examples of typical crayfish cardiac activity are shown in Fig. 2.3. The amplitude and the shape of heartbeat pattern slightly changes over time. The unique heartbeat pattern of individual crayfish varies with size, age, physiology and species (Burnett et al. 2013). Our observations show that in general, cardiac activity can be considered as having two peaks: a primary peak that defines the HR and a secondary peak that sometimes (more often at low HR) appears in the diastolic phase between primary peaks.

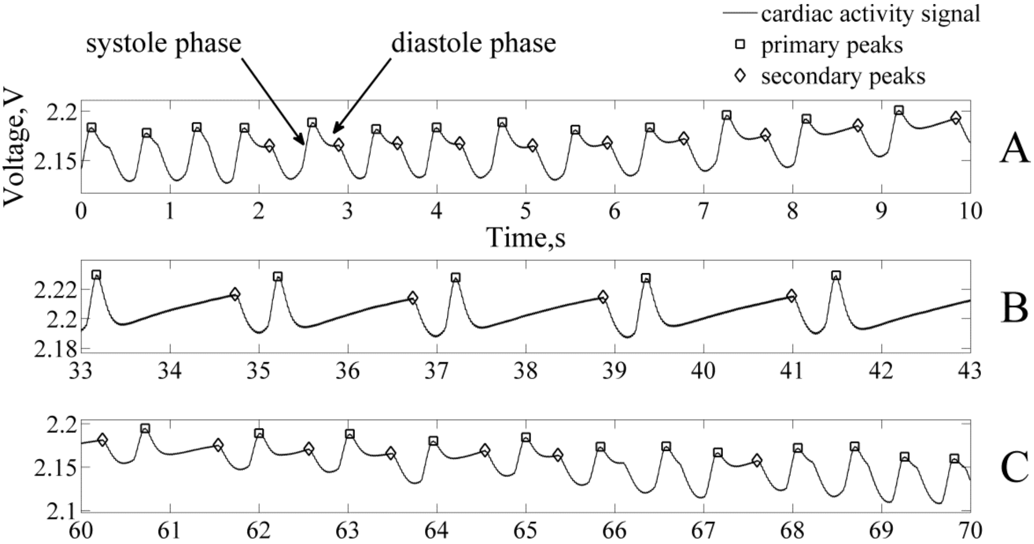


Fig. 2.3. Examples of cardiac activity signal recorded from one crayfish during 70 s interval. (A) Appearance of secondary peak. (B) Large amplitude secondary peak. (C) Disappearance of secondary peak.

The appearance of primary and secondary peaks in the cardiac signal provides the basis of the data processing sequence implemented in the NICCBAM system SW. Generally, it can be divided into three steps: preprocessing, detection of signal local extrema and classification of primary and secondary peaks (Fig. 2.4), and calculating chronotropic and inotropic parameters of cardiac signal.

At the preprocessing step, the high frequency noise of the signal is reduced by applying SW implementation of a RC low-pass filter. The filter parameters can be adjusted manually to keep the noise level visually lower than the peak detection threshold, without excessive distortion to signal shape. Typically used parameters result in the filter cutoff frequency of ~2.65 Hz. SW implementation of an RC filter generally allows tuning the filter parameters more easily and in a wider range in comparison to typical HW implementations that are usually designed without any tuning, largely to lower the cost.

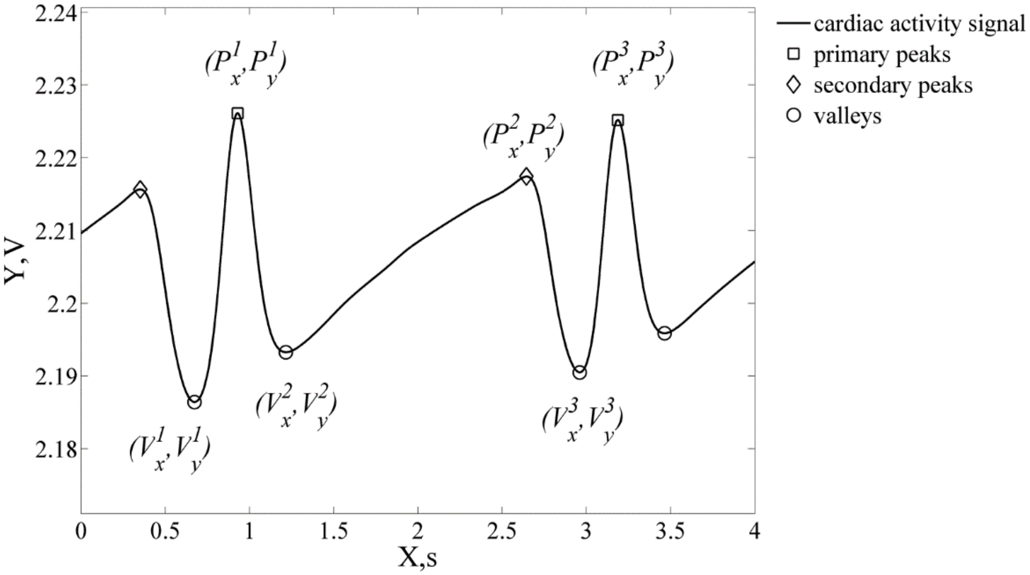


Fig. 2.4. Local extrema points of the crayfish cardiac activity signal used in data processing sequence.

Detection of signal local extrema at the second data processing step is done using the algorithm similar to the one implemented in function “findpeaks” in Mathworks Matlab. Briefly, the algorithm calculates the voltage differences between the tested data point and its neighbor points. The point is marked as extremum in case the absolute difference exceeds the specified voltage threshold. Typically used threshold value is 2 mV.

Classification of primary and secondary peaks is done by analyzing the shape of the peaks. Visual analysis of raw crayfish cardiac data shows that the shape of the secondary peaks generally differs from the shape of the primary peaks (Fig. 2.3). The rising slope of the secondary peak is gentler and the height is lower. These peculiar features of secondary peaks allow distinguishing them in SW by first calculating two-peak shape parameters: normalized peak height (*PHN*) and normalized peak rising slope (*PRSN*) using formulae in Table 2.1, and then assessing empirically obtained classification criterion *K*:

$$\frac{1}{1.146\,(PHN + 0.244)} - PRSN - 0.085 = K \qquad (2.1)$$

If the value of criterion *K* is positive, the peak is classified as secondary; otherwise it is classified as a primary peak. Obtaining of the classification criterion *K* is described in details in Section 3.3.

Table 2.1. Calculation of chronotropic and inotropic parameters of crayfish cardiac activity. P_x^1, P_y^1, P_x^2, P_y^2, P_x^3, P_y^3, V_y^1, V_x^2, V_y^2, V_x^3, V_y^3 – coordinates of the local extrema on the crayfish cardiac activity curve.

Parameter	**Description**	**Formula**
HR	heart rate	$60 / \left(P_x^3 - P_x^1\right)$
AMP	signal amplitude	$P_y^1 - min\left\{V_y^1, V_y^2\right\}$
PPSN	normalized previous peak slope	$-\left(\left(V_y^2 - P_y^1\right) / AMP\right) / \left(V_x^2 - P_x^1\right)$
NPSN	normalized next peak slope	$\left(\left(P_y^3 - V_y^3\right) / AMP\right) / \left(P_x^3 - V_x^3\right)$
PHN	normalized peak height	$\left(P_y^2 - min\left\{V_y^2, V_y^3\right\}\right) / AMP$
PW	peak width	$V_x^3 - V_x^2$
PWN	normalized peak width	$\left(V_x^3 - V_x^2\right) / \left(P_x^3 - P_x^1\right)$
PRSN	normalized peak’s rising slope	$\left(\left(P_y^2 - V_y^2\right) / AMP\right) / \left(P_x^2 - V_x^2\right)$
PFSN	normalized peak’s falling slope	$-\left(\left(V_y^3 - P_y^2\right) / AMP\right) / \left(V_x^3 - P_x^2\right)$
PSR	peak slopes ratio	$PRSN / PFSN$
PS	peak start	$V_x^2 - P_x^1$
PO	peak offset	$P_x^2 - P_x^1$
PE	peak end	$V_x^3 - P_x^1$
PSN	normalized peak start	$PS / \left(P_x^3 - P_x^1\right)$
PON	normalized peak offset	$PO / \left(P_x^3 - P_x^1\right)$
PEN	normalized peak end	$PE / \left(P_x^3 - P_x^1\right)$

At the final step, chronotropic and inotropic parameters of the cardiac activity are calculated separately for the primary and secondary peaks. The set of parameters is presented in Table 2.1.

Because of the need for combined analysis of the data from different crayfish which have generally different amplitudes of cardiac signal oscillations, normalization was introduced for the parameters *PPSN, NPSN, PHN, PRSN, PFSN* characterizing height and slopes of the peaks.

If the secondary peak was not present between subsequent primary peaks, only the *HR* and *AMP* parameters were calculated, parameters *PHN*, *PW*, *PWN* were set to zero and calculation of the rest of the parameters was skipped.

2.4.2. Motion detection

The NICCBAM system's motion detection module is primarily used for monitoring of crayfish BA. It can provide additional information on the functional state of crayfish independent of cardiac activity. Also the data from the module are used to facilitate cardiac activity data processing by excluding from consideration the periods of high crayfish locomotive activity. Rapid crayfish movements can result in varying of the signal offset or even short-time spikes in the signal that in turn can reduce the accuracy of cardiac activity parameter calculation.

The crayfish motion detection is based on the algorithms from OpenCV SW library. Briefly, the pixels belonging to moving object are detected based on the per pixel threshold values which are defined by the continuously updated statistical model of the background. The model is comprised of average and standard deviation (SD) values for each image pixel obtained using the standard running average and running variance methods (Piccardi 2004), respectively. Continuous updating of the background model improves the motion detection accuracy in case of varying ambient day-time illumination. Moreover, the algorithm eliminates the need in buffering of previous data (camera images): the model is updated based on the current image only.

The crayfish is considered to be moving if the number of moving-object pixels detected on the current image by the algorithm is higher than predefined threshold value. The threshold value depends on the size of the crayfish on the image and camera resolution. It can be interactively adjusted to improve the detection accuracy for the current system setup and size of the animal by comparing the manual analyzing of the video stream by operator and the results obtained using the automatic algorithm. The default threshold value of 100 pixels used by NICCBAM system was determined experimentally as a rounded average number of crayfish pixels manually estimated from 10 images with different moving individuals of commonly used size.

The algorithm is capable of detecting the motion of up to 16 crayfish simultaneously in real-time, with a frame rate of 1 frame per second (FPS) on the middle range PC without using the graphical card computing capabilities. The example of moving crayfish detected by the SW algorithm is shown in Fig. 2.5.

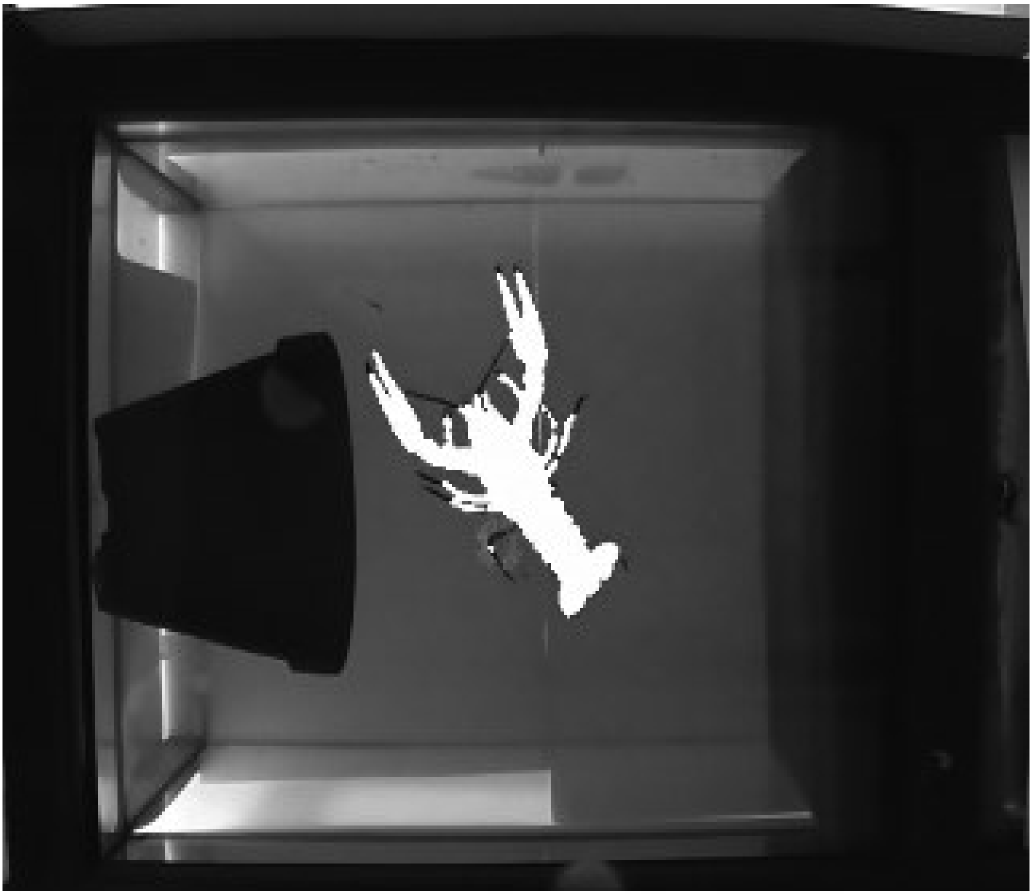


Fig. 2.5. Example of moving crayfish detected on the camera image. The image pixels classified by the motion detection algorithm as belonging to moving object are marked with white color.

2.5. Current system setup

The NICCBAM system shown in Fig. 2.6 is currently installed in South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, Vodňany, Czech Republic. It started operating from the beginning of 2014. Intellectual property rights on the system and on the cardiac sensor are protected by the registered national Czech Republic patent #305212 entitled "Method of behavioral monitoring of crawfishes and/or mollusks and behavioral system for monitoring behavior of crawfishes and/or mollusks" (see Appendix C) and utility model #27114 entitled "Non-invasive sensing element" (see Appendix D).

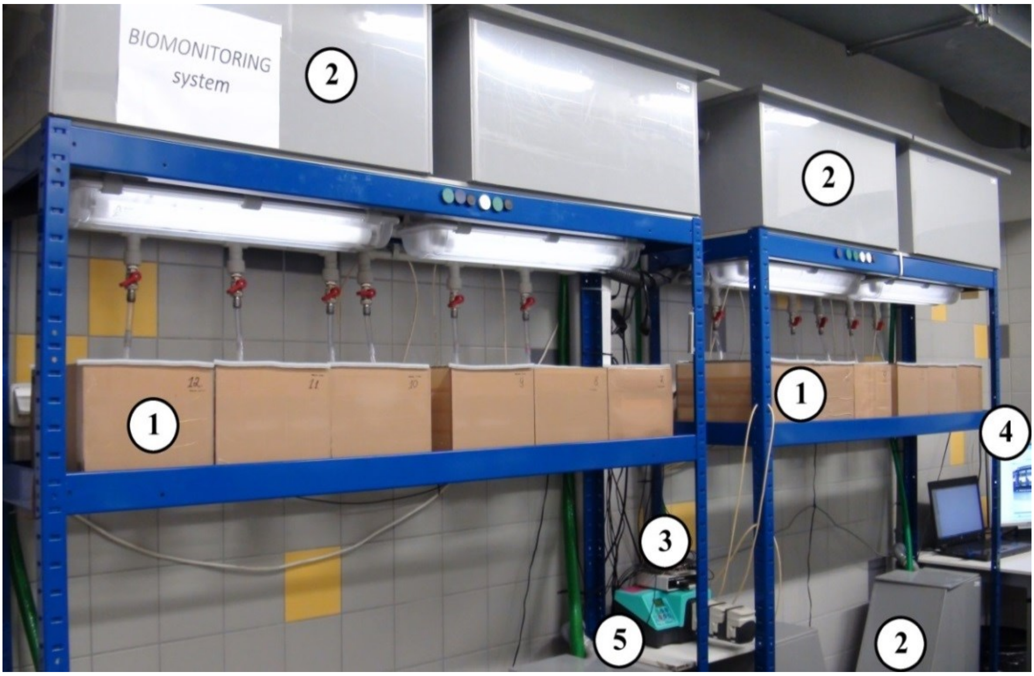


Fig. 2.6. Photo of the NICCBAM system current setup. (1) 12 crayfish aquaria. (2) Water reservoirs of the recirculation units. (3) TEDIA UDAQ-1416CA ADC. (4) PC with running SW. (5) Watson-Marlow peristaltic pump.

Current system setup is capable of monitoring up to 12 crayfish simultaneously in real-time 24 h a day. Each of the 12 aquaria (Fig. 2.6) contains one crayfish with attached NIR optical sensor (Fig. 2.7) and the crayfish security shelter (Fig. 2.8).

Fig. 2.7. Photo of the crayfish with attached NIR optical sensor.

The analog signals from the NIR optical sensors are digitized by UDAQ-1416CA ADC from TEDIA Company and transferred to the PC for processing over the USB interface. The example of crayfish cardiac activity information displayed by the SW to the PC's operator is shown in Fig. 2.9A.

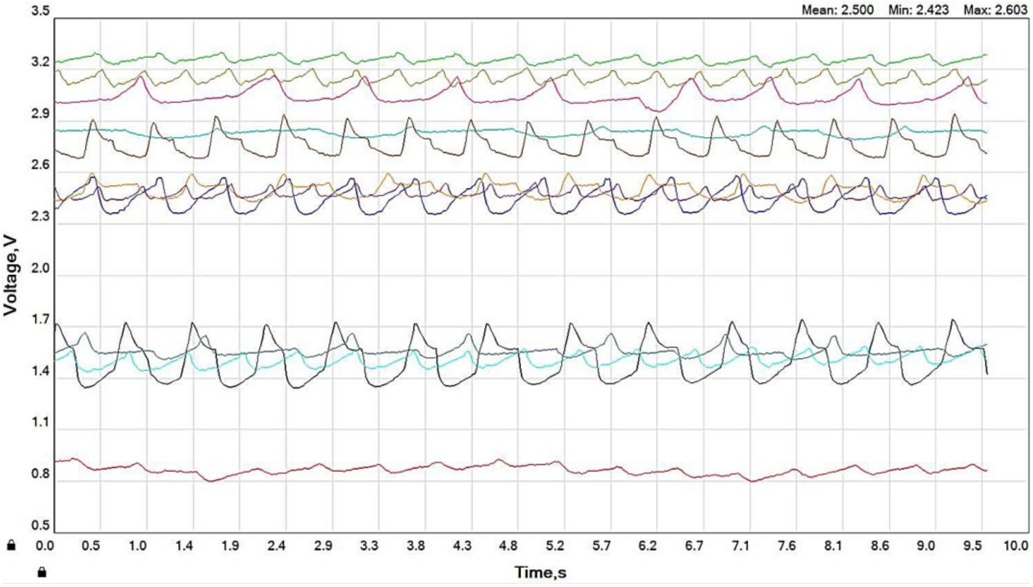
The walls of the aquaria are covered with non-transparent tape to prevent visual interaction among the neighbor crayfish and between crayfish and working operator. The bottom sides of the aquaria are transparent allowing crayfish motion detection (Fig. 2.9B) via the two installed IP8161 cameras and two TV6700 external illuminators from Vivotek and ABUS companies, respectively. In operating mode the cameras output video streams are set to grayscale mode and configured to a resolution of 1600 x 1200 pixels with 8 bits per pixel brightness range.

Each three consecutive aquaria are combined into a separate recirculation unit. Alternatively, each separate unit can be switched to the flow-through water circulation mode.

Fig. 2.8. Photo of the crayfish in the security shelter installed in the aquarium.

The system is also equipped with peristaltic pump from Watson-Marlow for transferring the water with the dissolved treatment into the aquaria during the experiments. The use of a peristaltic pump allows dosing out the treatment at a very low rate to avoid crayfish disturbance.

The operating principle of the system allows ethophysiological monitoring of crustaceans and molluscs. Up to now the system was successfully tested with *Pacifastacus leniusculus, Procambarus clarkia* and *Astacus leptodactylus* crayfish species.



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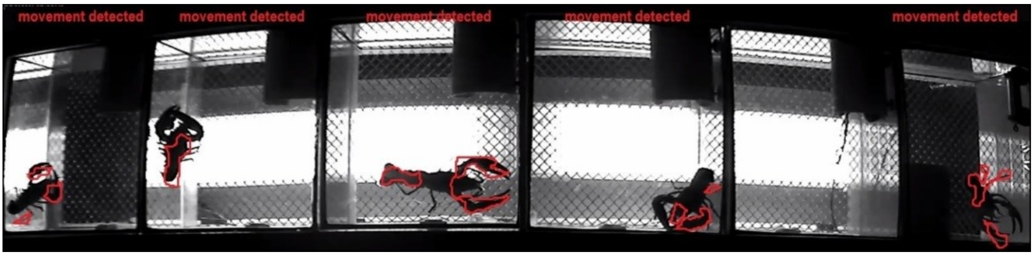


B

Fig. 2.9. Examples of the information displayed to the operator by the NICCBAM system SW. (A) Crayfish cardiac activities. (B) Crayfish motion detection.

3. Assessment

This chapter presents the results of the NICCBAM system evaluation experiments. The first experimental dataset described and analyzed in Section 3.1 was used to demonstrate the proper system operation, i.e. the system capabilities of recording crayfish cardiac activity in general. The second experimental dataset described in Section 3.2 and analyzed in the subsequent sections was used to show and prove the benefits of the novel cardiac signal shape analysis method over the other state of the art methods.

3.1. Combined recording of cardiac activity with NICCBAM and ECG systems

For the assessment of the NICCBAM system, an experiment on simultaneous recording of crayfish cardiac activity with both NICCBAM and well-known ECG systems was conducted. The ECG system used in the experiment consisted of two fine insulated wires connected to P5 Series AC preamplifier from Grass Technologies Company. The output of the preamplifier was connected directly to the input of the NICCBAM system's ADC to allow synchronous recording of the signals from both systems.

The male signal crayfish *Pacifastacus leniusculus* (Dana, 1852) used in the combined recording experiment (male; total length, 112 mm; carapace length, 56 mm; weight, 58 g) was caught in a baited trap in April 2015 in Vysočina Region, Czech Republic. The experiment was conducted in August 2015. The crayfish was kept in an outside pond under near-natural conditions prior to the experiment, then one day prior to beginning it was moved to a 10 L (water volume 7 L) glass aquarium for acclimatization. Two hours prior to recording, the NICCBAM optical sensor was attached using two-component, rapid epoxy adhesive to the dorsal area of the carapace over the heart. Then the two wires for ECG recording were inserted through the holes drilled in the carapace and cemented in place with instant adhesive. The photo of the crayfish with connected sensor and wires is shown in Fig. 3.1.

The recording of signals from both systems lasted 16 min. The data were processed to reduce the noise level: signal filtering was done with a zero-phase algorithm and the 8th order Butterworth low-pass filter with 10 Hz cutoff frequency implemented in Matlab functions "filtfilt" and "butter", respectively. Additionally, the signals were offset by subtracting the mean values, and the signal from the NICCBAM system was inverted for better visualization. A portion of the cardiac activity record is shown in Fig. 3.2.

Fig. 3.1. Photo of the signal crayfish with connected NIR optical sensor and two wires for combined recording of cardiac activity with NICCBAM and ECG systems.

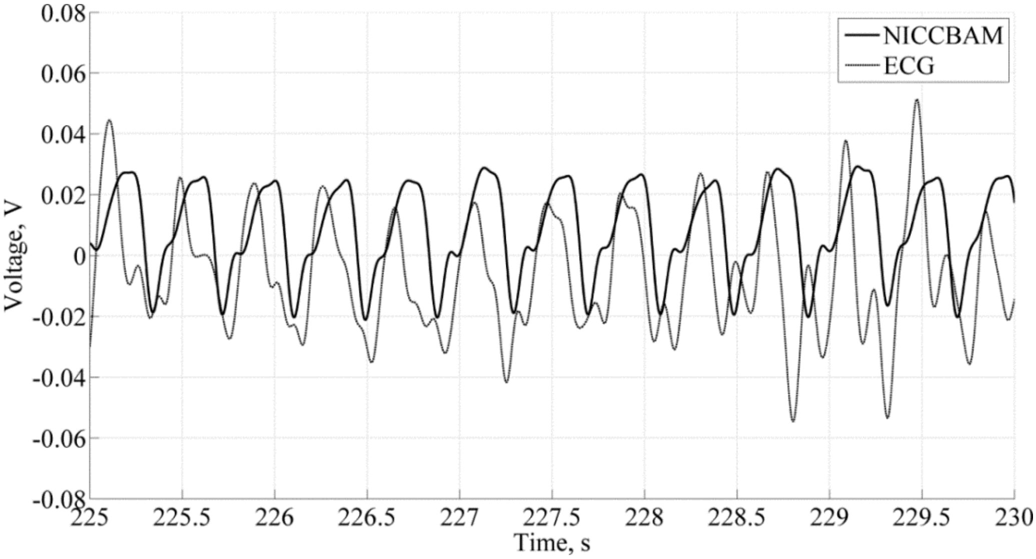


Fig. 3.2. Example of the signal crayfish cardiac activity synchronously recorded with NICCBAM and ECG systems.

Analyzing the data manually showed that both signals oscillated with approximately the same main frequencies, i.e. the HRs measured by both systems matched. Approximately constant time shift observed between the two signals can be explained by a delayed mechanical response (contraction) of the heart muscle to electrical stimulation. The ECG signal had smaller peaks between the main higher amplitude oscillations (Fig. 3.2). These peaks can be the cause of the secondary peaks observed in the signal from the NICCBAM system.

3.2. Experimental dataset

The experimental data for the assessment of the system and SW algorithms were obtained from signal crayfish *Pacifastacus leniusculus* (Dana, 1852), that were caught in baited traps in April 2014 in Vysočina Region, Czech Republic. The experimental group consisted of 12 adult individuals (half males, half females; mean total length, 109 ± 2 mm; mean carapace length, 55 ± 1 mm; mean weight, 57 ± 5.4 g).

The experiments were conducted at the end of March 2015. For acclimatization, crayfish were maintained for one week in individual 10 L (water volume 7 L) glass non-transparent aquaria, each equipped with a shelter. Prior to manipulations, selected crayfish were marked with individual numbers by permanent water-resistant marker. A week prior to beginning, sensors were attached to the dorsal side of the crayfish carapace directly over the heart using two-component, rapid epoxy adhesive. Throughout the experimental period, water temperature was 19.3-19.5 ºC, pH was 7.5-7.8, dissolved oxygen level was 9.0-9.5 mg/L, and the illumination regime was set and automatically maintained at 12 h of light and 12 h of dark. Crayfish were fed twice a week on *Chironomidae* larvae; food residue and excretory wastes were removed during the water exchange. No mortality occurred during the experimental period and the following two weeks.

The dataset contained 209 h of raw cardiac activity from 12 crayfish during seven experiments with the following treatments:

1. control,
2. food odor,
3. injured crayfish odor,
4. opposite sex crayfish odor,

5. predator odor,
6. chloramine-T,
7. ammonia ($NH_3 + NH_4^+$).

At each experiment except for the control, 50 ml of tap water with dissolved odor/chemical was pumped simultaneously into each aquaria using Watson-Marlow peristaltic pump at a flow rate of 25 ml/min. In case of the control experiment, 50 ml of pure tap water was pumped into each aquaria.

Treatments with numbers from 2 to 5 tested natural stimuli that can appear in water, while treatments with numbers 6 and 7 were artificial water contaminants; chloramine-T is a commonly used aquatic biocide and ammonia is one of the most important contaminants due to its toxic nature and ubiquity in surface water systems (Bloxham et al. 1999).

The water solution for the “food odor” experiment was prepared by mashing 1 g of *Chironomidae* in 1 L of tap water and then filtering out big food particles. For the “injured crayfish odor” experiment the treatment was obtained by keeping the crayfish together with its ablated thoracic leg in 1.8 L of tap water for 24 h. The treatment for the “opposite sex crayfish odor” experiment was obtained by keeping male and female crayfish in separate vessels containing 2 L of tap water for 24 h. The water from the male crayfish was during the experiment added to the aquaria with female crayfish and vice versa. The treatment for the “predator odor” experiment was obtained by keeping two pikeperch in 250 L of water for 48h. The final concentrations of chemicals in the aquarium water during the “chloramine-T” and “ammonia” experiments were 10 mg/L and 1 mg/L, respectively.

The specific concentration of chloramine used in the experiment is 2.5 times higher than the maximum concentration allowed by USA National Primary Drinking Water Regulations (www.epa.gov/safewater) and is commonly used in industry and aquaculture for water disinfection. The concentration of total ammonia nitrogen ($NH_3 + NH_4^+$) used in the experiment is twice higher than the drinking water concentration recommended by National Academy of Science (USA) and adopted by many European countries (http://public.health.oregon.gov).

One hour before each experiment the water flow through the aquaria was stopped. The recording of raw cardiac activity of each crayfish started 30 minutes before the treatment and stopped 30 minutes after treatment. After recording was finished, the water in aquaria was changed.

Each of the experiments (except for the control) was repeated three times during one day with ~ 3 h intervals between repetitions. The control experiment was also repeated three times but the repetitions were conducted on different days and at different time during the day. Before the “food odor” experiment the crayfish were not fed for 48 h.

First five experiments were held using all 12 experimental crayfish. The last two “chloramine-T” and “ammonia” experiments were held simultaneously on the last day of the experimentation period: six crayfish (three male and three female) received the chloramine-T treatment and six crayfish (three male, three female) received ammonia treatment. The above experimental procedure was chosen to exclude the possible long-term influence effects of potentially harmful chloramine-T and ammonia chemicals on crayfish physiological state in the rest of the experiments. The reduced number of crayfish used during the last two experiments was a result of the limited number of animals available at the time of the experiments.

On seven occasions, several hours before the start of an experiment, the sensor was accidentally detached from the crayfish as the result of its movement. After the sensor was reattached, the next 3 h of the crayfish data was considered unreliable and it was discarded from the final dataset (see Appendix A).

The dataset described above was processed by specially developed SW to find local extrema of the cardiac signal and roughly classify between primary and secondary peaks. The resulting ~ 980 000 of classified peaks were manually checked and found peak classification errors were corrected.

3.3. Classification of primary and secondary peaks

Large amplitude secondary peaks of the crayfish cardiac signal can be mistakenly considered as primary peaks; this would result in errors during *HR* calculation (Depledge and Andersen 1990; Burnett et al. 2013). In previous studies, the error rate was minimized by carefully adjusting the HW peak counting threshold according to particular cardiac signal shape/amplitude parameters and/or inverting the cardiac sensor output signal (Depledge and Andersen 1990). Unfortunately, no quantitative information on the accuracy of peak counting and classification algorithms was presented in the previous studies (Depledge and Andersen 1990; Fedotov et al. 2000; Burnett et al. 2013). In the current study, a classification algorithm based on analysis of the peak's shape was proposed, and its accuracy was addressed.

The manually chosen parameters *PHN* and *PRSN* were calculated using formulae in Table 2.1 for the complete dataset described above. The parameter data and manually verified classification data were used to optimize classification criteria, and for the assessment of peak classification accuracy.

The scatter plot of *PHN* and *PRSN* parameters calculated from the dataset is presented in Fig. 3.3. Note that the primary and secondary peaks are relatively well separated into two clusters in the plot. The chosen analytical criterion for classification of primary and secondary peaks was in the form of the following parametric equation:

$$\frac{1}{a(PHN+b)} + PRSN + c=0 \tag{3.1}$$

in which *PHN* and *PRSN* are the classified peak's parameters as defined in Table 2.1; *a*, *b* and *c* – optimization parameters.

The values of optimization parameters were determined using a Nelder-Mead simplex algorithm implemented in Matlab function "fminsearch" to minimize the number of classification errors in the dataset. The resulting values of 1.146, 0.244 and -0.085 for optimization parameters *a*, *b* and *c*, respectively, gave a classification error of less than 0.2% on the complete dataset (see Eq. 2.1).

A single peak classification error (assuming neighbor peaks are classified correctly) can result in approximately doubled or halved *HR* at a given point in time. This in turn can considerably affect the mean and variance of *HR*, especially if the period of time used for calculation of statistics is short.

3.4. Correlation between pairs of cardiac activity parameters

A secondary peak often appears in the crayfish cardiac activity signal at periods of low *HR*, while it disappears at high *HR*. To examine the relationship between *HR* and amplitude of the secondary peak, as well as the relationship between other pairs of cardiac activity parameters, a set of inotropic and chronotropic parameters was calculated from the complete dataset described above using formulae in Table 2.1.

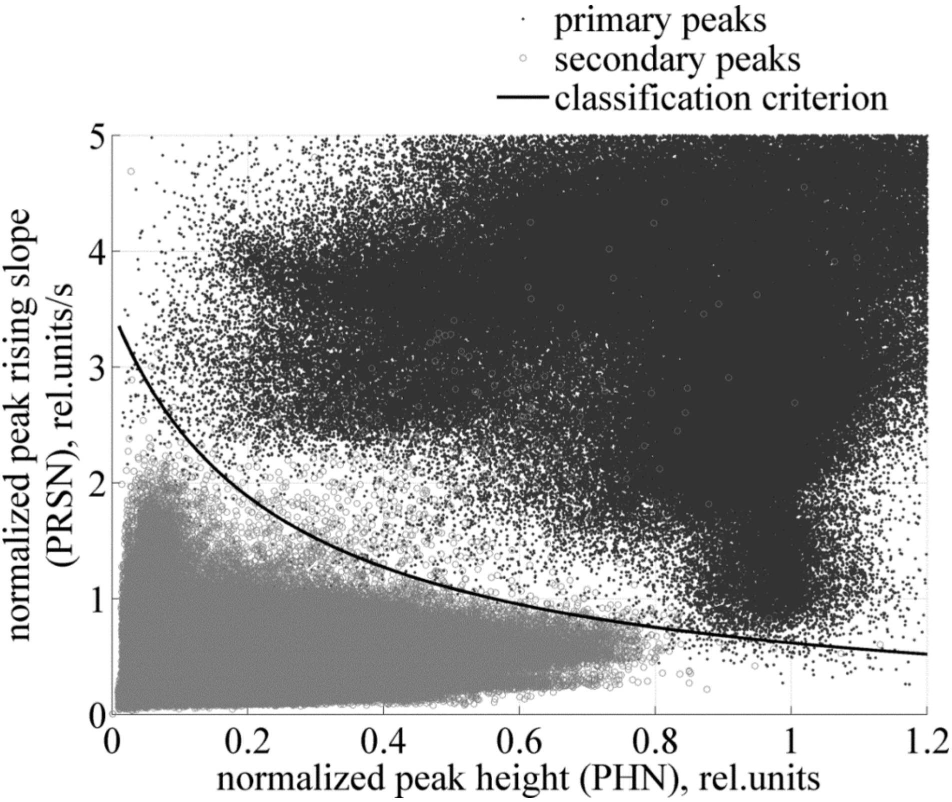


Fig. 3.3. Classification of primary and secondary peaks of the crayfish cardiac activity data based on peak's shape parameters: normalized peak height (*PHN*) and normalized peak rising slope (*PRSN*). Calculation of *PHN* and *PRSN* was done using formulae in Table 2.1.

The resulting Pearson product-moment and Spearman's rank correlation coefficients between all possible pairs from the whole set of chronotropic and inotropic parameters of cardiac activity are shown in Fig. 3.4. Calculation of the high number of correlations can be influenced by the Type I error. Therefore, the correlation significance test was performed. A p-value threshold maintaining 95% confidence in the total set of 120 correlation tests was set to 0.00042 after applying Bonferroni correction. Testing the hypotheses of no correlations, showed correlation significantly different from zero for all the parameter pairs except for {*AMP* : *PE*} pair for Pearson correlation and {*PEN* : *PPSN*} pair for Spearman correlation. Scatter plots of HR versus other parameters of cardiac activity for the complete dataset (see Section 3.2) and for 30 min record are shown in Fig. 3.5 and Fig. 3.6, respectively.

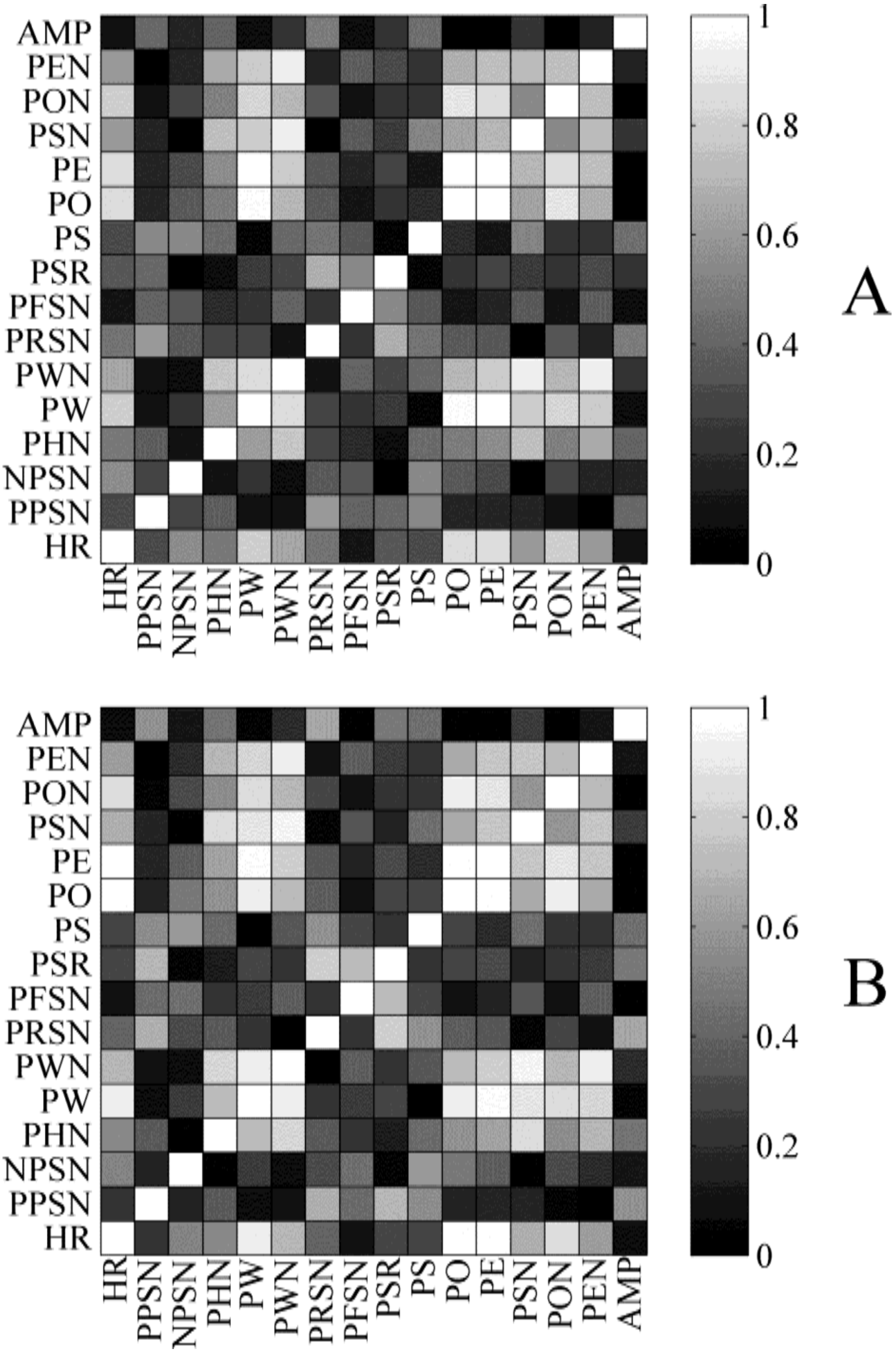


Fig. 3.4. Absolute values of correlation coefficients between pairs of crayfish cardiac activity parameters defined in Table 2.1 that can carry additional independent information on the crayfish state: (A) Pearson product-moment, (B) Spearman's rank.

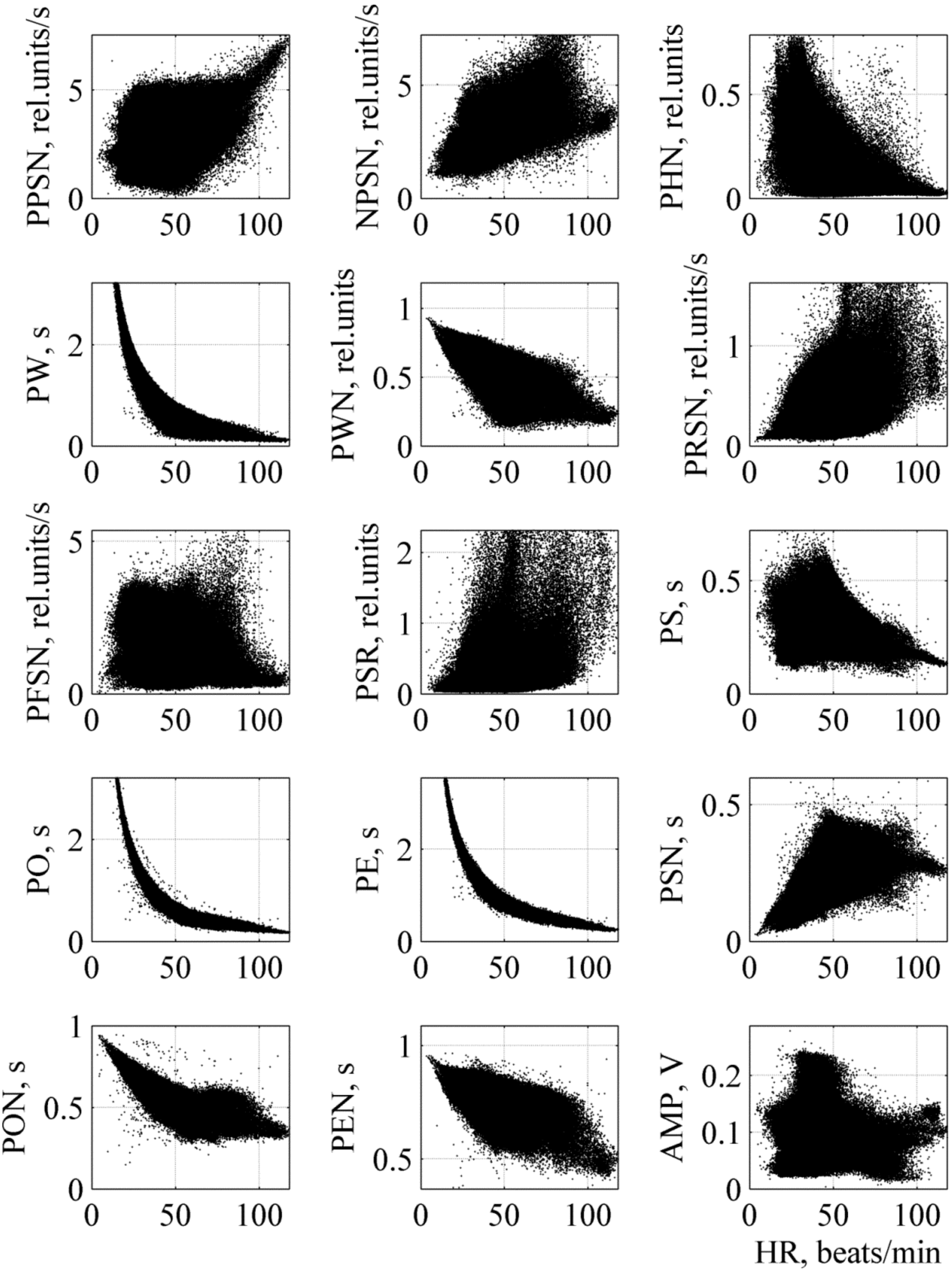


Fig. 3.5. Correlation between *HR* and other inotropic and chronotropic parameters of crayfish cardiac activity defined in Table 2.1 that can carry additional information on crayfish state other than what is provided by *HR* data. The plots contain all the data from the dataset described in Section 3.2.

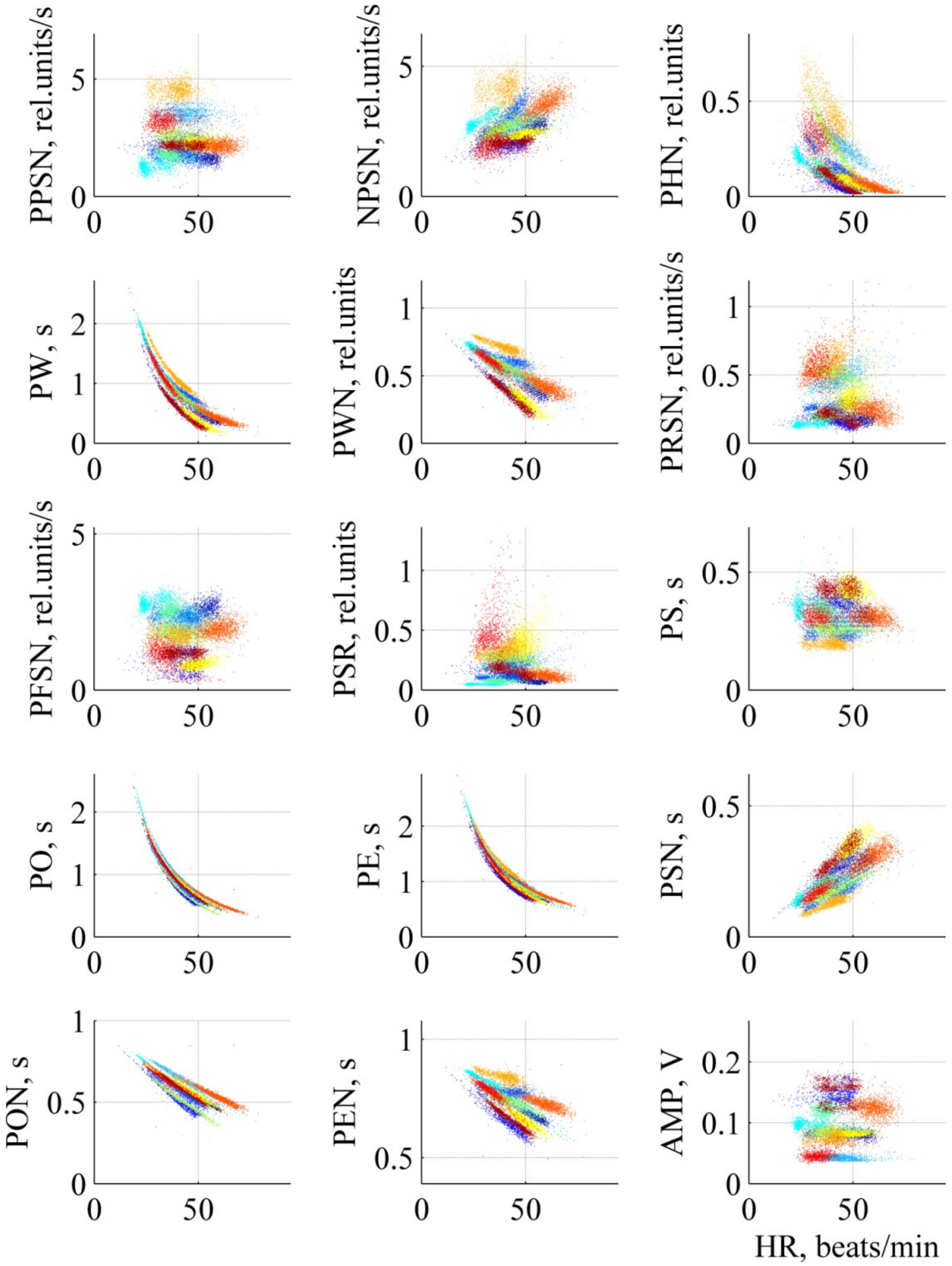


Fig. 3.6. Correlation between *HR* and other inotropic and chronotropic parameters of crayfish cardiac activity defined in Table 2.1 that can carry additional information on crayfish state other than what is provided by *HR* data. The plots contain the data from 12 crayfish simultaneously recorded during 30 min. Different colors were used to mark the data from each individual crayfish.

The *HR* was highly correlated with *PW, PO, PE* parameters (Fig. 3.4 - Fig. 3.6). Both Pearson product-moment and Spearman's rank correlation coefficients measuring linear correlation and monotonic relation between pairs of variables, respectively, gave absolute values greater than 0.80. Both Pearson and Spearman pairwise correlation coefficients between *PW, PO, PE* parameters were even higher, giving values greater than 0.92. Normalized chronotropic parameters *PWN, PON, PEN* were also highly correlated with *HR* and among each other.

Parameters describing the slopes of primary and secondary peaks (*PPSN, NPSN, PRSN, PFSN, PSR*), height (*PHN*) and starting time (*PS*, *PSN*) of secondary peaks and signal amplitude (*AMP*) were less correlated with *HR* and among themselves. Particularly, *AMP* parameter showed the lowest correlation to *HR* and overal correlation to all other parameters.

3.5. Effect of selected odors and chemicals on crayfish cardiac activity parameters

It was shown above that inotropic and chronotropic parameters of crayfish cardiac activity changed over time. However, it is an open question whether these parameters carry additional information on crayfish functional state independent of HR, or if they change randomly. It is also an open question as to whether monitoring of these parameters can be used for assessment of water quality. These questions were answered by analyzing the results of seven experiments described in Section 3.2.

Each crayfish cardiac activity recording was partitioned into two 12 min parts: the first part started 12 min before and the second started 3 min after the beginning of the experimental treatment. The initial 3 min of recorded data were considered unreliable because of the possible acoustic (turning on the pump) and/or visual disturbance caused by manipulations with the experimental equipment, and were excluded from the analysis. Manual analysis of the experimental data showed that typical crayfish response appeared within maximum 15 min after the start of the treatment, while later it exponentially decreased.

3.5.1. Analysis by statistical tests

It can be noticed in Fig. 3.6 that some of the cardiac activity parameters are not normally distributed. To check, a total of 6604 Kolmogorov-Smirnov tests were performed for each of the parameters in each of the 12 min parts of the data, except that for 84 cases where specific parameter values were not calculated because of the absence of secondary peaks in the original cardiac signal. The calculation was done using "kstest" function from Mathworks Matlab.

Performing multiple hypotheses tests can result in appearance of Type I errors ("false positives") so the significance level during the tests should be adjusted to control the familywise error rate. In the current set of tests, the used p-value threshold maintaining 95% confidence was set to $7.57 \cdot 10^{-6}$ after applying Bonferroni correction.

The results of the tests showed that the parameters differed significantly from normal distributions in 6403 (~ 97%) cases. It means that in general, cardiac activity parameters are distributed non-normally and that subsequent statistical analyses should not assume distributions normality.

For testing of statistical differences in crayfish cardiac activity during the experiments, a set of Mann-Whitney U (Wilcoxon rank-sum) tests was performed for pairs of cardiac activity parameter data vectors: before vs. during the treatments. The calculation was done using "ranksum" function from Mathworks Matlab. In the current set of 112 tests, the p-value threshold, maintaining 95% confidence was set to $4.46 \cdot 10^{-4}$ after applying Bonferroni correction. The results of the tests are summarized in Table 3.1.

The least expressed changes of cardiac activity parameters were observed during the "control" and "opposite sex crayfish odor" experiments. Statistically significant differences during the "control" experiment were revealed only in *HR*, *PS* and *AMP* cardiac activity parameters, while for the "opposite sex crayfish odor" experiment the null hypothesis could not be rejected for all of the parameters.

The results of the tests for the "food odor" experiment revealed that all of the cardiac activity parameters changed significantly. During other four experiments, only a few parameters showed no significant changes: *PW*, *PSR*, *PSN* and *PON* for "injured crayfish odor", *NPSN* and *AMP* for "predator odor", NPSN and AMP for “chloramine-T” and HR and PS for “ammonia” experiments.

Table 3.1. Statistically significant difference revealed in pairs of cardiac activity parameter data vectors (before vs. during the treatment) at each of the experiments (Mann-Whitney, $p < 4.46 \cdot 10^{-4}$).

Parameters	**control**	**food odor**	**injured crayfish odor**	**opposite sex crayfish odor**	**predator odor**	**chloramine-T**	**ammonia**
HR	yes	yes	yes	no	yes	yes	no
PPSN	no	yes	yes	no	yes	yes	yes
NPSN	no	yes	yes	no	no	no	yes
PHN	no	yes	yes	no	yes	yes	yes
PW	no	yes	no	no	yes	yes	yes
PWN	no	yes	yes	no	yes	yes	yes
PRSN	no	yes	yes	no	yes	yes	yes
PFSN	no	yes	yes	no	yes	yes	yes
PSR	no	yes	no	no	yes	yes	yes
PS	yes	yes	yes	no	yes	yes	no
PO	no	yes	yes	no	yes	yes	yes
PE	no	yes	yes	no	yes	yes	yes
PSN	no	yes	no	no	yes	yes	yes
PON	no	yes	no	no	yes	yes	yes
PEN	no	yes	yes	no	yes	yes	yes
AMP	yes	yes	yes	no	no	no	yes

3.5.1. Descriptive statistical analysis

Manual analysis of distributions of cardiac activity parameters showed that they were generally unimodal (see Fig. 3.6). In relatively rare cases the distributions were found to be bimodal that can be explained by natural transitions between functional states during the sampling period.

In the current study, mean and SD measures were chosen for characterization of the distributions. They were calculated for each of the 16 cardiac activity parameters (see Table 2.1) from each part of the data (see Appendix A). The mean and SD of parameters for each individual crayfish were then normalized by the mean value of the corresponding parameters before the treatment and averaged over the group of crayfish using the following equations:

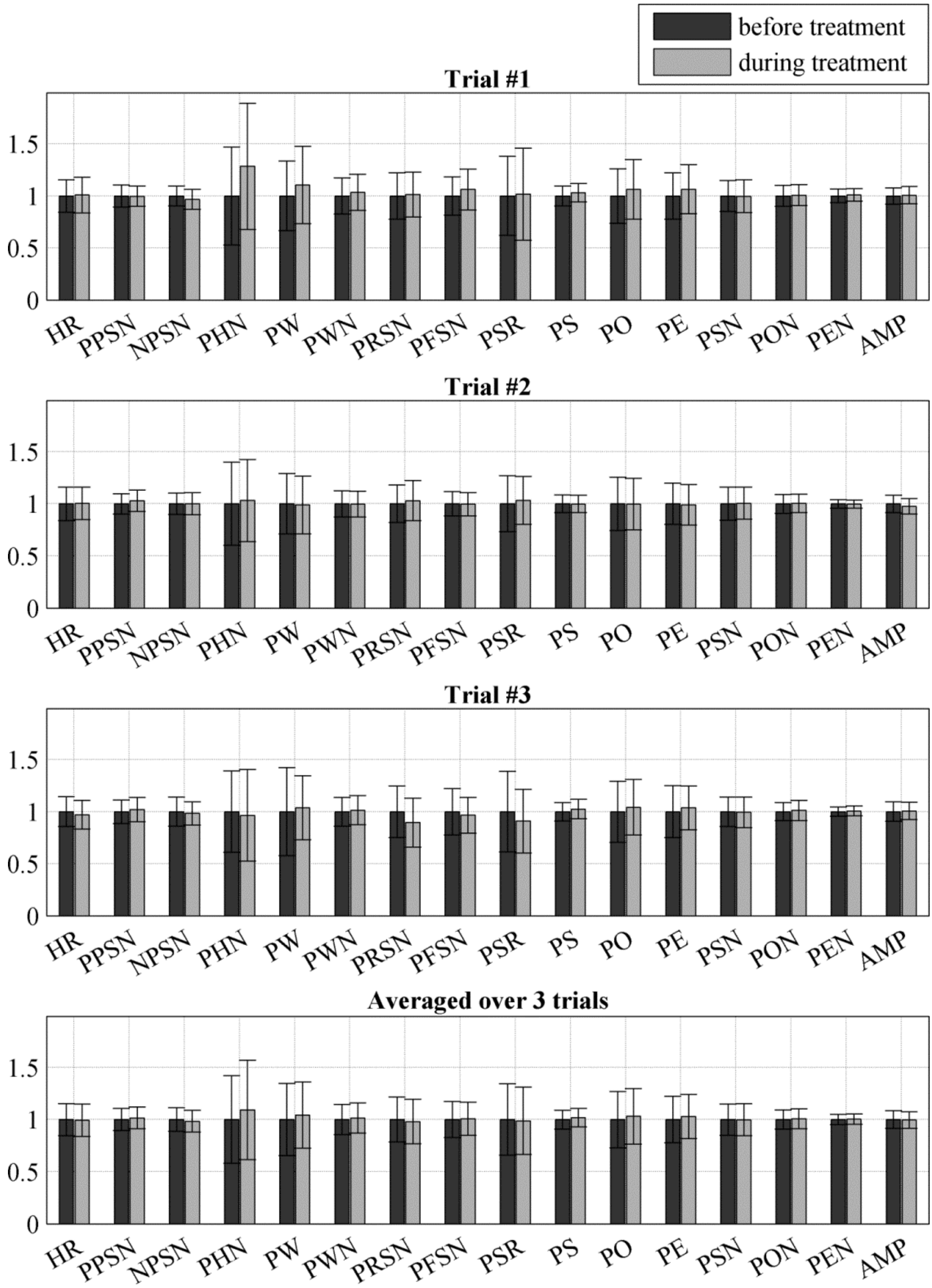
$$M^j = \frac{\sum_{i=1}^{N} M_i^j / M_i^j}{N} = 1 \tag{3.2}$$

$$\acute{M}^j = \frac{\sum_{i=1}^{N} \acute{M}_i^j / M_i^j}{N} \tag{3.3}$$

$$SD^j = \frac{\sum_{i=1}^{N} SD_i^j / M_i^j}{N} \tag{3.4}$$

$$\acute{SD}^j = \frac{\sum_{i=1}^{N} \acute{SD}_i^j / M_i^j}{N} \tag{3.5}$$

in which $M^j, SD^j, \acute{M}^j, \acute{SD}^j$ – normalized mean and SD values of *j*-th parameter over the group of crayfish before and during treatment, respectively; N – number of crayfish used in the experiment; M_i^j, SD_i^j, $\acute{M}_i^j$, $\acute{SD}_i^j$ – normalized mean and SD values of *j*-th parameter for *i*-th crayfish before and during treatment, respectively. The normalization of the parameter’s mean and SD values was done to allow proper averaging of the data originating from different crayfish generally having different distribution means (see Fig. 3.6). Also it improves the visualization of all the parameters having different scales on a single graph. The relative changes in mean and SD of crayfish cardiac activity parameters (see Table 2.1) in response to selected odors and chemicals are shown in Fig. 3.7 - Fig. 3.13 and are summarized in Table 3.2 and Table 3.3.



Trial #1

Trial #2

Trial #3

Averaged over 3 trials

Fig. 3.7. Relative changes in mean and SD of crayfish cardiac activity parameters in response to adding of pure tap water during the control experiment averaged over 12 crayfish.

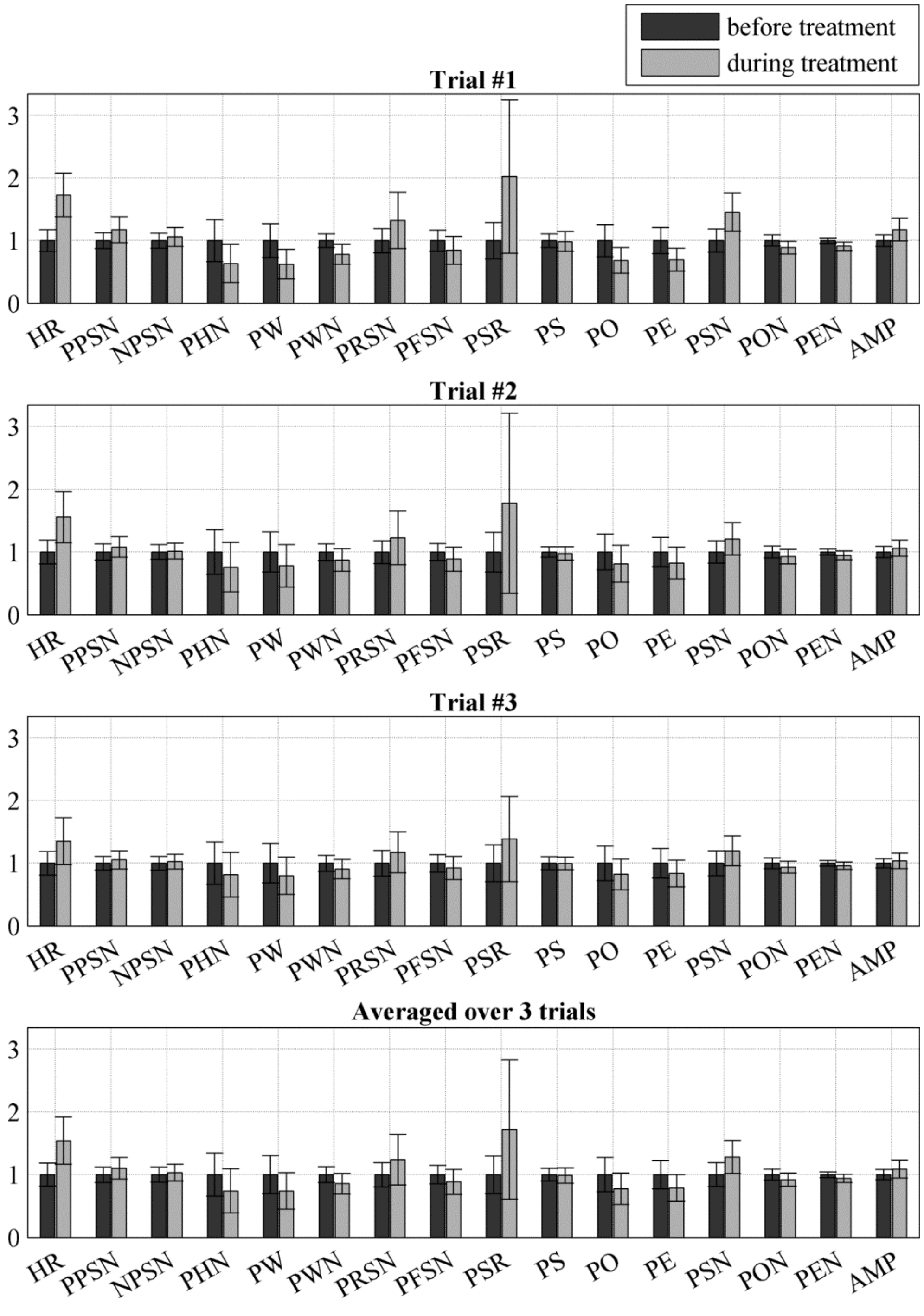


Fig. 3.8. Relative changes in mean and SD of crayfish cardiac activity parameters in response to food odor averaged over 12 crayfish.

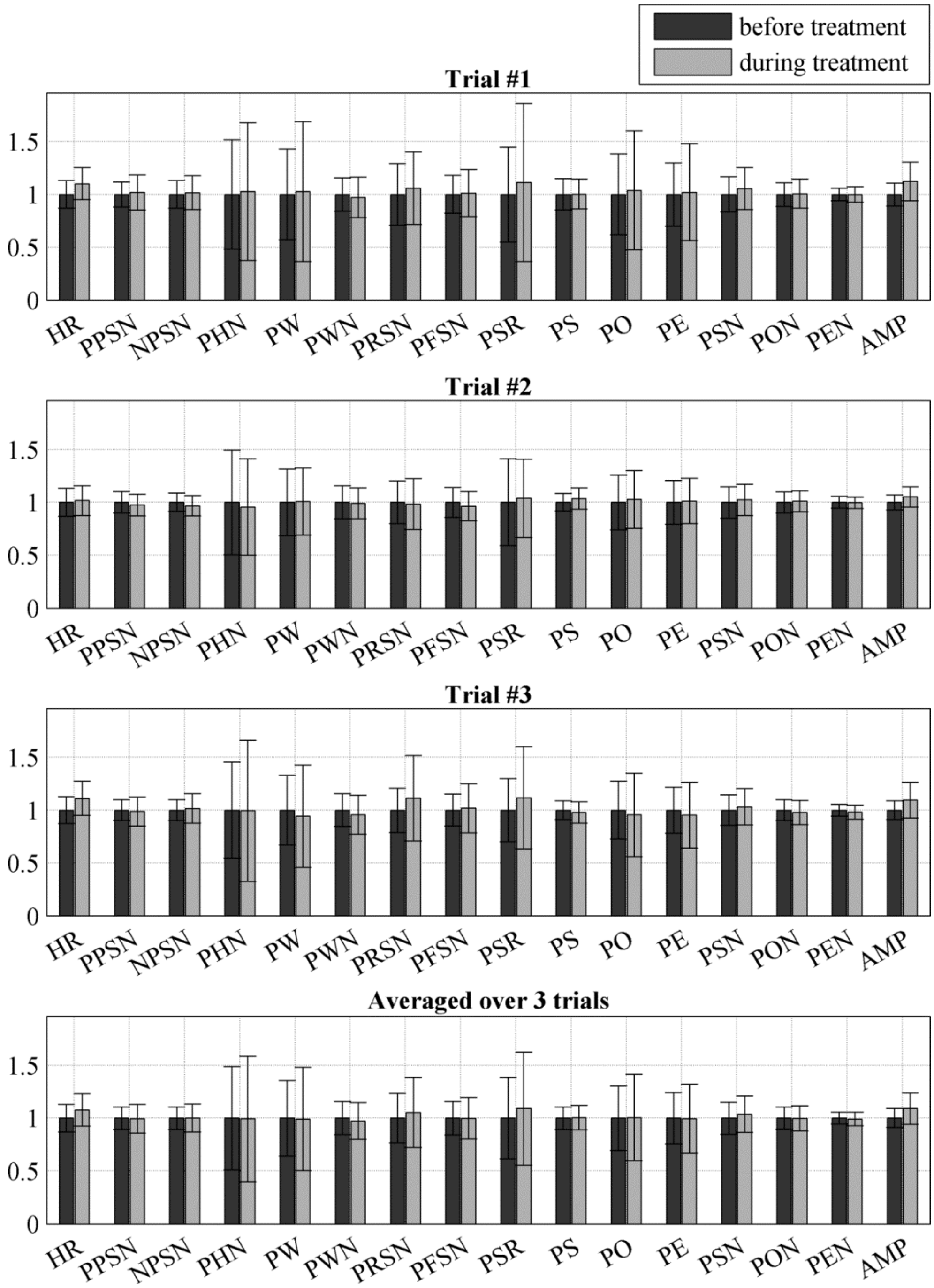


Fig. 3.9. Relative changes in mean and SD of crayfish cardiac activity parameters in response to injured crayfish odor averaged over 12 crayfish.

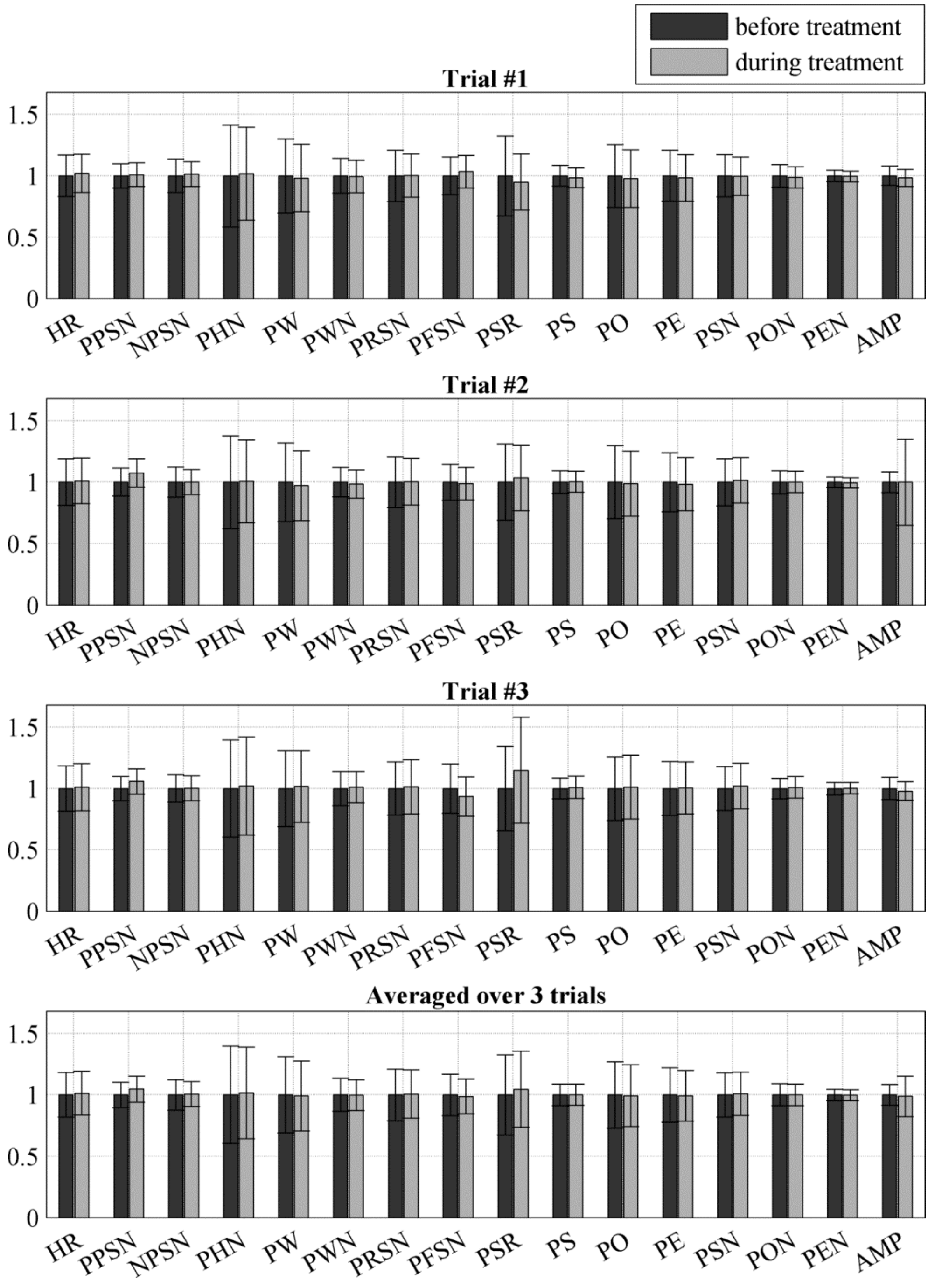


Fig. 3.10. Relative changes in mean and SD of crayfish cardiac activity parameters in response to opposite sex crayfish odor averaged over 12 crayfish.

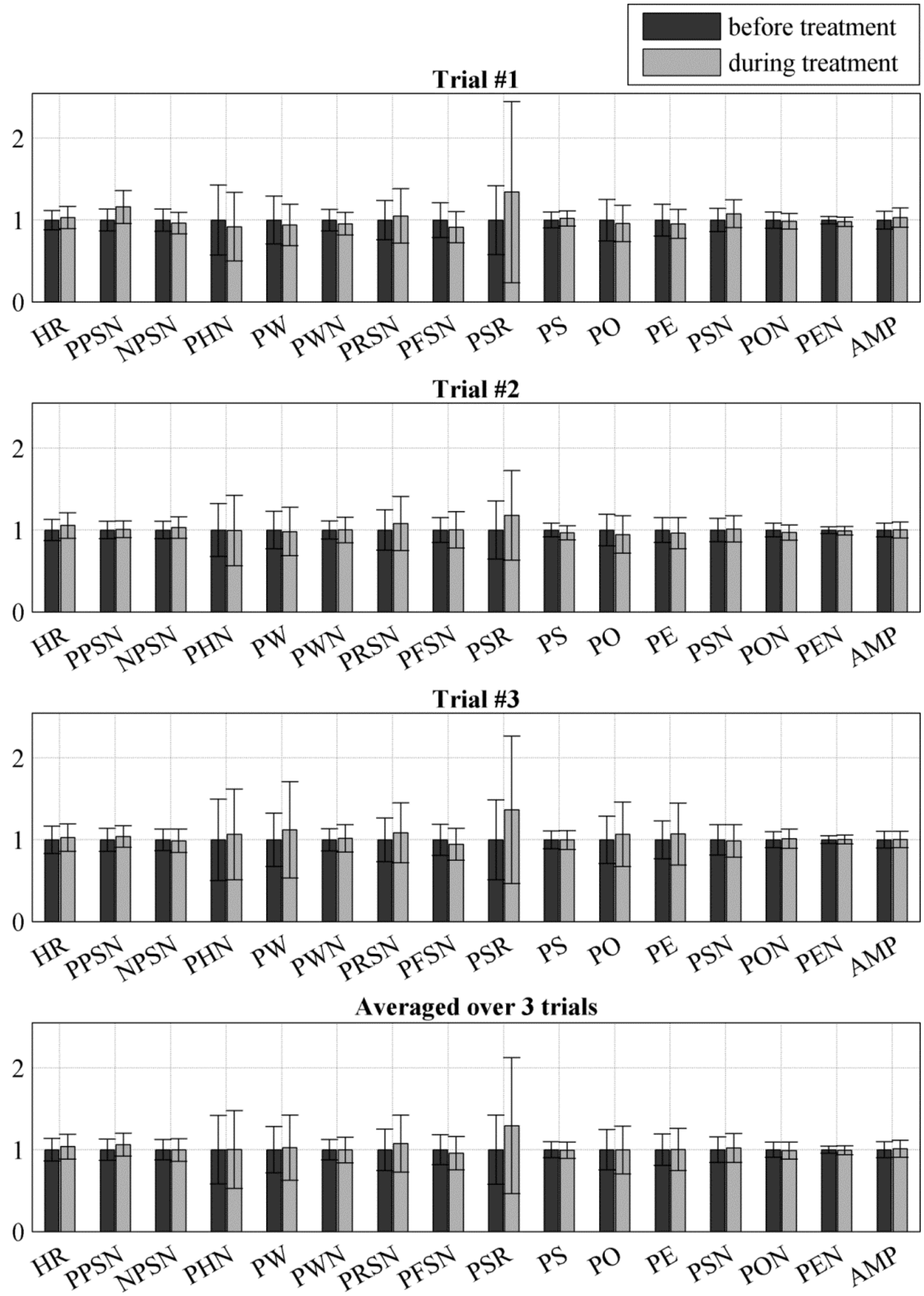


Fig. 3.11. Relative changes in mean and SD of crayfish cardiac activity parameters in response to predator odor averaged over 12 crayfish.

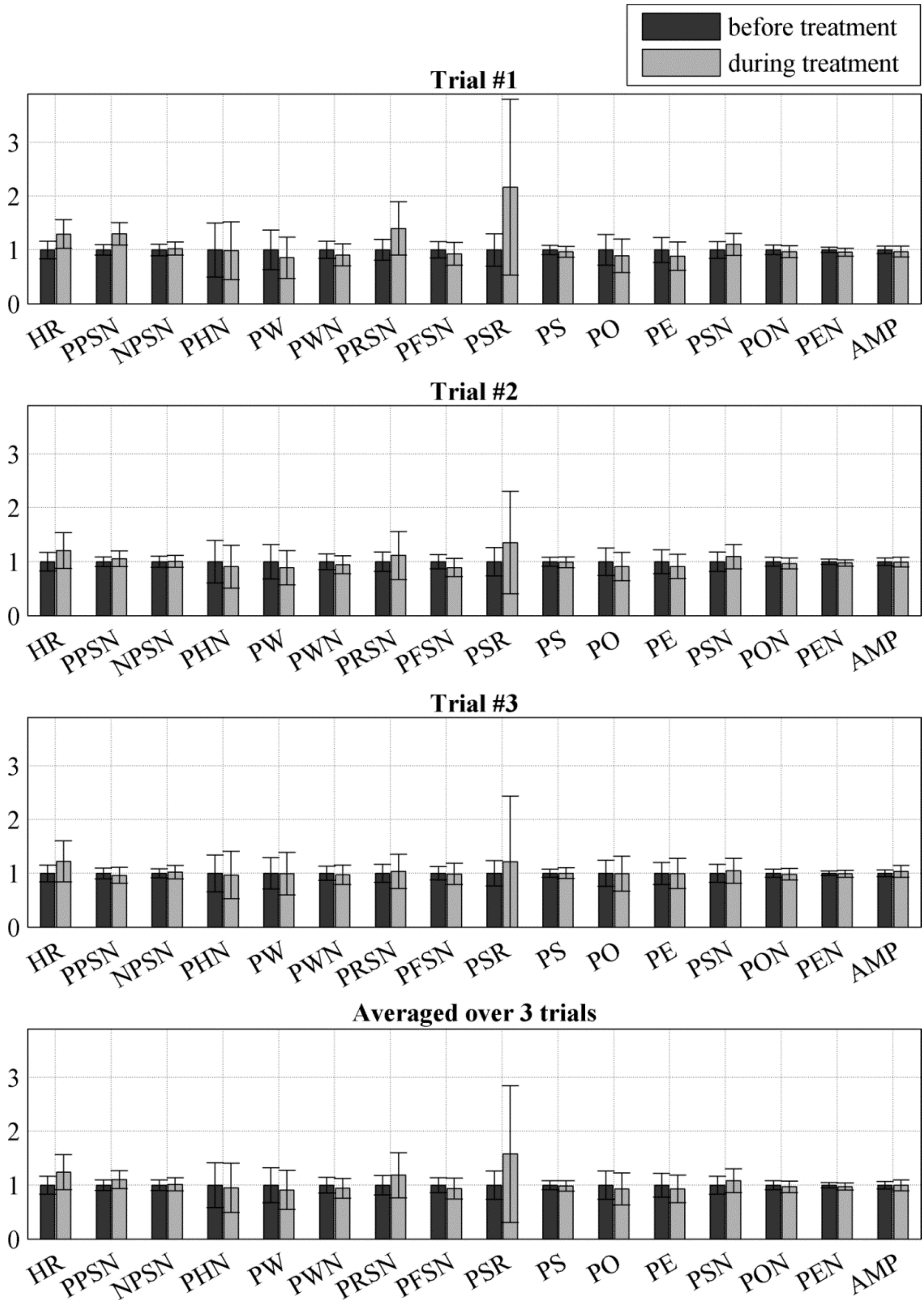


Fig. 3.12. Relative changes in mean and SD of crayfish cardiac activity parameters in response to chloramine-T in concentration 10 mg/L averaged over six crayfish.

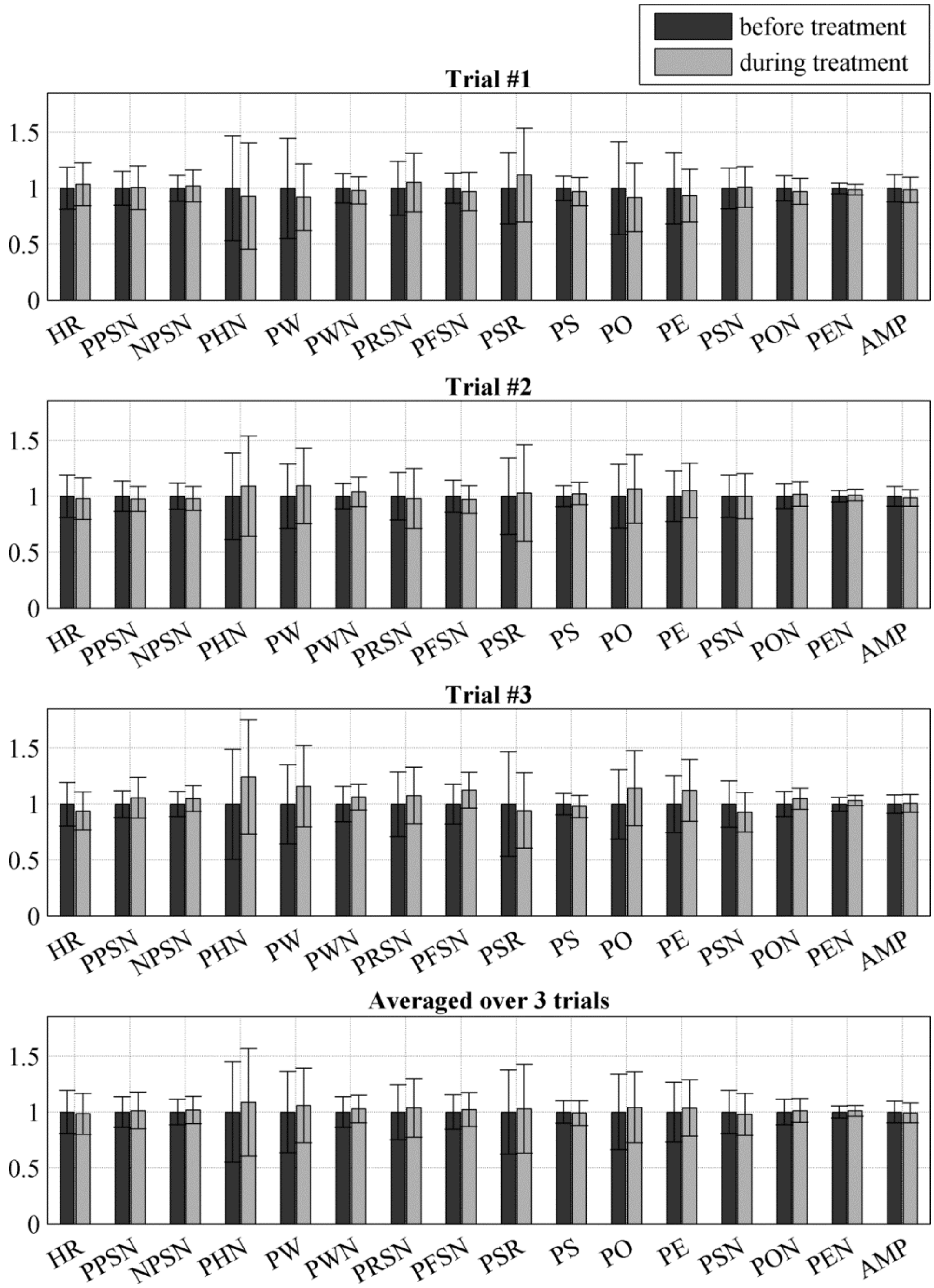


Fig. 3.13. Relative changes in mean and SD of crayfish cardiac activity parameters in response to ammonia in concentration 1 mg/L averaged over six crayfish.

Table 3.2. Relative changes in mean of crayfish cardiac activity parameters in response to selected odors and chemicals expressed in percents.

	control	**food odor**	**injured crayfish odor**	**opposite sex crayfish odor**	**predator odor**	**chloramine-T**	**ammonia**
HR	-0.6	54.1	7.8	1.4	3.7	24.1	-1.6
PPSN	1.6	10.1	-0.7	4.7	6.1	10.4	1.3
NPSN	-1.6	3.3	0.1	0.6	-0.4	1.7	1.7
PHN	9.2	-26	-0.8	1.5	0.4	-4.7	8.7
PW	4.2	-26.2	-0.9	-0.9	2.5	-8.7	5.7
PWN	1.4	-14.4	-2.7	-0.3	-0.4	-5.9	2.7
PRSN	-1.9	23.8	5.4	0.7	7.5	18.3	3.7
PFSN	0.8	-11.4	-0.1	-1.4	-4.3	-6.4	2.2
PSR	-1.2	72.1	9.1	4.5	29.4	57.8	3
PS	1.7	-1.4	0.4	-0.1	-0.8	-1.5	-0.9
PO	3.2	-22.5	0.6	-0.7	-0.4	-6.9	4.2
PE	2.9	-21.2	-0.6	-0.9	0.2	-7	3.6
PSN	-0.1	28.2	3.7	1.1	1.9	8.1	-2
PON	0.7	-8.1	-0.2	-0.1	-1	-2.8	1.3
PEN	0.5	-6	-0.8	-0.2	-0.8	-2.5	1.1
AMP	-0.3	9	9	-1.3	1.2	-0.2	-0.7

Table 3.3. Relative changes in SD of crayfish cardiac activity parameters in response to selected odors and chemicals expressed in percents.

	control	**food odor**	**injured crayfish odor**	**opposite sex crayfish odor**	**predator odor**	**chloramine-T**	**ammonia**
HR	1.5	105	16.9	-2.2	10.5	99.8	-4.8
PPSN	-0.8	39.8	26.9	2.5	9.8	72.8	19.9
NPSN	-7.6	12.8	25.1	-17.7	10.2	22	6.2
PHN	13.9	2.7	21.4	-6.1	13.3	10.6	6.6
PW	-8.4	-4.1	36.7	-8.1	40.7	11.9	-8.3
PWN	-0.2	32.6	11.5	-6.1	23	27.7	-8.6
PRSN	-0.8	108.2	42.2	-6.9	36.8	133.6	5.6
PFSN	-8.7	34.8	25.6	-15.6	10.3	41.4	-1
PSR	-5.8	269.9	39	-5	95.3	379.5	5.4
PS	-1.4	21.9	7.6	-1.2	1.2	19.6	9.9
PO	-1.2	-8.8	34.8	-6.4	17.9	14.5	-5.7
PE	-4.7	-4.2	35.7	-7.6	33.6	16.2	-5.3
PSN	1.9	42	13.3	-2.8	11.9	32.2	-2.9
PON	3.3	15.2	12.8	-2.9	10.6	29.3	-4.5
PEN	-0.8	50.2	16	-5.3	22.8	44.3	-9.9
AMP	-6.1	68.2	65.8	94.2	5.6	49	-9.7

The results of the “control” experiment represent the reference level of the changes in cardiac parameters during the treatment for other six experiments. Comparing the result of other experiments to the “control” one can allow revealing the changes in the cardiac parameters caused by experimental treatment only and excluding the effects caused by manipulations with the equipment during the experiment.

It can be seen in Fig. 3.7 and in Table 3.2 and Table 3.3 that the mean and SD values of cardiac parameters at the “control” experiment changed only slightly during the treatment. The biggest changes were observed for mean and SD of *PHN* parameter during the 1st trial while during the 2nd and the 3rd trials the effect was much less evident. The average mean and SD values of the *PHN* parameter over the three experimental trials were 9.2% and 13.9%, respectively.

In general, the results of the “food odor” experiment (see Fig. 3.8) showed the biggest changes in cardiac parameters during the treatment in comparison to the other six experiments. The only parameter that showed relatively small mean value change in all of the trials was *PS*. However, its average SD value over all trials showed an increase by 21.9%. The *PSR* parameter changed the most in all the three trials and its average mean and SD values increased by 72.1% and 269.9%, respectively.

In the “injured crayfish odor” experiment (see Fig. 3.9), mean values of the *HR*, *PWN*, *PSR*, *PSN* and *AMP* parameters changed in all the three experimental trials. The average results over the three trials showed the biggest changes by approximately 9% in the mean values of *PSR* and *AMP* parameters. The largest SD increase was by 65.8%, observed for the *AMP* parameter.

The treatment effect during the “opposite sex crayfish odor” experiment (see Fig. 3.10) on the mean values of cardiac parameters was relatively small. The biggest averaged over three trials increase by 4.7% and 4.5% in the mean values were obtained for the *PPSN* and *PSR* parameters, respectively. However, the mean value of the *PSR* parameter increased only during the 2nd and 3rd experimental trials. The biggest averaged increase in SD by 94.2% was observed for the *AMP* parameter.

The results of the “predator odor” experiment (see Fig. 3.11) showed the noticeable changes in mean values of the *HR*, *PPSN*, *PRSN* and *PSR* parameters in all the three experimental trials. The biggest averaged over the three trials increase by 29.4% and 95.3% for mean and SD values, respectively, was obtained for the *PSR* parameter.

The results of the "chloramine-T" experiment (see Fig. 3.12) showed noticeable changes in mean and SD values of all the parameters except for *NPSN*, *PHN*, *PS* and *AMP* in all the three experimental trials. The biggest averaged increase by 57.8% and 379.5% for mean and SD values, respectively, was obtained for the *PSR* parameter.

The results of the "ammonia" experiment (see Fig. 3.13) showed no noticeable changes in the mean values of cardiac parameters during the treatment in comparison to the "control" experiment. However, the averaged changes in the SD values of *HR*, *PPSN*, *PWN*, *PRSN*, *PS*, *PO*, *PEN* and *AMP* parameters were noticeably bigger than for the "control" experiment, but the same changes were not observed in each of the experimental trials separately.

Effect of repetitive treatment with chloramine-T in concentration 10 mg/L on the selected parameters of crayfish cardiac activity

Application of NICCBAM system as a basis BEWS requires demonstrating its sensitivity to repetitive appearance of chemicals in water. The less expressed effect of a subsequent impact of a chemical on a crayfish cardiac activity resulting in the decrease of system's sensitivity can be caused by adaption of crayfish to the chemical and/or damaging of the crayfish's chemoreceptors by the chemical.

The effect of the repetitive treatment with chloramine-T on crayfish cardiac activity parameters is shown in Fig. 3.14. The data for the graph was obtained during the "chloramine-T" experiment described above (see Section 3.2).

It can be seen in Fig. 3.14 that the effect of chloramine-T on the mean values of cardiac activity parameters like *PPSN*, *PW*, *PWN*, *PRSN*, *PSR*, *PO*, *PE* and *PSN* noticeably decreased from trial #1 to trial #3. During the last trial #3 the mean values of all the parameters except for *HR* and *PSR* did not change noticeably and were almost the same as before the treatment. However, the same effect was not observed for the SD values of the parameters.

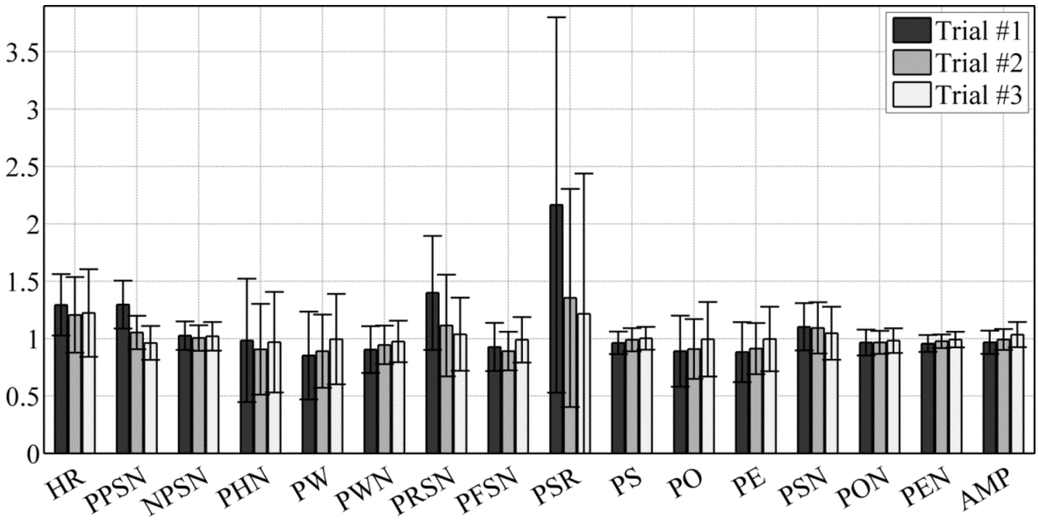


Fig. 3.14. Normalized mean and SD of crayfish cardiac activity parameters after repetitive treatment with chloramine-T in concentration 10 mg/L averaged over six crayfish.

4. General discussion

The NICCBAM system described here is relatively inexpensive and easily to manufactured in comparison to similar systems, e.g. CAPMON (Depledge and Andersen 1990), SIBWQM (Kholodkevich et al. 2008) and improved CAPMON (Burnett et al. 2013). The approximate price of the system's HW without PC but including the motion detection module containing two NIR cameras and two NIR illuminators is 950 EUR. The total price of the components and materials required for manufacturing of one sensor is 1-2 EUR depends on the ordering quantity. The NIR optical sensors that can be connected to the crayfish within a few minutes do not affect the crayfish behavior and do not restrict its movements. Cardiac and behavioral activities of up to 16 crayfish can be monitored simultaneously 24 hour/day. Also, the system allows recording and storing raw cardiac activity data for further manual or semiautomatic analysis.

Implementing most of the signal preprocessing and filtering in SW not only simplifies the HW design but also minimizes the signal distortion that can be caused by improperly adjusted HW parameters. Also, it grants the operator more flexibility and easier control over the data processing sequence.

The NICCBAM system cardiac activity monitoring method based on NIR optical sensor was evaluated using the well-known ECG method. The evaluation showed that HRs measured by both methods matched, i.e. it can be concluded that the NICCBAM system was actually registering the cardiac activity. Also the manual analysis of the ECG signal showed that it has a complex multiple peaks shape that can be the cause of the observed secondary peaks in the signal from the NICCBAM system.

The complexity of the crayfish cardiac activity signal results in errors in calculating HR and other cardiac activity parameters. The algorithm proposed here, based on analysis of peak shape, simplifies the classification of peaks and improves the accuracy of data processing. It also can be implemented as a modification to the previously developed systems (Depledge and Andersen 1990; Kholodkevich et al. 2008; Burnett et al. 2013) for improving accuracy.

One of the benefits of the proposed system is the capability to analyze the shape of the cardiac activity signal. Previously developed systems were focused on calculating the HR from the cardiac activity, while shape analysis of the signal can provide additional useful information on crayfish functional state.

This information can be used in various crayfish model based studies, e.g. on the effect of drugs on crayfish cardiac activity (Panksepp and Huber 2004), as well as in studies using aquatic organisms for biomonitoring of water quality (Depledge and Galloway 2005).

One of the intrinsic drawbacks of the optocoupler-based cardiac activity monitoring approach is the appearance of brief spikes in the signal which are caused by rapid crayfish movements and that affect the accuracy of cardiac data processing. The NICCBAM system addresses this problem by utilizing the data from the motion detection module. The implemented data processing algorithm provides an option to exclude the periods of high crayfish locomotive activity from consideration during cardiac activity data processing.

The analysis of correlation between HR and other chronotropic and inotropic parameters of crayfish cardiac activity (Fig. 3.4 and Fig. 3.5) showed that the slopes of the peaks, the normalized amplitude and starting time of the secondary peak and absolute amplitude of the signal can potentially carry additional information beyond that provided by HR. Whether these parameters describe some internal variables of the crayfish cardiac system or they change randomly will be the focus of future studies.

The statistical analysis of the data from the experiments on the effect of selected odors and chemicals on cardiac activity showed that this method can has promise for detecting water quality changes and can be applied in BEWS. No significant changes in the parameters revealed during the “opposite sex crayfish odor” experiment can be explained by the fact that the experiment was conducted outside of the reproduction period for the signal crayfish; therefore the odor was not so intensive and attractive for the tested crayfish.

The descriptive analysis of experimental data (Fig. 3.7 - Fig. 3.13) showed that most changes in parameters were observed for the food odor and chloramine-T treatments, while the changes caused by opposite sex crayfish odor and ammonia treatments were almost negligible. One of the possible reasons for the small effect of the ammonia on the crayfish cardiac activity is that 1 mg/L concentration of the chemical is near or below the crayfish sensitivity threshold. The relatively small effect of opposite sex crayfish odor can be explained by the date of the experiment that was out of the signal crayfish reproduction period.

Repetitive treatment of crayfish with the same chemical/odor can result in a reduced response in the cardiac activity as it was shown in Fig. 3.14 for the case of chloramine-T. This should be taken into account for application of the NICCBAM system as a basis BEWS. E.g., after crayfish were exposed to chemical they should be replaced with new ones (not impacted by the chemical) for further system operation. However, additional experiments should be held to study the crayfish sensitivity decrease after repetitive treatments as well as the sensitivity recovery period.

The general analysis of the NICCBAM system data processing method evaluation results showed that some of the cardiac activity shape parameters can be particularly promising for crayfish model based studies:

- *PSR* (mean and SD) and *PRSN* (SD) parameters showed more considerable relative changes than *HR* during the experiments with selected odors and chemicals treatments (see Table 3.2 and Table 3.3). Their monitoring can be potentially used for detection of water quality changes, e.g. in BEWS application.
- *AMP* (mean and SD) parameter showed more considerable relative change than other parameters during the experiment with injured crayfish odor treatment. Therefore, it can be potentially used for detection of this particular odor in water.
- *PPSN*, *PHN* and *PFSN* parameters were among the group that showed the most accurate results in the analysis by statistical tests (see Table 3.1). These parameters can be potentially used for detection of water quality changes by statistical tests method, e.g. in BEWS application. Moreover, being weakly correlated with *HR* (see Fig. 3.4) they can potentially carry additional information on crayfish functional state. However, this should be studied additionally.

NPSN, *PHN*, *PS* and *PSN* parameters didn’t show promising results in the experiments but being weakly correlated with *HR* (see Fig. 3.4) they still can be potentially useful for characterization of crayfish functional state. However, additional experiments are needed to prove that.

PW, *PWN*, *PO*, *PE*, *PON* and *PEN* parameters were highly correlated with *HR* (see Fig. 3.4) and were changing similarly during the treatments experiments. Therefore, these parameters can be considered redundant and can be excluded from later analyses.

5. Conclusions

The NICCBAM system is an inexpensive tool allowing simultaneous monitoring of cardiac and behavioral activities (motion detection) of multiple crayfish. It can be used as BEWS and/or in stand-alone crayfish model based ethophysiological studies. In comparison to similar state of the art systems like CAPMON (Depledge and Andersen 1990), SIBWQM (Kholodkevich et al. 2008) and improved CAPMON (Burnett et al. 2013) it is based on the novel cardiac activity shape analysis method providing additional information on crayfish functional state, is less complicated in manufacturing, requires less HW parts and is equipped with crayfish motion detection module. Moreover, most of the system's data processing is implemented in SW, allowing the user to tune it for particular crayfish species and experimental conditions to obtain better quality signal, thus improving the data processing accuracy and making the system more versatile than competitor ones.

The results of the odors/chemicals treatment experiments showed that monitoring of some of the crayfish cardiac activity parameters can be promising for analysis of crayfish state during physiological studies:

- parameters that change more extensively than HR can allow easier detection of changes in crayfish functional state;
- specific patterns of relative changes in the groups of selected parameters during different types of treatments can potentially allow qualitatively distinguishing among the treatments and/or crayfish functional states.

5.1. Limits of the approach

The currently used relatively rigid plastically insulated wires that connect the crayfish sensor with the system can sometimes twist affecting movement or even limiting the area that crayfish can reach in the aquarium. The problem can be partially resolved by using wires with varnish insulation. Such wires are typical less rigid than the plastic coated ones but caution should be taken to select wire with sufficient thickness; wires that are too thin can be easily cut by crayfish claws.

The process of attaching (gluing) the sensor to the crayfish carapace takes only a few minutes but still causes considerable stress to the animal, mainly because the crayfish is exposed to the air and its movement is restricted.

It can take several hours for crayfish to recover after manipulation. To reduce the stress, a special socket for the sensor can be developed to simplify its attachment. It could be glued to the crayfish after molting and allow easy mechanical insertion and withdrawal of the sensor in water. This sensor/socket design can considerably reduce the time needed for the experimental preparation. Moreover, a stronger adhesive can be used to permanently fix the socket on the crayfish and decrease the chances of sensor/socket detachment during operation.

5.2. Future work

The NICCBAM system was initially developed for cardiac activity monitoring, but having the same operating principle as other similar systems, e.g. CAPMON, after minor modifications it can be applied for crayfish ventilatory activity monitoring as well. Moreover, up to now, it has been successfully tested only with the selected crayfish species, but probably it also can be applied for ethophysiological monitoring in other crustaceans and mollusks.

Until now, the combined cardiac and behavioral activities information recording was done only during the system testing. The simultaneous data recording from both cardiac and motion detection modules during the ethophysiological experiments with multiple crayfish are planned.

One of the promising approaches for studying of crayfish cardiac activity is the analysis of the signal frequency spectrum obtained by applying Fourier transform to the raw cardiac data. Magnitude and/or phase spectra corresponding to different shapes of cardiac activity signal can be compared to find typical spectrum patterns characterizing different crayfish functional states. These typical spectra can be later used for classification of the cardiac activity effects caused by different external stimuli.

The NICCBAM system can be extended with ECG recording module. Combined recording of cardiac pacemaker electrical activity and corresponding cardiac muscle response by ECG and PPG methods, respectively, allow obtaining extended information on functioning of crayfish cardiac system. It can be particularly useful in studies using crayfish model for drug testing.

5.2.1. Camera-based cardiac and behavioral activities monitoring in crayfish

Our recent experiments on recording of freely moving crayfish in water with NIR camera showed the possibility of obtaining its cardiac activity by analysis of the recorded image sequence only. During the experiments we used MT9M001 monochrome complementary metal-oxide-semiconductor camera sensor and the lamp with 850 nm peak wavelength and 2.4 W of radiant power placed above the aquarium containing one *Pacifastacus leniusculus* crayfish. The total length of the crayfish and the water layer depth in the aquarium were 110 mm and 8 cm, respectively.

The special data acquisition and semiautomatic image processing algorithms developed in Matlab were capable of obtaining 10 bit uncompressed images with resolution 640x480 and 30 FPS frame rate from the camera and extracting the cardiac activity data. The cardiac activity was obtained by averaging the pixel intensity values over the user specified area on the crayfish carapace above the heart. The example of cardiac activity signal is shown in Fig. 5.1. The recording was done when the crayfish was stationary.

This truly noninvasive approach allows obtaining the cardiac activity in one or several crayfish simultaneously and without causing any disturbance or stress to them. Before practical application, the crayfish tracking algorithm to allow adjustment of the position of the monitored area on the image according to crayfish movements should developed. The crayfish tracking data obtained from the algorithm also can be used separately for crayfish BA monitoring. The disadvantages of the approach in comparison to NICCBAM system are low cardiac activity sampling rate which is equal to camera frame rate and higher noise level. The sampling rate can be increased by using higher frame rate capable cameras (e.g. 60 FPS) while the noise level can be reduced by using better quality and/or higher bit per pixel resolution image sensors.

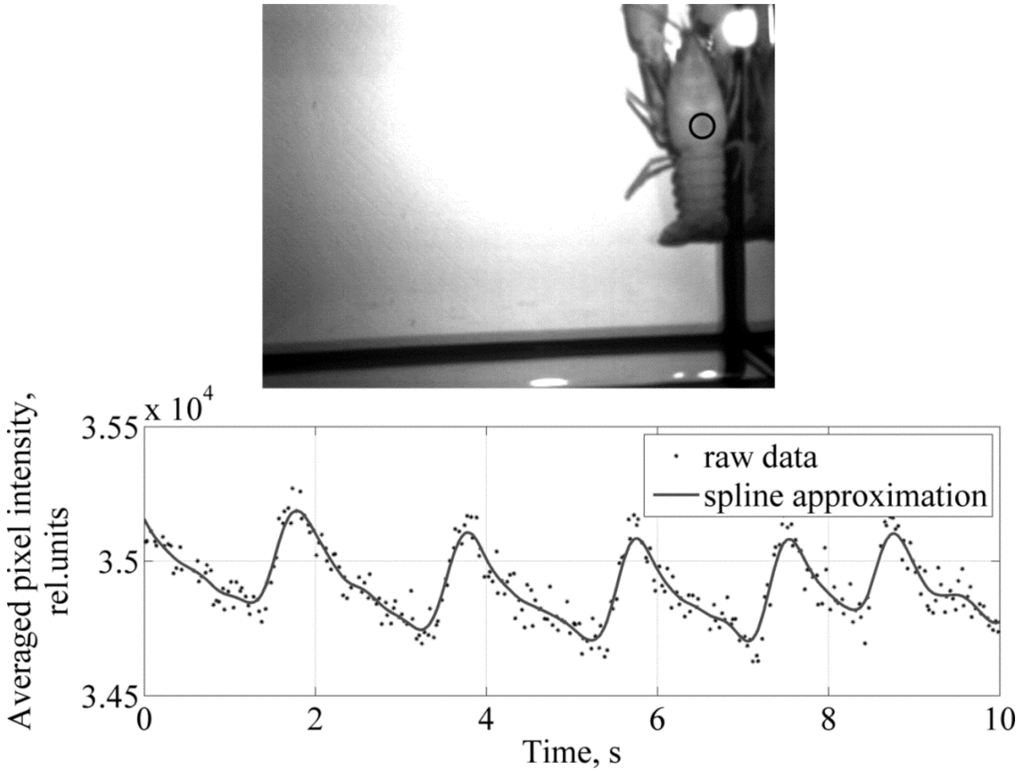


Fig. 5.1. Camera-based cardiac activity monitoring in crayfish. The black circle on the top image defines the area over which the pixel intensitities are averaged to obtain the cardiac activity curve shown at the graph below.

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Appendix A: Experimental data used in descriptive statistical analysis

Table A.1. Mean and SD values of crayfish cardiac activity parameters before (1st row) and during (2nd row) the treatment with pure tap water (“control” experiment)

Crayfish #	1	2	3	4	5	6	7	8	9	10	11	12
Trial #1												
HR	72.6±7.42	31.5±7.71	36.5±7.26	55.9±6.32	31.8±5.34	34.3±6.92	51.2±6.44	49.3±3.30	103±10.8	n/a	37.2±3.94	44.8±12.6
	68.3±7.27	32.5±7.94	36.6±6.19	58.6±7.06	30.1±4.05	33.6±6.80	52.0±5.73	49.3±2.74	65.7±11.0	n/a	60.6±19.3	38.6±5.99
*PPSN**	176±26.6	210±17.6	311±36.9	340±26.8	165±19.8	146±16.1	279±24.2	190±18.8	582±86.3	n/a	312±35.7	207±12.2
	178±19.3	220±17.8	337±24.7	333±30.3	164±15.5	160±21.2	255±23.5	195±18.3	459±31.3	n/a	313±49.7	214±12.4
*NPSN**	261±21.1	165±16.8	189±19.4	277±25.9	208±13.0	266±29.9	226±16.0	225±17.6	358±45.5	n/a	179±24.2	171±14.3
	234±28.5	160±13.7	183±15.3	271±28.1	206±9.73	249±29.7	240±17.5	224±15.9	324±39.5	n/a	162±31.4	175±13.9
*PHN**	4.90±3.87	13.1±6.34	20.1±7.32	15.8±5.33	12.7±3.84	15.8±6.94	14.8±7.41	5.03±1.56	4.25±3.68	n/a	18.6±5.85	1.79±0.83
	5.14±5.58	12.7±5.79	19.5±6.55	12.9±4.93	13.3±3.21	15.0±6.55	13.4±7.42	5.99±1.71	13.8±7.09	n/a	13.6±7.48	4.02±1.55
*PW**	46.3±21.6	107±44.5	93.4±29.3	46.1±14.8	111±27.9	113±40.1	51.5±13.0	32.5±7.06	18.4±10.9	n/a	86.1±17.0	57.3±17.1
	47.4±37.2	100±38.9	90.4±26.4	40.1±14.7	119±23.9	112±38.3	51.1±11.3	31.2±6.04	44.5±14.9	n/a	75.1±20.9	59.5±17.1
*PWN**	38.5±7.91	49.8±9.12	53.5±7.62	41.5±6.33	56.4±6.24	60.0±6.35	41.9±6.19	26.1±4.21	27.6±10.9	n/a	52.2±5.63	34.8±6.95
	35.8±9.01	47.9±8.64	52.5±6.94	37.3±7.80	58.2±5.18	58.4±6.60	42.9±5.05	25.4±3.92	45.2±9.89	n/a	47.8±8.52	36.1±6.63
*PRSN**	17.8±5.03	16.9±5.06	33.1±6.21	54.0±8.97	14.8±2.81	18.2±2.04	52.2±8.62	44.1±18.0	77.7±20.1	n/a	62.4±10.4	14.1±2.88
	18.8±7.15	17.2±3.23	31.9±5.61	54.3±11.6	14.2±1.75	17.1±2.15	51.7±9.67	53.9±18.3	65.7±14.7	n/a	66.5±11.7	15.1±2.41
*PFSN**	229±14.2	123±14.7	161±19.2	159±28.1	232±24.7	275±13.8	182±18.9	89.7±12.5	53.5±25.4	n/a	75.5±19.0	27.0±11.4
	216±33.0	116±12.0	153±19.4	147±29.8	232±16.9	262±21.4	188±16.0	89.3±11.6	93.1±28.4	n/a	61.3±24.4	37.2±10.2
*PSR**	7.82±2.39	14.8±13.0	20.8±4.66	35.2±9.33	6.53±2.22	6.66±0.83	28.9±5.20	52.2±34.2	181±96.4	n/a	88.5±31.3	57.4±18.2
	9.30±5.88	15.6±13.9	21.4±7.20	38.9±13.6	6.17±0.94	6.60±1.16	27.7±5.77	61.7±24.1	85.9±61.9	n/a	133±75.6	43.7±14.9
*PS**	31.3±2.80	39.8±2.66	23.7±2.45	27.1±2.04	34.7±3.12	32.6±2.63	25.4±1.63	46.5±4.06	16.8±2.42	n/a	32.5±6.12	48.1±3.01
	33.7±3.24	39.3±2.40	23.5±2.16	28.2±2.50	34.9±3.25	34.3±3.12	24.9±1.55	46.3±4.00	20.7±1.42	n/a	32.2±4.60	46.7±2.96
*PO**	59.0±21.2	122±45.7	88.4±28.7	58.2±14.7	122±27.5	120±41.3	52.8±11.6	60.1±7.43	24.9±7.63	n/a	94.0±16.7	98.4±17.5
	62.9±37.1	117±39.9	87.3±25.4	54.9±14.0	129±23.8	123±39.3	49.6±10.5	59.1±6.28	45.7±12.1	n/a	86.2±18.0	95.3±17.0
*PE**	77.6±21.9	147±44.7	117±29.4	73.3±14.9	146±27.8	145±40.3	76.9±13.1	78.9±7.55	35.2±12.8	n/a	119±18.1	105±17.5
	81.1±36.9	140±38.8	114±26.1	68.2±14.5	154±23.9	146±38.4	76.1±11.4	77.5±6.29	65.2±14.9	n/a	107±20.9	106±17.7
*PSN**	27.8±4.64	20.1±4.17	14.4±3.02	25.2±3.11	18.3±3.22	18.6±3.80	21.2±2.40	37.7±3.17	27.1±2.33	n/a	20.0±3.01	30.2±3.61
	28.6±5.05	20.3±4.12	14.3±2.83	27.3±3.93	17.5±2.70	19.2±4.07	21.4±2.35	37.9±3.02	22.1±3.50	n/a	21.2±4.06	29.2±3.36
*PON**	49.8±5.56	57.4±7.99	50.4±7.62	52.7±4.77	62.1±5.08	64.2±6.02	43.1±4.58	48.5±2.92	39.0±5.43	n/a	57.1±3.47	60.5±4.29
	49.0±6.70	56.5±7.43	50.7±6.25	51.7±5.08	63.4±4.32	64.5±5.81	41.6±4.24	48.2±2.63	47.0±5.91	n/a	55.3±5.32	58.6±3.83
*PEN**	66.3±3.79	69.8±5.23	67.9±4.96	66.6±3.73	74.7±3.26	78.6±2.70	63.0±4.14	63.9±2.26	54.6±9.88	n/a	72.2±3.50	65.0±3.63
	64.5±4.50	68.2±4.67	66.8±4.38	64.5±4.38	75.7±2.79	77.6±2.76	64.2±3.08	63.3±1.99	67.3±6.73	n/a	69.0±5.71	65.4±3.57
*AMP**	9.57±0.75	12.3±0.95	7.60±0.50	3.92±0.32	9.36±0.57	11.6±0.49	8.81±0.61	6.69±0.42	12.5±1.72	n/a	4.47±0.50	18.1±1.30
	9.15±0.64	12.3±0.80	7.43±0.39	4.16±0.41	9.28±0.43	11.7±0.67	8.97±0.59	6.47±0.41	9.06±0.96	n/a	6.19±1.07	18.2±1.20
Trial #2												
HR	59.1±7.17	44.0±7.88	41.1±7.36	43.2±7.02	27.8±5.19	37.4±6.12	41.1±7.92	49.7±5.75	37.9±6.71	64.4±8.74	34.3±5.25	43.7±7.01
	60.2±7.03	42.8±7.63	41.1±7.28	42.8±6.20	28.1±4.95	38.7±6.00	43.3±9.19	52.3±7.89	38.3±6.32	61.2±6.80	33.6±3.87	42.0±6.69
*PPSN**	160±18.0	205±15.9	183±32.5	349±27.5	125±19.7	175±16.2	259±24.2	225±14.8	446±32.2	217±21.3	328±27.9	219±13.3
	169±18.3	220±19.0	195±32.2	348±24.8	123±21.3	176±19.5	283±26.0	232±17.5	449±32.8	231±22.0	329±28.2	215±12.4
*NPSN**	275±20.0	201±21.9	311±59.3	276±27.2	289±28.0	254±17.7	266±27.6	239±11.0	403±47.4	369±38.3	208±36.4	209±11.8
	271±18.7	203±28.1	300±55.5	276±25.8	288±26.1	256±18.5	265±26.2	239±10.1	406±47.1	374±42.7	214±39.1	209±12.1
*PHN**	4.69±2.04	6.08±4.60	16.5±6.95	26.9±5.83	17.4±4.99	13.6±4.40	28.0±13.0	7.58±2.39	44.0±11.6	5.77±2.85	31.3±8.11	8.51±4.60
	4.44±1.65	6.36±4.46	17.2±7.30	27.8±5.45	16.7±4.88	13.6±4.59	28.0±13.8	7.85±2.18	42.9±11.3	7.18±3.50	32.1±5.15	9.19±4.82
*PW**	45.1±9.87	64.8±40.4	92.9±25.2	79.7±23.8	153±37.0	91.5±23.1	87.7±25.6	39.5±10.6	118±27.0	42.3±8.85	109±20.9	54.1±19.9
	43.4±7.10	64.4±38.2	92.0±24.8	79.7±21.0	150±33.9	86.4±21.6	82.2±27.9	36.9±9.97	116±28.5	44.9±9.53	112±20.9	57.4±20.6
*PWN**	39.5±4.61	40.6±8.64	60.7±4.73	54.7±6.73	67.9±5.55	54.6±5.46	56.6±6.34	31.4±5.83	71.8±3.74	42.1±5.05	60.3±5.14	36.2±8.58
	38.6±4.02	40.4±8.65	60.1±4.76	54.8±6.00	67.3±5.37	53.5±5.22	54.7±7.57	30.1±5.75	71.2±4.29	44.0±5.23	61.2±4.14	37.4±8.53
*PRSN**	18.1±3.38	15.7±3.79	26.9±3.09	47.7±7.62	13.8±1.25	19.6±3.41	49.9±6.23	33.3±9.83	55.4±8.48	24.9±5.75	60.6±10.2	20.3±4.34
	18.0±3.49	15.8±4.50	27.9±3.52	47.5±6.45	13.6±1.52	20.4±2.87	53.1±8.30	39.6±14.8	56.1±7.97	26.9±5.25	57.7±8.86	20.3±4.12
*PFSN**	266±20.5	122±17.1	234±23.3	215±27.1	283±19.2	253±21.4	182±18.0	81.6±9.88	168±25.9	203±24.8	125±27.8	118±10.4
	257±19.3	105±20.1	233±24.1	217±27.6	281±20.7	265±16.3	179±19.9	78.8±10.3	167±23.0	210±21.0	128±19.9	128±11.4
*PSR**	6.86±1.37	13.4±8.26	11.7±2.45	22.5±3.97	4.88±0.50	7.92±3.07	27.7±4.28	41.4±15.2	33.5±6.03	12.4±2.99	51.4±17.8	17.3±4.11
	7.05±1.48	15.7±6.70	12.1±2.12	22.1±3.85	4.87±0.76	7.75±1.26	30.1±5.83	50.4±16.9	34.1±6.03	12.9±2.50	45.9±8.24	16.0±3.66

*PS**	31.9±2.66	37.0±2.40	24.9±3.11	27.3±2.76	34.9±3.14	34.5±2.88	26.3±1.78	42.8±2.84	20.1±1.62	30.1±2.97	30.7±2.63	42.7±2.84
	32.0±3.00	37.8±2.78	24.8±2.66	27.4±1.98	35.2±4.20	33.8±2.21	26.2±1.82	41.9±3.15	20.4±1.62	29.3±2.60	30.5±2.51	43.2±2.73
*PO**	57.8±10.3	78.3±41.5	87.0±27.4	91.5±24.2	162±37.9	105±24.3	81.3±25.6	68.4±10.7	105±27.4	52.8±9.05	114±19.4	84.3±19.8
	56.7±7.30	80.7±39.7	86.7±26.3	91.4±21.4	159±34.6	99.8±23.0	78.2±27.4	65.3±10.8	104±28.2	55.2±9.17	117±20.7	87.8±20.5
*PE**	77.0±10.2	102±40.6	118±25.2	107±24.0	188±36.7	126±23.5	114±26.1	82.3±10.5	138±27.1	72.5±9.22	139±20.7	96.9±19.7
	75.4±7.26	102±39.0	117±24.6	107±21.3	185±33.7	120±22.4	108±28.6	78.8±10.9	137±28.4	74.2±9.20	142±21.3	101±20.3
*PSN**	28.3±2.91	25.5±4.32	17.0±3.43	19.6±3.52	16.2±3.46	21.2±3.16	17.9±3.27	34.7±3.58	12.6±2.20	30.5±3.44	17.4±2.69	30.2±4.83
	28.7±2.83	25.9±4.35	16.9±3.41	19.5±2.88	16.5±3.50	21.6±2.84	18.6±3.51	35.0±3.22	13.1±2.43	29.3±3.60	17.1±2.20	29.7±4.81
*PON**	50.7±3.71	49.8±7.61	56.4±6.07	63.1±5.45	71.8±5.05	62.6±5.16	52.2±6.95	54.8±3.70	63.1±5.03	52.7±3.80	63.7±3.53	57.5±5.38
	50.5±2.95	51.4±7.44	56.3±5.94	63.1±4.96	71.4±4.80	61.9±4.83	51.9±7.55	53.8±4.11	63.4±5.19	54.4±3.61	64.1±3.61	58.4±5.34
*PEN**	67.8±2.29	66.1±4.67	77.7±2.21	74.3±3.62	84.0±2.21	75.8±2.64	74.5±3.30	66.1±2.65	84.4±1.99	72.6±2.28	77.8±2.83	66.4±3.98
	67.3±1.90	66.3±4.66	77.1±2.26	74.3±3.42	83.8±2.09	75.1±2.62	73.3±4.34	65.2±3.10	84.3±2.23	73.3±2.14	78.3±2.34	67.2±3.93
*AMP**	8.03±0.53	14.0±1.13	8.35±0.70	4.25±0.32	9.72±0.53	12.6±0.79	8.59±0.59	8.23±0.29	7.67±0.89	12.3±1.57	4.48±0.55	13.1±1.25
	8.12±0.56	14.1±1.04	8.27±0.77	4.26±0.31	9.86±0.64	12.1±0.94	8.31±0.66	8.16±0.33	7.48±0.77	10.9±0.96	4.25±0.44	12.9±0.57
Trial #3												
HR	78.1±6.73	34.7±6.09	n/a	42.2±8.84	39.3±12.8	40.9±4.46	47.0±6.72	77.2±6.34	46.2±5.87	90.1±7.95	30.9±2.94	61.3±8.80
	78.4±5.65	34.3±6.39	n/a	42.2±8.49	32.5±6.19	39.7±6.21	52.6±8.37	73.7±8.57	46.0±6.58	80.7±7.25	29.7±4.06	59.3±7.41
*PPSN**	195±0.00	214±16.1	n/a	340±24.1	97.5±24.9	133±15.4	194±44.3	204±23.6	344±23.7	386±64.2	325±30.1	225±11.2
	246±31.0	209±17.9	n/a	344±23.3	88.7±17.7	148±22.0	225±40.0	190±21.1	350±25.6	336±38.6	316±23.8	224±11.6
*NPSN**	236±0.00	179±15.3	n/a	216±24.8	308±33.5	249±17.2	312±41.9	284±44.3	329±25.9	557±341	216±22.2	205±16.5
	240±21.0	183±17.6	n/a	222±25.8	318±30.5	258±35.4	316±38.0	274±33.2	320±25.6	456±121	204±20.0	199±11.6
*PHN**	8.32±0.00	8.87±3.87	n/a	14.0±3.56	10.8±6.05	10.5±3.28	21.2±10.6	9.26±2.55	22.9±7.45	6.85±7.81	39.4±6.39	2.04±0.69
	4.75±4.36	8.68±4.28	n/a	16.3±3.20	12.6±4.72	12.3±8.75	18.9±11.4	11.7±6.52	22.0±7.82	4.67±2.53	35.3±8.16	1.79±0.37
*PW**	39.6±0.00	84.0±28.6	n/a	78.7±31.0	112±52.5	76.2±11.8	74.5±16.5	31.7±9.00	77.6±15.1	24.2±6.55	123±17.8	33.8±73.6
	32.8±17.1	88.1±35.6	n/a	79.3±33.9	129±37.3	84.8±25.5	63.2±18.9	43.6±7.36	77.7±18.4	30.0±6.04	131±23.6	25.6±8.94
*PWN**	35.0±0.00	45.3±6.98	n/a	50.9±9.17	60.9±10.5	50.8±4.00	56.5±5.07	33.6±4.38	58.4±4.44	34.7±6.28	62.5±3.95	21.9±8.77
	30.5±8.26	46.5±7.80	n/a	51.3±8.78	66.2±5.76	53.2±4.89	52.4±6.31	38.7±3.05	57.6±5.29	37.8±4.98	63.0±5.32	21.0±5.35
*PRSN**	30.8±0.00	14.7±2.34	n/a	41.1±7.28	13.8±3.29	20.4±3.26	52.6±10.6	97.7±58.0	46.4±5.83	64.2±44.2	53.5±8.48	16.3±3.53
	23.3±4.75	14.3±3.61	n/a	42.8±7.97	13.0±1.84	20.3±8.80	59.4±13.4	56.1±42.7	44.6±6.33	35.4±16.7	47.8±6.42	16.7±3.44
*PFSN**	191±0.00	96.4±13.0	n/a	114±23.1	277±20.6	266±21.8	212±29.4	151±46.5	150±18.9	98.4±94.1	135±19.5	31.3±8.99
	180±32.3	101±16.8	n/a	128±22.9	270±16.5	262±38.1	205±26.7	173±31.9	140±19.7	68.8±23.7	122±24.3	28.4±7.77
*PSR**	16.1±0.00	15.7±5.16	n/a	37.7±11.2	5.02±1.28	7.70±1.57	25.2±5.84	69.3±37.6	31.4±5.32	89.9±58.7	40.8±17.8	62.9±72.5
	13.4±4.16	15.1±8.82	n/a	34.6±9.53	4.84±0.74	7.70±1.13	29.6±8.15	32.5±20.1	32.6±6.81	55.4±29.8	42.2±20.2	63.3±21.8
*PS**	31.8±0.00	40.8±2.59	n/a	29.5±2.08	30.5±3.94	32.5±2.54	23.6±2.80	29.9±3.67	23.6±1.67	24.4±3.93	31.0±3.80	42.9±1.90
	30.5±2.19	40.9±3.17	n/a	29.2±2.00	31.3±3.59	32.9±2.87	23.3±2.28	33.0±6.07	24.0±1.80	27.8±2.90	31.8±2.45	43.6±2.09
*PO**	58.8±0.00	103±29.4	n/a	95.8±30.8	114±53.3	83.4±12.5	62.7±16.4	42.9±10.6	73.1±15.3	37.7±6.43	124±16.8	67.5±66.2
	50.7±16.4	106±37.4	n/a	95.3±33.0	129±38.5	93.3±27.3	54.0±17.6	57.9±12.6	74.3±18.4	42.3±5.74	133±22.0	61.1±9.12
*PE**	71.4±0.00	125±28.6	n/a	108±31.1	143±53.9	109±11.9	98.1±16.4	61.6±12.1	101±15.2	48.6±7.23	154±17.8	76.7±73.7
	63.3±16.6	129±36.4	n/a	108±33.9	160±37.1	118±26.6	86.5±19.3	76.6±11.8	102±18.7	57.9±6.66	162±23.9	69.2±8.66
*PSN**	28.1±0.00	23.1±3.64	n/a	20.6±4.22	18.9±5.39	22.0±2.51	18.4±3.22	32.4±2.30	18.2±2.51	35.3±5.23	15.9±2.25	36.3±4.45
	30.3±4.38	23.0±3.84	n/a	20.4±4.13	16.9±3.70	21.4±2.85	20.1±3.41	29.4±3.10	18.4±2.71	35.4±3.75	15.7±2.20	36.7±3.09
*PON**	52.0±0.00	55.9±5.79	n/a	62.9±6.32	61.7±10.1	55.6±3.80	47.2±5.61	45.6±4.03	54.9±4.78	54.3±4.69	63.1±3.04	51.0±4.50
	48.4±5.57	56.2±6.80	n/a	62.6±6.28	65.9±6.00	58.7±4.88	44.6±5.94	51.1±5.70	55.0±5.35	53.6±4.68	64.5±3.79	51.0±3.42
*PEN**	63.2±0.00	68.5±3.58	n/a	71.5±5.17	79.8±5.44	72.8±2.05	74.9±2.48	66.0±2.77	76.6±2.26	70.1±3.74	78.5±2.26	58.2±4.57
	60.8±4.17	69.4±4.33	n/a	71.7±4.86	83.1±2.29	74.6±2.42	72.5±3.59	68.0±2.75	76.0±2.84	73.2±3.16	78.7±3.41	57.8±2.61
*AMP**	10.3±0.66	14.2±0.93	n/a	4.52±0.38	10.2±0.90	10.5±0.55	7.67±0.66	3.22±0.13	8.21±0.53	7.70±2.55	4.33±0.37	14.8±0.88
	9.58±0.65	14.1±0.92	n/a	4.38±0.39	9.82±0.49	10.6±0.95	7.78±0.68	3.17±0.23	8.33±0.55	8.90±1.49	4.50±0.34	15.0±0.80

*parameter values in the table are multiplied by 100

Table A.2. Mean and SD values of crayfish cardiac activity parameters before (1st row) and during (2nd row) the treatment with food odor

Crayfish #	1	2	3	4	5	6	7	8	9	10	11	12
Trial #1												
HR	50.5±8.32	34.7±9.54	23.2±5.67	42.4±7.41	25.3±4.66	38.5±8.57	39.8±5.82	56.7±5.03	31.9±5.14	62.3±7.62	31.3±4.65	n/a
	73.8±13.2	56.5±18.5	39.2±16.5	73.5±14.4	53.0±14.4	51.9±13.9	93.7±13.4	57.6±6.09	112±10.8	74.4±9.22	31.2±4.10	n/a
PPSN*	183±27.9	281±16.4	368±50.2	345±27.8	105±15.7	106±22.6	324±26.2	248±38.8	457±39.6	210±38.4	335±37.6	n/a
	234±36.1	289±22.7	336±62.0	315±63.3	213±48.9	149±44.3	326±41.3	258±39.7	600±101	218±34.4	331±33.2	n/a
NPSN*	255±32.3	192±21.7	270±34.4	240±32.8	276±17.9	300±33.4	241±20.2	230±16.5	414±45.2	432±105	196±29.1	n/a
	306±29.5	191±25.7	220±49.8	314±93.2	319±29.1	298±28.9	307±40.4	233±16.7	360±41.1	471±83.8	187±23.3	n/a
PHN*	7.16±3.34	10.7±5.22	50.4±11.9	19.9±4.93	22.2±5.98	12.6±4.84	29.7±8.71	5.34±1.22	59.1±13.0	15.4±10.1	29.8±7.04	n/a
	6.37±2.93	5.60±3.99	26.8±16.2	11.6±7.40	15.2±8.17	9.41±4.81	11.5±8.57	7.19±1.73	4.16±3.43	5.26±3.44	26.7±7.36	n/a
PW*	57.8±17.0	104±50.7	213±54.5	77.3±24.5	178±42.4	102±36.1	84.9±20.7	25.4±5.31	148±26.7	51.7±11.8	123±22.8	n/a
	41.9±12.1	70.3±46.7	127±74.0	34.6±16.9	72.9±36.7	69.1±30.7	37.9±15.1	25.6±6.72	17.1±9.04	38.6±7.64	124±22.7	n/a
PWN*	44.0±5.52	51.8±11.0	77.2±5.08	51.7±7.78	71.9±5.00	60.2±5.68	54.4±5.64	23.9±3.68	76.2±3.10	51.6±5.83	62.5±4.76	n/a
	39.2±6.61	40.7±15.5	59.2±16.1	35.7±9.86	51.9±11.2	49.7±10.7	38.8±7.55	24.2±4.03	27.6±6.45	44.0±4.99	62.2±5.36	n/a
PRSN*	19.3±3.35	17.0±5.71	28.5±3.25	41.5±7.38	15.1±2.19	17.1±2.24	54.4±6.09	113±33.0	57.3±7.92	54.3±20.8	49.1±6.68	n/a
	25.8±4.82	21.2±7.80	40.5±21.8	75.6±40.5	32.3±10.9	22.0±6.20	62.8±18.5	131±35.7	85.8±27.6	28.9±9.34	47.1±6.50	n/a
PFSN*	230±32.6	74.3±15.7	249±31.9	168±34.8	318±31.7	288±22.2	146±18.9	43.6±11.2	180±21.7	260±74.6	115±24.4	n/a
	193±49.7	49.8±15.6	168±66.7	170±65.8	251±66.7	285±35.0	146±39.8	55.7±11.8	58.7±25.7	207±37.0	106±24.6	n/a
PSR*	8.57±2.12	24.3±18.5	11.6±2.27	25.8±6.95	4.81±0.89	5.97±0.93	37.8±6.92	273±98.0	32.1±5.93	20.9±4.85	45.7±18.3	n/a
	15.8±11.7	48.3±27.6	31.2±27.0	52.8±42.2	14.5±8.71	7.93±2.77	45.9±20.4	242±73.8	177±104	14.2±4.62	48.9±21.5	n/a
PS*	31.8±3.06	34.2±2.41	22.1±2.79	28.7±2.44	30.6±3.56	30.8±2.90	27.9±2.11	40.7±5.17	21.0±1.84	24.2±4.58	30.2±4.51	n/a
	30.7±2.52	34.5±3.57	28.3±11.8	27.8±6.24	28.6±3.54	29.7±3.11	23.9±1.99	39.3±4.90	16.2±2.62	27.4±3.43	30.6±4.49	n/a
PO*	69.1±17.6	117±51.5	206±55.2	92.7±24.6	180±42.3	105±37.7	85.2±20.6	53.5±8.05	132±27.3	51.2±11.8	128±22.8	n/a
	55.9±11.0	92.9±44.8	136±68.0	51.1±17.4	80.8±34.0	76.1±29.4	42.1±12.6	51.8±9.22	23.5±8.55	44.9±8.02	129±20.7	n/a
PE*	89.7±17.6	138±50.9	235±54.5	106±24.6	209±42.1	133±36.8	113±21.2	66.1±8.11	169±26.7	75.9±11.9	154±23.3	n/a
	72.6±11.9	105±47.1	155±72.2	62.4±18.2	102±36.5	98.8±32.1	61.8±16.2	64.9±9.37	33.3±10.8	65.9±8.19	154±22.1	n/a
PSN*	25.0±3.45	19.4±5.05	8.52±2.31	20.2±3.74	12.9±2.86	19.1±3.51	18.4±2.55	38.4±3.31	11.2±2.07	24.5±4.75	15.6±2.48	n/a
	29.7±3.94	24.3±7.06	16.4±8.24	30.5±6.36	23.0±6.17	23.5±4.97	26.0±3.84	37.6±3.10	27.3±2.26	31.5±3.70	15.7±2.92	n/a
PON*	52.8±4.48	59.3±9.58	74.5±6.00	62.5±5.90	72.6±4.86	62.0±5.88	54.6±5.34	50.3±4.02	67.8±4.54	50.9±4.53	64.6±3.79	n/a
	52.8±3.73	57.2±9.48	65.2±10.9	53.8±7.50	58.8±7.88	55.6±8.04	44.0±4.74	49.2±4.27	38.6±5.05	51.2±3.85	65.1±3.44	n/a
PEN*	69.1±2.66	71.2±6.09	85.7±2.95	71.9±4.38	84.8±2.35	79.3±2.55	72.8±3.39	62.3±3.22	87.4±1.40	76.0±2.53	78.1±3.02	n/a
	68.9±3.24	65.0±8.68	75.6±9.47	66.1±5.98	74.9±5.43	73.2±6.21	64.9±4.28	61.9±3.41	54.9±6.19	75.5±2.71	77.8±3.10	n/a
AMP*	6.73±0.71	11.8±1.02	2.94±0.22	4.21±0.42	5.99±0.21	13.7±0.63	7.90±0.52	5.11±0.22	6.55±0.56	6.43±1.63	3.01±0.35	n/a
	7.22±1.00	13.4±1.76	3.03±0.64	5.80±1.58	5.11±0.40	12.6±1.25	8.97±1.33	4.89±0.34	10.6±1.60	11.6±2.10	3.13±0.36	n/a
Trial #2												
HR	62.5±10.3	32.7±4.30	35.5±9.32	43.8±7.87	25.0±5.18	39.4±11.1	36.4±9.57	45.6±7.35	34.4±5.41	60.8±8.02	29.9±4.35	38.7±6.58
	57.6±9.17	57.1±16.0	64.3±19.9	59.5±15.8	40.3±11.5	37.4±7.96	44.1±11.9	48.7±7.77	91.0±23.4	63.6±9.65	74.0±24.6	71.1±19.7
PPSN*	214±26.5	202±13.8	149±50.2	365±26.5	118±16.9	134±38.6	266±34.3	231±22.2	453±33.8	232±18.6	307±26.5	228±9.92
	156±23.6	224±22.0	236±81.6	339±42.0	187±34.4	125±17.0	247±33.1	230±24.0	521±76.6	225±20.1	302±27.5	240±18.3
NPSN*	274±23.7	207±14.8	287±63.5	261±33.3	284±27.6	276±28.0	257±29.9	206±13.0	363±52.2	350±31.6	208±20.3	186±43.2
	261±25.3	234±32.1	257±49.3	281±50.3	283±22.3	297±33.3	264±29.7	214±15.2	357±62.8	358±33.0	174±22.2	211±24.6
PHN*	5.68±3.21	10.6±3.80	18.4±10.7	24.0±5.17	17.9±4.99	13.5±6.67	38.5±17.4	8.54±1.97	42.0±7.68	6.21±2.99	30.7±4.33	5.46±1.57
	5.22±2.60	8.23±5.90	13.4±7.96	16.3±8.32	13.8±6.29	14.7±6.40	29.5±15.6	8.38±2.58	14.8±15.6	5.92±3.24	19.3±6.48	2.62±1.64
PW*	43.8±13.9	100±33.7	118±44.6	77.9±28.9	179±42.3	99.1±40.1	112±39.9	47.0±14.8	132±31.0	43.8±9.43	132±28.0	64.7±30.1
	48.9±13.1	72.5±47.5	75.5±37.2	52.8±31.1	97.7±41.3	106±45.8	87.1±31.5	41.1±13.9	49.2±51.8	44.1±11.3	132±43.6	35.7±20.0
PWN*	38.4±6.02	52.3±5.47	63.1±7.19	53.4±7.72	71.2±5.50	57.3±8.70	61.4±8.03	33.6±7.24	72.7±3.66	41.8±5.10	63.5±4.85	38.6±9.73
	40.5±5.78	45.1±10.0	50.8±10.2	43.2±12.1	56.4±10.7	60.4±5.88	56.0±8.88	31.0±7.05	42.0±18.9	42.2±5.84	61.3±11.1	27.1±9.22
PRSN*	21.5±5.06	14.4±2.22	20.8±4.02	48.7±7.89	12.0±1.49	18.9±3.24	49.0±5.44	43.7±15.4	51.1±7.77	24.1±4.36	44.2±5.80	18.4±3.77
	18.5±4.01	18.8±12.7	30.3±9.78	64.4±24.1	19.5±5.74	19.0±2.80	51.8±8.98	46.3±16.5	102±45.6	23.2±6.06	47.7±23.1	18.0±4.40
PFSN*	201±32.3	130±11.9	263±28.9	182±30.1	282±23.6	279±29.5	213±19.7	69.2±12.8	149±22.6	177±16.5	113±15.1	71.2±18.0
	256±27.7	114±29.6	192±52.0	165±54.4	240±43.0	293±25.2	210±31.3	75.8±13.1	84.4±45.3	183±17.7	77.7±21.3	41.1±19.0
PSR*	11.3±5.13	11.5±7.66	7.94±1.63	27.3±5.53	4.30±0.69	6.92±1.65	23.3±3.55	63.8±19.3	35.7±21.9	13.7±2.55	39.9±10.8	26.5±9.55
	7.32±1.87	23.3±74.1	17.7±8.65	44.8±26.6	8.60±3.72	6.54±1.09	25.5±8.71	61.5±19.4	179±141	12.7±3.71	72.6±63.7	53.5±27.3
PS*	31.9±2.42	39.3±2.18	27.4±3.87	27.1±1.90	34.0±3.63	31.1±2.74	26.0±2.00	43.0±3.78	22.0±1.72	31.4±2.48	32.6±2.73	43.8±2.00
	32.3±2.92	36.7±2.73	27.2±3.59	28.0±4.13	32.8±4.08	30.9±2.57	25.5±2.28	42.4±3.94	19.2±2.86	31.3±2.71	33.2±2.73	41.5±5.80

_PO*_	58.3±13.8	115±34.9	115±47.3	90.0±29.0	185±42.9	104±41.1	105±40.0	75.5±15.9	124±31.0	56.6±9.51	137±27.6	99.7±30.6
	60.7±12.8	87.6±48.6	79.8±37.3	68.4±30.1	108±39.2	110±47.3	81.5±30.1	69.0±15.4	52.8±46.1	56.4±11.2	141±40.7	69.7±21.3
_PE*_	75.7±14.3	139±34.0	145±44.6	105±29.1	213±41.9	130±41.3	138±40.6	89.9±15.9	154±30.9	75.2±9.51	164±28.4	108±30.4
	81.2±13.2	109±48.1	103±37.7	80.8±31.2	131±41.1	136±46.9	113±32.3	83.5±15.3	68.4±53.5	75.4±11.3	165±44.5	77.1±21.7
PSN*	28.9±3.52	21.4±2.93	16.2±4.69	19.7±3.56	14.2±3.45	19.6±4.33	15.5±3.76	31.8±3.83	12.6±2.19	30.5±3.41	16.2±2.40	28.2±4.81
	27.4±3.51	25.5±5.06	20.5±5.05	25.9±6.47	20.9±5.54	18.9±3.34	17.7±3.75	33.2±3.77	24.0±7.05	30.6±3.86	17.0±4.95	34.3±5.33
PON*	51.5±4.03	60.4±4.99	61.0±8.93	62.2±6.16	73.7±5.15	60.6±8.27	57.3±8.91	54.6±5.49	68.2±4.19	54.2±3.69	65.9±4.21	61.1±6.09
	50.5±4.16	55.6±7.78	54.0±8.76	57.9±7.88	63.6±7.87	63.2±6.05	52.3±8.38	52.8±5.67	49.3±12.8	54.2±4.04	66.3±6.73	55.3±5.19
PEN*	67.3±2.99	73.7±2.78	79.3±3.38	73.1±4.42	85.4±2.23	77.0±4.61	76.9±4.43	65.4±4.00	85.3±1.79	72.3±2.24	79.6±2.72	66.7±5.05
	67.9±2.82	70.6±5.66	71.3±6.09	69.1±6.38	77.4±5.53	79.3±2.95	73.7±5.51	64.2±4.00	66.1±13.0	72.8±2.59	78.2±6.38	61.4±4.44
AMP*	8.36±0.89	15.0±0.67	6.18±1.19	4.15±0.38	10.1±0.54	12.3±0.86	8.90±0.80	7.12±0.26	7.03±0.77	11.9±0.82	4.92±0.62	21.7±1.31
	8.25±0.79	15.3±2.32	6.78±1.78	4.64±0.73	8.82±0.77	12.2±0.73	9.35±1.12	7.10±0.30	9.62±1.65	12.3±1.10	6.18±0.59	20.2±1.44
Trial #3												
_HR_	51.5±7.33	29.7±6.43	28.2±5.95	40.0±6.73	25.1±4.34	27.5±10.2	38.1±6.91	44.9±6.39	35.8±6.42	49.7±6.92	29.5±4.57	37.5±6.31
	56.0±10.3	36.3±9.33	38.5±8.94	58.7±12.1	40.4±20.8	29.0±5.77	50.4±10.8	42.6±3.14	77.5±14.3	52.5±9.75	48.9±28.9	48.1±13.7
PPSN*	141±19.6	232±15.0	249±36.5	350±26.5	113±19.3	176±30.5	219±23.6	221±31.7	446±37.0	226±18.3	297±27.0	218±10.3
	141±24.5	232±18.4	294±50.0	326±48.3	146±42.8	158±15.2	281±44.4	212±24.4	477±62.3	227±21.0	294±24.3	225±11.6
NPSN*	261±26.4	201±18.2	276±34.4	247±31.6	280±22.8	258±37.3	291±32.4	197±17.1	355±60.2	283±32.5	155±15.3	179±14.0
	265±22.9	198±18.1	262±27.4	273±76.1	274±21.2	276±26.5	280±33.3	201±12.9	432±82.5	287±33.6	153±14.2	181±12.6
PHN*	5.60±2.60	13.2±3.97	37.5±13.5	23.5±5.63	17.3±4.39	22.9±11.8	34.1±11.9	7.54±1.95	41.3±13.1	8.33±4.28	17.0±4.13	4.12±1.06
	5.48±3.32	9.62±4.53	28.5±9.22	15.2±9.74	16.0±6.63	18.8±5.92	26.0±15.0	8.30±1.62	17.5±10.5	8.45±4.36	16.2±3.75	2.85±1.64
_PW*_	55.0±14.8	124±56.6	168±54.8	87.1±25.6	176±42.6	177±88.4	103±25.8	42.2±15.1	123±31.4	55.6±16.5	132±22.2	66.7±24.5
	54.3±16.2	92.8±45.4	106±31.0	48.5±27.4	148±68.3	146±47.9	68.0±27.3	46.7±12.5	42.2±16.3	55.4±31.2	136±23.7	59.5±20.5
PWN*	43.2±5.50	56.1±8.03	74.0±4.66	55.4±7.16	70.6±5.22	67.0±9.98	62.7±5.00	30.3±7.97	70.4±6.19	43.8±6.11	61.8±4.58	39.1±8.50
	43.1±5.90	48.4±10.2	63.0±7.54	40.8±12.9	63.9±13.3	66.3±4.65	51.0±9.68	32.6±6.17	48.3±10.6	43.5±6.81	62.1±4.52	37.0±8.21
PRSN*	17.3±3.28	15.3±1.94	28.8±5.55	42.4±6.62	12.0±1.94	16.2±6.45	50.0±5.06	51.3±23.3	52.5±9.52	21.8±3.84	41.8±5.93	17.0±2.65
	16.8±3.49	15.8±5.48	37.7±6.44	68.5±37.2	14.8±4.99	16.1±2.84	62.8±15.6	42.3±13.1	97.2±32.3	22.6±4.86	39.3±5.51	17.5±2.62
PFSN*	265±25.8	91.8±10.5	270±25.4	171±33.6	279±19.7	245±30.3	232±19.0	62.6±14.4	145±25.4	176±17.3	70.2±14.4	47.1±8.64
	263±29.7	82.0±19.6	244±28.6	155±61.6	261±37.9	248±18.9	208±26.0	61.6±11.0	124±42.2	180±19.3	67.2±13.2	34.5±15.4
PSR*	6.55±1.23	16.9±3.80	10.6±1.85	25.6±5.44	4.33±0.86	6.99±5.19	21.8±4.07	84.6±38.2	37.5±10.6	12.5±2.29	63.8±25.5	37.4±10.3
	6.60±3.08	20.7±9.98	15.7±3.30	52.4±35.9	6.11±3.60	6.61±2.55	30.8±8.98	71.5±29.4	98.7±78.0	12.7±3.50	61.3±19.4	61.9±29.2
_PS*_	32.4±3.47	40.3±2.80	19.4±4.52	28.3±2.32	34.5±3.39	35.3±2.86	24.8±1.78	45.8±6.99	21.8±2.12	33.5±2.87	33.9±4.76	45.8±2.44
	32.0±3.32	39.9±3.33	20.9±3.04	28.8±5.53	34.1±3.39	34.5±2.82	24.6±1.72	47.5±5.52	19.4±1.96	33.4±3.06	34.2±2.70	44.5±2.44
_PO*_	65.1±15.1	142±57.7	154±55.2	100±25.7	182±42.1	187±89.7	93.2±25.9	75.1±15.8	113±27.5	70.5±16.8	142±21.3	103±25.3
	64.3±16.0	112±45.6	99.2±30.1	64.5±25.8	157±65.8	155±49.6	65.3±24.1	80.3±11.2	41.3±13.7	70.9±30.6	146±22.8	95.9±20.6
_PE*_	87.3±15.1	165±56.6	188±54.3	115±25.8	210±42.1	212±88.1	128±26.3	88.0±16.2	145±30.5	89.1±16.6	166±22.7	113±25.2
	86.3±16.4	133±45.6	127±30.5	77.4±27.4	183±68.9	181±48.5	92.5±27.6	94.2±11.1	61.5±16.3	88.8±31.4	170±23.7	104±20.8
PSN*	26.0±3.48	19.9±4.24	9.12±2.86	18.8±3.34	14.5±3.11	15.9±5.57	15.6±2.60	33.9±5.07	13.1±3.04	27.3±3.77	16.1±2.35	28.3±4.27
	26.2±3.67	22.9±4.93	13.1±3.43	27.0±6.87	17.3±6.12	16.4±2.83	19.8±3.79	33.7±4.62	23.5±4.07	27.7±4.26	15.9±2.14	29.0±4.20
PON*	51.3±4.49	64.6±6.64	67.1±5.74	64.1±5.51	73.2±4.28	71.2±8.97	56.3±5.78	54.6±5.33	64.6±4.50	56.0±4.71	66.6±3.17	61.6±5.35
	51.2±4.71	59.5±7.70	58.8±7.02	56.2±8.51	68.9±9.83	70.2±4.69	49.3±7.06	56.5±2.98	47.8±7.24	56.3±4.97	66.9±3.42	60.7±4.86
PEN*	69.2±2.70	75.9±3.91	83.1±2.25	74.2±4.10	85.1±2.33	82.9±4.54	78.3±2.84	64.1±4.48	83.5±3.46	71.1±2.82	77.9±2.80	67.4±4.37
	69.3±2.83	71.3±5.57	76.1±4.46	67.9±7.35	81.2±7.33	82.8±1.94	70.9±6.25	66.3±2.38	71.8±7.62	71.2±3.05	78.0±2.76	66.0±4.20
AMP*	8.13±0.68	13.4±0.62	3.04±0.21	4.03±0.35	9.70±0.70	11.0±0.78	8.66±0.56	5.93±0.30	7.18±0.89	12.0±0.98	5.91±0.65	21.9±1.17
	8.85±1.05	13.9±1.00	3.14±0.75	4.97±1.01	9.28±0.64	11.6±0.51	8.61±1.40	5.79±0.20	8.16±1.71	12.1±1.22	5.98±0.49	20.9±1.50

*parameter values in the table are multiplied by 100

Table A.3. Mean and SD values of crayfish cardiac activity parameters before (1st row) and during (2nd row) the treatment with injured crayfish odor

Crayfish #	1	2	3	4	5	6	7	8	9	10	11	12
Trial #1												
HR	83.2±6.27	63.8±9.40	39.0±4.57	64.4±6.59	36.5±7.96	45.8±7.04	61.4±6.91	n/a	65.0±7.13	79.4±7.00	44.9±6.08	48.2±8.73
	84.9±8.55	63.9±10.9	47.4±9.78	95.7±8.19	41.6±8.13	45.3±5.85	59.7±6.80	n/a	72.1±12.1	97.3±9.32	43.2±3.98	47.9±7.62
*PPSN**	87.8±23.8	190±14.2	290±29.8	337±30.5	168±29.6	140±12.4	269±28.3	n/a	422±30.6	260±39.2	362±38.2	219±16.6
	111±42.2	204±15.5	244±40.8	304±86.7	173±23.6	140±11.5	289±27.9	n/a	435±39.7	245±73.9	370±34.9	225±11.7
*NPSN**	392±87.1	204±28.7	256±21.1	278±27.1	218±16.7	262±21.1	246±19.1	n/a	453±45.2	472±146	249±33.9	185±21.3
	346±75.2	194±33.6	284±38.1	332±107	226±13.7	269±16.0	245±14.3	n/a	414±66.6	512±166	234±25.6	197±14.9
*PHN**	6.79±4.51	6.88±5.76	22.1±5.49	10.9±4.38	10.1±3.38	7.49±3.27	8.89±6.29	n/a	18.8±8.26	7.93±7.26	29.4±8.77	1.65±0.69
	7.95±10.2	6.52±5.82	15.6±6.54	7.80±4.78	8.41±3.77	7.71±2.65	11.5±6.75	n/a	14.7±9.11	11.1±11.0	26.9±6.04	2.49±0.86
*PW**	43.2±20.3	76.3±113	92.9±17.1	35.0±9.03	93.1±24.9	67.0±17.9	37.3±14.5	n/a	53.0±10.8	36.8±8.99	74.9±14.3	52.7±40.9
	54.5±32.5	149±295	75.7±30.5	24.0±12.6	74.0±26.4	67.3±12.3	39.2±9.96	n/a	47.1±16.6	37.7±16.7	76.7±13.2	41.4±15.9
*PWN**	43.9±7.14	40.8±12.1	59.1±3.87	35.6±5.76	52.0±7.70	48.3±4.79	35.7±5.69	n/a	56.2±5.02	45.7±5.63	53.4±5.35	32.5±10.6
	45.6±10.3	41.8±18.9	54.9±7.04	30.8±9.43	47.0±8.25	49.1±4.26	37.0±5.40	n/a	51.0±8.62	47.2±7.24	54.2±4.80	29.6±7.41
*PRSN**	33.8±15.7	19.2±16.0	33.2±3.35	53.2±10.7	14.3±3.26	17.8±2.40	49.6±12.0	n/a	76.2±11.7	52.4±22.6	76.8±11.8	15.1±3.80
	22.1±9.95	17.6±14.1	34.0±5.44	86.1±52.0	15.7±2.24	18.2±2.16	55.1±12.0	n/a	69.4±16.3	61.5±25.6	81.0±13.6	16.3±3.14
*PFSN**	394±75.0	138±22.0	204±16.3	148±31.5	227±47.9	250±20.1	218±24.6	n/a	145±24.0	226±61.4	118±28.9	24.1±5.87
	352±61.0	127±35.1	209±21.0	161±74.1	248±30.4	250±16.2	212±20.9	n/a	122±33.3	250±98.4	109±20.4	30.9±7.16
*PSR**	8.37±3.03	15.3±19.9	16.4±2.01	38.0±13.0	7.13±7.18	7.16±1.04	23.2±6.88	n/a	53.7±11.5	23.4±7.85	71.0±33.0	66.7±24.6
	6.19±2.00	23.0±62.1	16.5±3.99	65.7±47.2	6.45±1.37	7.30±0.92	26.4±7.20	n/a	63.0±37.9	25.8±10.8	78.8±31.5	55.3±15.2
*PS**	22.3±15.0	35.2±1.93	24.0±2.00	26.9±2.31	36.7±4.35	31.7±2.19	24.3±1.71	n/a	18.3±1.26	21.1±4.13	27.5±2.44	45.6±5.30
	27.2±7.10	35.6±2.19	23.3±2.00	24.7±6.91	34.6±3.07	31.5±1.94	24.2±1.34	n/a	18.9±1.80	20.9±7.86	27.2±2.85	44.2±2.50
*PO**	45.8±32.2	90.9±114	91.0±17.4	48.6±8.75	109±24.0	73.4±18.9	41.7±14.1	n/a	42.7±9.64	35.6±8.62	77.1±12.5	90.7±40.3
	62.2±31.6	159±273	70.7±30.4	37.5±14.8	89.1±26.2	73.4±13.1	44.0±9.24	n/a	40.5±14.8	38.5±19.5	78.9±11.8	77.0±16.4
*PE**	65.5±32.5	112±113	117±17.0	61.9±9.16	130±24.4	98.7±18.4	61.6±15.1	n/a	71.3±10.8	57.9±9.69	102±13.9	98.3±42.5
	81.7±31.3	185±295	99.0±30.6	48.7±17.0	109±26.8	98.8±12.7	63.4±10.1	n/a	66.1±16.9	58.5±20.0	104±13.3	85.6±16.5
*PSN**	22.0±6.54	25.7±6.16	15.6±2.18	27.9±3.03	21.3±4.16	23.5±2.82	24.0±2.53	n/a	19.8±2.51	26.5±4.49	19.9±2.68	31.9±5.77
	25.0±6.75	24.6±8.75	17.9±3.24	32.9±6.22	23.3±4.05	23.4±2.49	23.4±2.37	n/a	21.5±3.80	26.9±7.00	19.5±2.40	33.1±3.88
*PON**	44.1±11.0	51.3±9.67	57.8±3.91	49.7±4.10	61.3±5.16	52.9±4.72	40.2±4.71	n/a	45.1±4.45	44.0±4.32	55.1±3.29	59.4±6.06
	52.3±9.97	52.8±13.6	51.1±6.73	48.9±8.36	57.2±6.16	53.5±4.11	41.7±4.15	n/a	43.9±6.04	47.3±8.17	55.9±3.14	56.3±4.51
*PEN**	65.9±7.04	66.5±6.32	74.7±2.03	63.5±3.53	73.4±3.87	71.8±2.60	59.8±3.57	n/a	76.0±2.98	72.2±3.08	73.3±3.26	64.4±5.31
	70.7±5.57	66.5±10.3	72.8±4.45	63.8±9.22	70.3±4.48	72.4±2.28	60.5±3.36	n/a	72.6±5.43	74.1±4.11	73.7±3.10	62.8±3.77
*AMP**	7.83±1.05	15.7±1.17	8.70±0.69	4.30±0.44	9.66±0.90	13.9±0.70	9.63±0.94	n/a	7.55±0.71	6.69±1.54	3.85±0.61	18.6±1.32
	8.93±1.45	15.6±1.25	9.87±1.36	6.91±1.63	10.4±0.84	13.6±0.68	9.27±0.93	n/a	8.16±1.44	9.56±4.21	3.68±0.35	18.6±1.04
Trial #2												
HR	79.8±4.96	36.9±6.39	34.6±2.81	59.9±6.47	44.2±6.31	37.7±7.80	52.8±4.80	61.6±9.76	64.2±10.6	n/a	42.1±5.89	36.6±4.70
	79.2±6.22	37.6±6.00	42.4±8.09	78.9±11.6	37.3±5.25	38.6±6.64	53.0±5.61	55.2±8.92	54.4±6.16	n/a	38.5±3.31	40.3±5.72
*PPSN**	141±19.0	212±15.6	342±23.2	328±26.0	136±17.2	154±23.3	221±18.1	260±42.2	399±37.7	n/a	354±28.8	209±13.0
	126±23.6	193±16.1	345±37.9	322±39.2	134±14.0	140±17.4	249±25.3	223±23.9	442±36.4	n/a	338±30.2	209±12.3
*NPSN**	296±23.8	187±14.0	233±13.2	280±24.5	225±14.3	256±26.5	255±17.5	295±22.9	409±59.7	n/a	224±29.8	197±13.5
	282±33.9	195±23.4	229±21.1	318±53.6	225±12.6	258±26.7	249±17.9	255±12.8	362±44.4	n/a	171±20.7	202±14.0
*PHN**	6.36±9.26	9.56±4.71	25.8±3.41	12.5±5.02	5.66±2.68	12.9±6.72	12.4±5.95	7.88±2.17	17.7±9.07	n/a	29.7±11.8	8.20±2.65
	6.94±6.50	8.40±4.36	18.6±7.30	10.1±7.43	8.75±2.99	11.0±5.10	13.2±6.61	7.33±2.19	21.1±7.20	n/a	17.6±5.76	6.75±2.66
*PW**	48.9±34.4	77.6±26.7	110±12.7	39.6±11.8	63.5±18.6	95.6±36.4	51.9±9.98	28.6±9.45	55.6±15.3	n/a	76.4±21.7	70.8±17.2
	51.0±21.4	74.7±23.8	80.8±22.5	32.7±28.6	84.9±20.1	89.4±35.6	51.1±10.6	33.3±8.70	63.6±12.6	n/a	83.8±12.9	59.7±16.2
*PWN**	39.8±11.5	44.4±7.54	62.6±2.45	38.0±6.73	43.8±6.72	55.4±7.04	44.8±4.55	27.9±5.24	54.9±8.77	n/a	51.4±8.89	41.6±6.33
	40.6±8.55	44.1±7.16	53.6±7.94	34.3±9.31	50.9±5.75	54.1±5.87	43.9±4.88	28.6±5.09	56.4±4.82	n/a	52.6±4.62	38.1±6.19
*PRSN**	18.7±5.56	16.8±3.11	31.2±2.72	51.4±11.4	13.9±2.44	18.3±2.50	50.7±8.27	85.4±35.7	65.3±11.8	n/a	80.4±14.1	14.7±2.60
	22.5±11.0	16.1±4.10	32.6±4.51	61.9±28.8	14.3±1.62	17.3±2.57	52.4±8.61	51.4±19.6	63.6±9.81	n/a	67.8±9.81	14.5±2.64
*PFSN**	270±24.1	136±13.4	192±12.7	162±29.3	258±21.6	258±14.5	214±15.8	72.0±17.6	136±36.8	n/a	111±32.7	92.9±9.09
	268±30.2	155±18.7	167±23.3	148±51.5	259±15.4	267±17.7	200±17.4	81.8±13.9	114±20.6	n/a	76.0±19.2	97.0±8.72
*PSR**	7.00±2.23	13.2±14.1	16.3±1.38	33.1±12.0	5.45±1.20	7.11±1.06	23.8±4.56	127±70.2	55.8±40.3	n/a	84.1±52.0	16.0±3.67
	8.37±3.81	10.6±4.95	19.7±2.91	50.4±39.0	5.56±0.74	6.48±1.07	26.5±5.43	63.8±29.3	58.2±19.1	n/a	97.8±42.4	15.1±3.35
*PS**	31.7±3.18	38.8±2.07	22.1±1.34	27.4±2.11	34.3±2.98	32.7±2.59	23.6±1.54	38.4±5.73	19.2±1.70	n/a	28.3±2.35	46.0±3.20
	34.4±7.73	39.3±2.36	22.9±2.09	26.9±3.30	35.1±3.06	32.9±2.72	24.0±1.45	42.5±4.56	20.7±1.56	n/a	29.8±2.69	44.9±2.85

PO*	62.1±35.0	96.1±27.8	105±13.2	53.0±10.9	75.0±17.8	104±37.7	47.2±8.95	52.9±12.9	46.6±11.9	n/a	78.3±17.2	103±17.5
	65.2±26.5	92.5±25.2	80.1±21.2	47.3±27.4	96.0±20.0	97.9±36.4	48.3±9.69	59.5±10.3	57.5±12.8	n/a	90.8±11.0	91.4±16.4
PE*	80.6±34.5	116±26.7	132±12.8	67.0±11.5	97.8±18.6	128±36.7	75.4±10.0	67.0±13.2	74.9±14.9	n/a	105±21.3	117±17.4
	85.4±26.8	114±23.8	104±21.7	59.6±29.2	120±20.2	122±36.2	75.1±10.5	75.8±10.6	84.3±12.6	n/a	114±12.4	105±16.4
PSN*	29.4±6.84	23.4±3.86	12.7±1.19	27.0±3.43	24.5±3.66	20.3±4.00	20.7±2.16	38.4±3.43	19.8±3.61	n/a	19.8±3.30	27.7±3.53
	28.7±4.66	24.2±3.59	16.0±3.46	30.9±4.79	21.6±3.20	21.1±3.49	21.0±2.23	37.1±3.33	18.7±2.47	n/a	18.9±2.29	29.4±3.50
PON*	52.0±8.97	55.4±6.42	60.1±2.71	51.2±4.47	52.1±4.85	60.5±6.56	40.8±3.87	52.0±5.59	46.1±5.25	n/a	53.2±5.05	61.3±4.08
	52.1±8.56	54.9±6.65	53.3±6.67	51.4±5.68	57.7±4.70	59.4±5.49	41.5±4.06	51.3±4.23	50.8±5.34	n/a	57.1±2.62	58.8±3.94
PEN*	69.2±5.13	67.8±3.89	75.3±1.46	65.0±3.78	68.3±3.47	75.8±3.26	65.5±2.84	66.3±3.72	74.7±5.50	n/a	71.3±5.95	69.4±3.11
	69.3±6.07	68.2±4.14	69.6±4.68	65.2±5.82	72.5±2.89	75.2±2.64	64.9±3.06	65.7±3.01	75.2±2.90	n/a	71.6±2.94	67.5±2.98
AMP*	9.36±0.59	13.8±0.62	7.51±0.28	4.28±0.37	10.6±0.68	11.9±0.73	9.16±0.50	7.53±0.41	8.09±1.04	n/a	3.80±0.51	18.8±1.11
	9.58±0.73	14.1±0.95	7.74±0.75	5.73±1.17	10.3±0.72	12.2±0.63	8.94±0.63	7.64±0.37	7.56±0.67	n/a	4.58±0.53	19.3±1.16
Trial #3												
HR	71.9±6.71	37.8±6.64	34.6±5.28	58.3±7.39	30.8±4.11	36.0±5.69	52.0±5.26	49.8±4.07	75.8±10.3	69.2±5.76	40.7±6.86	34.7±3.82
	77.2±5.89	41.2±8.08	41.0±11.4	75.5±10.6	34.2±5.90	38.7±6.38	53.1±5.61	49.7±6.31	84.4±7.25	87.9±11.2	39.2±4.26	39.8±6.27
PPSN*	180±18.2	214±13.4	351±28.0	333±31.1	146±22.4	142±18.1	201±18.8	202±20.8	466±45.6	270±39.5	329±24.9	213±11.6
	174±15.3	216±20.1	352±35.2	276±53.3	153±31.2	83.0±14.9	266±28.7	196±20.2	475±47.0	290±106	330±30.2	217±12.3
NPSN*	254±28.6	163±12.7	178±13.5	272±29.1	215±13.1	259±24.2	255±20.6	226±21.2	333±39.6	491±91.2	185±17.9	183±14.8
	233±27.2	165±15.0	178±19.1	332±53.6	216±14.7	289±32.3	237±19.0	222±19.7	310±42.6	508±216	190±26.8	187±16.7
PHN*	5.23±5.57	7.74±4.34	19.6±6.12	13.4±4.84	12.1±3.07	11.4±5.25	12.4±6.78	6.89±1.75	9.44±5.06	9.20±5.47	19.3±6.21	7.49±1.50
	9.60±8.40	7.14±4.59	15.9±6.40	10.7±7.61	10.3±3.51	10.2±4.62	12.3±6.67	5.67±2.33	5.27±2.93	15.6±23.1	19.9±5.45	5.47±1.77
PW*	53.4±41.0	70.1±29.2	96.9±25.5	41.7±15.8	116±22.8	98.3±37.1	54.9±11.6	31.0±10.8	33.8±12.0	44.4±6.83	78.4±19.7	74.7±18.1
	78.6±68.6	65.4±31.7	82.4±26.6	39.6±52.7	99.0±27.1	96.8±33.3	48.1±11.1	32.4±15.4	22.1±6.87	39.4±27.7	81.3±15.6	59.4±18.7
PWN*	39.2±10.6	39.9±8.28	53.7±6.26	38.4±7.32	58.1±4.81	55.9±5.76	46.4±4.66	25.2±4.72	39.4±8.42	49.8±5.00	50.5±7.27	42.1±5.76
	45.0±15.8	38.5±8.28	48.9±7.75	38.6±9.70	53.6±7.64	59.1±5.11	41.3±5.36	25.2±6.48	29.5±6.45	47.4±8.88	52.1±5.26	36.9±7.09
PRSN*	16.1±3.17	15.9±2.25	28.2±4.57	53.7±12.1	13.6±1.59	15.8±2.28	49.4±8.06	64.9±22.2	74.6±21.5	44.2±15.5	71.0±14.6	16.3±2.74
	19.6±5.74	16.4±3.92	28.5±5.52	57.1±28.6	14.0±2.18	15.6±2.29	51.3±10	51.1±19.4	77.2±25.6	96.8±88.0	67.4±11.4	16.8±3.42
PFSN*	236±23.6	114±10.8	139±18.6	154±28.9	234±20.4	243±16.4	215±17.1	82.8±12.3	84.4±30.9	187±36.6	87.9±20.4	62.3±8.45
	229±25.9	112±16.7	128±19.9	212±65.3	222±41.5	283±20.0	184±20.7	81.7±10.1	63.8±23.7	269±145	85.2±19.1	53.2±12.5
PSR*	6.85±1.27	14.0±2.48	20.7±4.50	36.1±11.4	5.85±0.96	6.53±1.04	23.2±4.48	79.8±29.1	110±77.2	23.9±7.31	90.4±51.7	26.6±6.03
	8.51±2.28	15.0±5.21	22.7±5.72	32.4±27.1	6.60±2.03	5.53±0.86	28.3±7.70	63.1±24.1	148±94.2	37.3±31.3	84.6±33.3	33.8±12.0
PS*	32.1±2.87	38.9±2.20	24.4±2.32	27.7±3.28	35.3±3.54	35.1±3.17	23.0±1.71	46.0±4.38	20.4±1.62	23.9±3.42	30.5±2.24	46.7±2.74
	32.4±2.63	37.9±2.02	25.1±2.69	26.2±2.65	35.8±3.83	29.4±3.31	24.8±1.69	47.1±5.25	20.2±1.89	20.5±5.95	30.5±2.75	45.6±2.90
PO*	67.1±42.8	87.9±29.2	96.6±24.5	55.3±16.3	125±23.5	108±38.2	47.1±10.7	59.1±12.1	37.9±10.4	44.2±6.72	87.3±17.2	108±18.4
	93.2±70.3	82.2±31.9	85.6±27.1	49.8±52.0	110±25.2	95.6±35.3	47.8±10.1	62.1±16.9	29.8±6.06	38.5±26.6	88.6±14.5	93.1±19.0
PE*	85.5±42.6	109±28.9	121±25.1	69.4±16.7	151±23.2	133±37.3	77.9±11.8	77.1±11.9	54.2±12.3	68.3±6.85	109±19.8	121±18.5
	111±70.5	103±31.5	108±26.7	65.8±52.7	135±26.6	126±34.3	73.0±11.2	79.5±16.8	42.3±7.74	59.8±28.2	112±15.4	105±19.1
PSN*	27.4±5.05	23.5±3.85	14.1±2.65	26.5±3.55	18.1±2.63	21.0±3.57	19.8±2.22	38.0±3.25	24.9±3.29	26.9±3.73	20.4±3.22	27.0±3.09
	24.3±7.50	23.9±3.85	15.9±3.42	29.2±4.96	20.2±3.86	18.8±3.14	21.7±2.37	38.1±4.14	27.5±2.39	25.9±6.47	19.9±2.81	29.4±3.70
PON*	50.8±8.72	50.6±6.51	53.5±5.36	51.4±5.32	62.8±4.33	61.5±5.43	39.7±4.24	48.3±3.79	44.8±5.57	49.5±4.31	56.7±4.23	61.5±3.71
	55.8±12.5	49.1±6.66	50.8±7.57	50.2±6.25	60.1±5.17	58.1±5.89	41.1±4.17	49.1±4.85	40.1±4.35	46.5±5.79	56.9±3.58	58.7±4.47
PEN*	66.6±5.70	63.4±4.63	67.7±3.86	64.9±4.45	76.1±2.52	76.9±2.47	66.2±2.97	63.2±2.78	64.3±5.81	76.7±2.60	70.9±4.34	69.1±2.92
	69.3±8.49	62.5±4.66	64.8±4.63	67.7±5.69	73.8±4.09	77.8±2.45	63.0±3.39	63.3±3.37	57.0±5.66	73.3±5.60	72.0±3.00	66.4±3.68
AMP*	9.42±0.68	12.6±0.87	7.28±0.33	4.12±0.38	9.49±0.62	11.9±0.50	9.14±0.58	6.64±0.40	9.61±2.36	6.45±1.12	5.01±0.43	18.3±1.14
	9.89±0.89	12.4±1.00	7.50±0.82	5.84±1.16	9.34±0.68	12.3±0.49	8.83±0.61	7.05±0.52	10.2±1.41	10.6±5.56	4.59±0.55	18.4±1.26

*parameter values in the table are multiplied by 100

Table A.4. Mean and SD values of crayfish cardiac activity parameters before (1st row) and during (2nd row) the treatment with opposite sex crayfish odor

Crayfish #	1	2	3	4	5	6	7	8	9	10	11	12
Trial #1												
HR	60.6±7.41	38.4±7.24	39.1±7.78	49.8±9.40	27.7±4.89	39.6±4.06	42.9±9.01	50.2±6.79	47.8±14.0	58.0±6.90	36.7±4.62	41.2±7.03
	58.7±6.76	37.1±8.01	39.1±7.57	49.4±8.26	28.7±5.11	38.2±4.91	59.4±8.31	49.8±6.13	44.8±7.92	57.5±6.46	35.0±4.29	43.4±7.20
*PPSN**	158±20.8	232±15.4	208±34.9	344±32.1	127±20.4	157±12.6	259±23.6	225±16.8	443±32.2	227±21.7	329±27.0	216±13.5
	156±18.6	235±16.1	208±29.4	352±30.7	124±18.1	172±15.6	241±26.2	222±16.9	490±35.2	227±22.4	329±32.1	217±13.7
*NPSN**	273±22.4	194±39.3	285±51.1	267±104	287±27.5	262±15.0	256±26.0	239±9.69	375±53.8	363±39.2	212±34.3	203±13.2
	275±23.6	188±17.8	283±49.0	278±34.9	288±28.6	249±16.5	258±22.3	237±9.38	459±55.3	355±35.0	218±31.2	202±12.9
*PHN**	4.45±1.68	7.09±4.34	19.3±8.51	19.1±7.24	17.5±4.89	10.7±3.13	26.1±13.4	7.80±2.05	31.6±15.6	7.93±3.83	25.5±9.39	8.97±4.23
	4.46±1.71	8.09±4.31	18.7±8.18	22.6±8.39	16.5±4.71	12.3±3.80	10.1±9.47	7.54±1.94	45.8±13.2	7.48±3.39	29.6±4.76	8.07±3.77
*PW**	44.1±7.63	73.0±34.8	99.1±29.4	61.4±26.9	153±34.9	80.6±16.2	81.6±25.0	38.8±10.1	89.4±38.1	47.7±10.8	96.5±21.1	60.1±21.8
	45.1±8.43	80.8±37.9	97.9±28.8	62.7±21.8	146±35.1	85.5±20.9	47.7±16.7	38.1±9.44	98.0±25.9	47.4±10.2	105±21.2	54.2±19.1
*PWN**	39.2±4.02	42.5±8.98	60.8±5.30	46.9±9.36	67.8±5.22	52.2±3.96	54.1±7.03	30.9±5.73	62.4±11.4	44.4±5.49	57.3±6.11	38.0±8.60
	39.8±4.24	44.5±9.60	60.3±5.48	48.7±8.59	66.8±5.59	52.6±4.61	41.9±6.99	30.3±5.36	69.8±5.66	43.9±5.24	59.7±4.80	35.7±8.24
*PRSN**	18.1±3.50	15.5±5.10	27.7±3.76	55.6±24.1	13.9±1.46	18.2±1.91	49.3±6.95	35.4±9.91	60.9±14.1	26.5±5.16	59.0±9.33	19.6±4.07
	17.7±3.45	15.4±2.30	27.2±3.67	57.6±12.2	14.0±1.30	19.0±1.92	43.4±11.8	34.3±9.73	72.8±10.7	25.1±4.72	57.3±7.94	20.3±4.27
*PFSN**	267±22.9	81.9±18.7	236±23.4	174±53.4	279±22.1	258±13.2	188±18.2	78.1±10.0	143±36.3	202±18.1	109±34.5	119±14.0
	271±22.5	83.3±17.8	234±25.8	185±41.9	279±21.5	255±18.0	183±23.1	79.1±8.35	187±26.5	200±18.5	122±19.1	112±14.0
*PSR**	6.81±1.31	19.7±6.57	11.8±1.82	34.9±19.5	5.03±0.72	7.07±0.78	26.5±4.73	45.5±11.6	49.5±35.4	13.2±2.75	67.9±53.9	16.8±4.33
	6.54±1.25	19.8±8.88	11.8±2.86	32.9±12.0	5.05±0.61	7.47±0.99	24.0±6.99	43.6±11.8	39.4±6.80	12.6±2.49	47.9±8.51	18.5±4.98
*PS**	31.6±2.93	39.1±2.22	25.1±3.19	28.4±3.19	35.0±3.32	34.4±2.11	26.5±1.89	43.3±3.32	21.2±1.86	30.9±2.67	30.5±2.46	44.0±3.17
	31.2±2.92	39.3±2.36	25.5±2.93	27.4±2.49	34.7±3.06	34.9±2.29	24.8±2.07	43.8±3.29	18.6±1.50	31.3±2.56	30.5±2.51	43.7±3.01
*PO**	56.3±7.98	91.9±36.0	95.3±31.1	76.8±26.5	162±35.8	92.9±17.1	77.7±25.1	68.8±10.6	83.8±32.8	60.0±10.8	104±18.8	91.8±22.0
	56.6±8.87	101±39.1	94.8±29.9	75.7±20.8	154±35.5	99.8±21.7	45.8±15.5	68.4±9.69	86.2±23.6	60.3±10.0	110±20.4	86.4±19.1
*PE**	75.7±7.98	112±34.8	124±29.3	89.7±26.9	188±34.7	115±16.6	108±25.6	82.1±10.5	111±37.8	78.6±10.7	127±20.7	104±21.9
	76.3±8.79	120±38.0	123±28.3	90.2±21.6	180±34.5	120±21.3	72.5±17.0	81.9±9.74	117±25.6	78.6±10.0	135±21.3	97.9±19.0
*PSN**	28.3±2.81	24.9±4.69	16.3±3.69	23.3±4.77	16.2±3.31	22.7±2.36	18.6±3.43	35.2±3.49	16.8±5.15	29.4±3.82	18.6±2.89	29.4±4.73
	27.8±2.82	23.8±4.92	16.6±3.80	22.5±4.20	16.6±3.53	22.0±2.59	22.7±3.08	35.5±3.36	13.9±2.89	29.5±3.57	17.8±2.52	30.3±4.62
*PON**	50.1±3.14	54.4±7.42	58.1±6.46	59.7±6.39	71.7±4.80	60.3±3.57	51.3±7.43	55.2±3.89	59.3±7.58	56.1±3.87	62.1±3.60	59.1±5.51
	50.0±3.44	56.5±7.83	58.1±6.24	59.5±5.90	70.7±4.94	61.6±3.93	40.3±5.99	54.9±3.41	61.3±5.06	56.1±3.64	63.0±3.74	58.0±5.07
*PEN**	67.6±1.93	67.4±4.45	77.2±2.34	70.3±5.08	84.0±2.06	74.9±1.89	72.7±3.84	66.1±2.80	79.2±6.50	73.9±2.18	75.9±3.56	67.4±4.09
	67.6±2.15	68.3±4.84	76.9±2.30	71.3±4.75	83.4±2.20	74.7±2.28	64.6±4.48	65.8±2.59	83.7±3.07	73.4±2.13	77.4±2.68	66.0±3.88
*AMP**	7.92±0.64	14.2±0.77	7.78±0.78	4.46±0.46	9.73±0.50	12.9±0.62	9.07±0.67	8.18±0.26	7.75±0.89	11.1±0.82	4.63±0.74	12.8±0.66
	7.98±0.58	14.3±0.73	7.89±0.71	4.24±0.42	9.78±0.54	12.3±0.74	9.91±0.81	8.16±0.28	6.89±0.69	11.3±0.75	4.21±0.35	12.1±0.69
Trial #2												
HR	54.6±7.77	30.1±7.23	31.6±9.23	36.0±6.77	23.0±3.58	30.9±7.73	42.5±8.65	46.1±7.22	32.2±6.00	48.6±7.42	31.4±6.34	95.4±11.2
	53.7±7.32	33.5±9.48	30.4±6.79	36.5±7.23	23.2±4.84	32.8±7.72	42.3±9.02	46.9±6.06	32.1±5.83	51.1±7.13	28.7±3.23	95.9±11.9
*PPSN**	154±23.7	235±16.3	265±52.6	356±23.0	117±16.0	149±13.7	199±36.0	221±20.9	406±29.6	211±18.8	312±27.4	n/a
	157±18.5	237±15.7	246±34.2	347±25.9	158±24.8	168±16.8	289±42.0	215±21.3	416±28.3	208±18.6	307±27.7	n/a
*NPSN**	260±23.2	173±30.1	257±41.4	259±25.7	273±23.0	250±24.1	281±50.2	216±12.9	387±45.0	317±47.5	180±25.5	n/a
	259±22.2	165±18.1	263±34.0	264±28.4	257±18.3	260±21.8	262±28.7	219±15.4	380±41.2	329±41.9	192±21.1	n/a
*PHN**	5.22±3.01	10.4±4.61	26.9±10.5	32.4±6.65	19.4±3.99	18.4±6.62	27.6±15.6	7.21±1.57	48.1±14.1	8.84±4.74	26.0±9.29	n/a
	5.42±2.26	6.56±3.27	28.3±9.43	32.1±7.73	22.1±4.96	18.3±5.84	29.1±14.9	7.73±1.78	47.5±13.0	8.04±5.06	31.0±4.48	n/a
*PW**	50.9±15.6	116±52.3	139±41.1	108±32.5	194±34.0	133±56.4	88.3±33.9	44.5±14.0	146±36.3	61.9±23.2	126±32.2	n/a
	51.5±11.0	100±48.5	144±39.3	107±36.4	191±43.3	123±44.9	84.6±34.2	41.1±10.4	145±34.5	57.8±16.9	137±23.2	n/a
*PWN**	41.3±5.65	52.0±9.76	66.8±6.79	61.1±6.74	72.3±3.76	62.6±6.51	57.6±6.40	32.5±6.72	74.8±4.13	47.2±6.31	61.8±7.30	n/a
	41.7±5.01	47.7±10.7	68.5±5.60	60.9±7.06	70.8±5.99	61.8±6.30	54.6±8.26	30.7±5.64	74.4±4.10	46.7±5.71	64.3±4.13	n/a
*PRSN**	17.7±3.75	14.7±4.84	25.3±6.62	40.9±5.91	11.9±1.43	17.5±1.88	52.0±9.26	39.3±15.0	46.0±7.10	21.1±3.92	52.3±9.69	n/a
	17.7±3.08	14.1±2.37	25.2±3.99	40.8±7.15	13.7±2.05	18.9±2.12	54.8±8.89	34.3±10.7	45.8±6.75	21.7±9.06	47.3±5.85	n/a
*PFSN**	255±26.5	76.9±20.6	212±27.5	217±26.2	263±16.8	265±14.8	215±28.4	63.6±13.1	177±23.3	177±24.9	92.2±25.5	n/a
	252±23.2	55.6±19.6	240±28.9	222±32.0	248±26.0	268±18.1	170±25.3	69.7±9.39	172±21.8	181±20.9	108±14.4	n/a
*PSR**	6.97±1.56	21.2±18.4	12.2±4.15	19.0±3.50	4.54±0.69	6.60±0.78	24.5±4.92	62.6±20.7	26.3±4.13	12.0±2.08	67.0±44.8	n/a
	7.09±1.37	29.0±12.5	10.6±2.31	18.7±3.86	5.63±1.53	7.06±0.84	33.2±8.49	49.9±16.1	26.9±5.19	12.0±4.33	44.5±8.69	n/a
*PS**	32.0±3.56	40.8±2.68	25.2±3.83	27.0±1.83	35.8±3.67	34.1±2.49	25.1±2.54	45.1±4.37	21.2±1.83	34.5±2.67	32.2±2.95	n/a
	32.1±3.07	40.9±2.67	24.2±2.84	27.0±2.09	36.5±3.46	33.1±2.33	26.9±2.19	45.8±4.07	21.5±1.91	33.4±2.71	32.8±2.83	n/a

*PO**	61.8±15.9	136±53.7	135±41.8	117±32.7	200±35.3	142±57.4	77.4±34.1	76.9±15.5	131±36.5	75.8±23.9	131±28.6	n/a
	62.6±10.9	124±50.2	139±39.9	115±36.3	202±43.1	133±46.0	81.1±32.8	73.3±10.7	132±34.5	69.9±17.3	141±22.1	n/a
*PE**	82.9±15.9	157±52.2	164±40.5	135±32.7	230±34.1	167±57.0	113±34.9	89.6±15.6	167±36.2	96.4±23.6	159±32.2	n/a
	83.6±10.9	141±48.8	168±39.1	134±36.6	228±43.4	156±45.7	112±35.0	86.8±10.7	167±34.3	91.2±17.1	170±23.6	n/a
*PSN**	26.7±3.57	20.2±4.87	13.1±4.02	16.2±3.09	13.7±2.42	17.2±3.63	17.5±3.43	33.9±3.69	11.4±2.43	27.5±4.03	16.5±3.09	n/a
	26.4±3.27	21.8±5.10	12.3±3.16	16.4±3.28	14.1±3.10	17.7±3.35	18.7±3.62	34.9±3.67	11.6±2.48	27.8±3.73	15.6±2.00	n/a
*PON**	50.4±4.59	62.0±7.90	64.7±7.51	66.7±5.62	74.6±3.80	66.9±6.04	50.0±7.56	56.7±4.91	66.8±5.44	58.2±4.97	64.6±4.47	n/a
	50.8±3.81	60.5±8.50	65.9±6.23	65.9±6.15	74.8±4.73	66.9±5.78	52.4±7.67	55.3±3.57	67.2±5.06	56.7±4.63	66.2±3.16	n/a
*PEN**	68.0±2.79	72.2±5.00	79.9±3.20	77.2±3.82	86.0±1.53	79.8±3.03	75.1±3.46	66.4±3.75	86.2±2.04	74.7±2.67	78.3±4.58	n/a
	68.1±2.34	69.6±5.71	80.8±2.74	77.2±3.98	84.9±3.10	79.5±3.12	73.4±4.99	65.6±2.66	86.0±2.06	74.6±2.49	80.0±2.50	n/a
*AMP**	7.90±0.62	13.2±0.75	6.45±0.65	4.14±0.31	9.42±0.52	12.3±0.54	8.15±0.97	7.96±0.32	7.56±0.72	12.3±0.95	4.69±0.52	13.1±2.17
	7.90±0.60	14.1±0.88	6.29±0.51	4.20±0.31	8.56±0.56	12.0±0.69	7.80±0.85	9.22±26.0	7.45±0.67	12.0±0.87	4.45±0.32	13.3±2.35
Trial #3												
HR	62.3±9.08	24.6±6.12	26.0±5.72	32.5±6.40	22.4±4.83	26.4±8.25	62.7±15.2	46.3±5.68	67.6±11.2	44.7±6.16	31.7±3.49	49.1±5.56
	66.2±8.16	25.4±5.41	24.0±5.06	32.1±6.05	32.4±13.0	27.8±6.02	44.4±7.37	45.4±4.23	66.2±13.3	45.7±6.19	31.4±4.11	45.8±4.84
*PPSN**	242±33.2	211±16.0	300±39.1	339±22.7	118±14.6	193±24.0	256±34.8	215±17.6	419±45.4	178±21.2	306±24.9	220±0.26
	212±33.6	210±16.2	302±41.9	340±23.6	221±21.6	182±19.6	241±28.5	217±16.4	408±47.9	192±21.2	295±33.8	227±2.18
*NPSN**	265±26.9	157±16.1	211±24.5	232±22.7	248±25.4	239±29.7	325±40.7	211±13.4	441±67.0	314±36.3	235±21.4	160±24.8
	262±24.3	158±12.5	216±29.2	227±22.7	215±12.7	235±22.5	367±43.1	210±12.6	432±78.1	318±36.8	230±29.9	171±8.32
*PHN**	5.84±3.36	14.7±4.44	27.0±10.7	29.7±6.35	18.2±5.33	26.1±10.6	25.6±14.4	7.46±1.53	17.0±11.3	7.81±4.31	35.4±5.66	1.84±0.82
	5.22±3.35	14.0±4.46	30.2±10.6	28.8±5.87	14.8±5.83	20.8±6.49	35.8±12.9	7.41±1.35	19.9±14.0	9.12±4.20	33.9±8.35	1.89±0.89
*PW**	41.8±14.9	150±48.8	169±50.6	122±39.4	204±56.5	178±90.2	63.8±21.6	40.4±9.83	50.7±18.7	69.3±17.4	120±22.2	21.2±4.81
	42.4±13.1	142±47.3	189±50.1	122±39.9	134±53.2	150±52.9	88.8±26.6	41.3±9.46	56.6±22.4	68.7±15.4	122±25.9	22.8±3.39
*PWN**	36.6±6.25	57.0±7.17	68.4±6.07	61.8±7.15	71.9±5.33	67.3±8.21	52.9±9.69	30.0±5.36	52.8±10.0	49.6±5.67	62.1±4.11	16.8±3.90
	37.4±5.73	55.8±7.46	71.5±4.90	61.7±6.95	58.1±11.9	64.6±5.74	62.6±5.61	30.6±5.15	54.8±11.7	50.5±5.35	61.9±5.35	18.1±2.19
*PRSN**	25.8±10.4	12.6±1.39	19.6±3.57	34.5±4.73	10.8±1.19	18.5±4.62	75.1±15.2	36.9±9.87	67.3±15.3	16.5±4.43	50.6±8.46	16.4±4.42
	22.8±5.23	12.8±1.53	19.5±3.88	33.9±5.33	14.7±5.59	16.9±1.93	71.4±15.8	38.5±10.8	67.5±18.2	19.8±3.95	48.8±8.43	13.9±2.98
*PFSN**	147±39.0	103±13.7	172±21.7	170±24.5	243±17.2	258±24.6	218±29.2	64.5±10.4	133±33.0	185±19.9	119±15.1	44.7±35.3
	179±36.7	100±14.2	177±21.4	164±23.7	131±20.4	248±15.6	218±19.0	61.9±9.32	141±40.3	181±16.9	115±27.6	26.0±11.3
*PSR**	21.4±23.5	12.5±2.07	11.5±2.20	20.7±4.17	4.47±0.62	7.19±1.68	34.6±6.88	58.6±17.9	55.1±27.2	9.01±2.37	43.1±11.0	47.5±27.6
	13.5±5.01	13.3±5.88	11.1±3.11	21.0±4.33	12.0±7.75	6.84±0.76	32.9±7.08	63.5±18.6	54.8±37.5	11.1±2.65	45.1±16.8	56.1±13.0
*PS**	31.9±2.53	43.0±2.87	25.6±4.38	28.9±2.05	34.8±3.47	33.6±3.02	22.3±1.75	46.2±4.02	19.6±1.60	36.4±3.37	32.7±2.65	44.8±0.85
	31.3±2.73	43.0±2.90	25.1±4.72	29.1±2.26	39.4±4.40	34.7±2.31	23.3±2.29	45.9±3.51	19.4±2.03	34.2±3.08	33.8±3.88	44.1±0.42
*PO**	55.9±14.4	168±50.5	165±49.5	131±39.4	207±57.1	188±92.2	55.1±17.7	73.4±9.61	44.9±15.1	82.2±18.0	122±21.7	56.7±0.14
	54.2±13.3	161±48.9	182±49.9	132±39.8	152±52.7	161±54.2	75.4±26.0	74.2±9.52	48.5±17.9	79.0±15.7	124±24.6	59.7±3.54
*PE**	73.8±15.2	193±49.1	194±48.6	151±39.5	239±56.3	212±91.2	86.1±21.7	86.5±9.75	70.3±18.3	106±17.6	152±22.6	66.0±3.96
	73.8±13.6	185±47.5	214±48.8	151±39.9	174±54.3	185±53.3	112±27.7	87.2±9.49	75.9±21.7	103±15.3	156±25.9	66.9±3.82
*PSN**	29.0±3.73	17.4±3.48	11.3±3.67	15.6±3.31	13.0±3.10	14.6±4.21	19.6±3.60	34.9±3.64	21.7±4.05	26.8±3.92	17.3±2.14	35.5±0.47
	28.4±3.35	18.0±3.70	10.1±3.13	15.6±3.17	19.2±5.46	15.9±3.22	17.1±2.68	34.6±3.36	20.3±4.80	25.8±3.81	17.6±2.88	35.0±0.65
*PON**	49.5±4.05	64.3±6.39	66.7±5.53	67.0±5.73	72.9±5.26	71.4±7.79	45.9±6.50	54.9±2.99	47.1±6.77	59.0±4.51	63.1±3.67	44.9±0.14
	48.0±4.45	63.8±6.44	68.8±4.89	67.2±5.54	67.0±8.68	69.7±5.36	52.8±5.86	55.4±3.08	47.4±7.57	58.2±4.24	63.4±4.24	47.4±1.48
*PEN**	65.6±3.07	74.3±3.80	79.7±2.61	77.5±4.00	85.0±2.36	81.9±4.13	72.6±6.41	64.9±2.41	74.5±6.55	76.4±2.43	79.4±2.42	52.3±3.43
	65.8±2.94	73.8±3.89	81.6±2.12	77.3±3.96	77.3±6.68	80.6±2.62	79.7±3.33	65.2±2.39	75.2±7.25	76.3±2.18	79.6±3.18	53.1±1.54
*AMP**	7.52±0.90	12.3±0.73	5.62±0.45	4.23±0.31	9.42±0.56	11.0±0.64	9.68±2.39	7.27±0.50	7.70±1.07	14.6±1.18	4.07±0.26	19.2±0.81
	8.21±0.86	12.5±0.49	5.54±0.49	4.24±0.29	7.95±0.47	11.7±0.62	7.43±0.48	7.41±0.30	7.92±1.20	12.9±1.09	4.23±0.53	19.6±0.76

*parameter values in the table are multiplied by 100

Table A.5. Mean and SD values of crayfish cardiac activity parameters before (1st row) and during (2nd row) the treatment with predator odor

Crayfish #	1	2	3	4	5	6	7	8	9	10	11	12
Trial #1												
HR	86.0±6.73	43.7±7.23	n/a	57.2±8.30	33.2±5.89	48.6±4.00	70.6±6.98	81.6±5.35	56.6±8.69	84.6±8.48	33.2±3.98	116±10.9
	82.7±4.00	41.4±5.83	n/a	53.2±8.63	43.3±12.4	49.2±5.21	65.5±5.35	82.4±5.98	80.2±11.0	81.5±7.85	33.5±4.40	100±10.9
PPSN*	298±40.4	211±19.3	n/a	353±25.0	85.6±17.0	144±15.2	198±49.0	n/a	379±33.5	377±65.6	315±28.1	n/a
	n/a	217±18.4	n/a	350±23.2	177±67.9	161±21.9	202±35.6	201±9.91	464±47.6	320±43.5	313±28.2	n/a
NPSN*	236±18.5	202±21.8	n/a	269±30.1	321±29.3	286±18.3	319±42.3	n/a	453±50.6	530±221	236±25.4	n/a
	n/a	196±18.2	n/a	261±28.8	302±30.1	282±18.0	327±37.9	315±8.41	430±92.1	469±128	229±30.6	n/a
PHN*	3.43±0.87	6.11±3.91	n/a	13.1±3.60	13.0±4.06	7.14±2.22	9.20±5.89	n/a	25.1±11.5	10.9±8.48	36.4±6.86	n/a
	n/a	6.36±3.40	n/a	14.5±3.49	12.1±5.11	7.05±2.95	11.5±6.28	6.98±0.09	13.7±9.83	6.32±4.21	33.2±9.79	n/a
PW*	30.5±13.2	61.0±36.3	n/a	43.8±19.1	124±28.4	57.7±7.91	40.3±8.49	n/a	68.0±15.1	31.6±5.81	112±20.8	n/a
	n/a	61.1±19.7	n/a	50.9±21.5	86.4±35.5	56.6±11.2	44.1±6.80	26.0±7.64	36.5±14.1	33.8±4.98	110±23.2	n/a
PWN*	29.5±7.33	39.5±7.15	n/a	39.3±8.16	65.8±5.45	46.1±3.24	44.3±5.04	n/a	61.9±4.94	42.5±5.43	60.6±4.74	n/a
	n/a	39.7±6.14	n/a	42.1±8.20	54.8±12.4	45.3±4.48	46.8±3.85	28.5±5.02	45.0±11.4	41.9±4.37	59.8±5.69	n/a
PRSN*	24.6±4.87	16.7±5.48	n/a	53.6±9.78	13.7±1.47	19.9±3.20	67.4±16.1	n/a	67.0±11.8	67.8±44.2	51.1±6.92	n/a
	n/a	16.3±4.16	n/a	48.2±8.09	22.9±14.9	19.6±2.99	70.9±16.7	85.7±50.4	83.8±27.9	41.2±17.2	49.7±6.29	n/a
PFSN*	96.0±48.9	104±21.2	n/a	131±23.8	301±18.4	276±16.7	251±37.5	n/a	180±25.5	151±72.7	146±18.5	n/a
	n/a	99.8±20.9	n/a	148±25.7	245±62.4	263±20.6	247±30.4	207±54.3	138±53.0	116±34.5	136±29.3	n/a
PSR*	32.2±17.1	18.3±21.8	n/a	42.2±11.3	4.58±0.61	7.25±1.39	27.5±8.48	n/a	38.0±9.31	45.9±22.5	36.1±16.2	n/a
	n/a	18.1±18.1	n/a	33.5±8.13	13.2±21.1	7.50±1.40	29.0±7.19	39.6±14.0	80.2±73.8	36.4±12.2	39.2±15.1	n/a
PS*	29.2±1.62	38.5±2.48	n/a	28.0±2.04	30.8±3.45	31.9±2.00	18.7±2.82	n/a	18.3±1.80	23.5±3.69	31.5±3.20	n/a
	n/a	38.8±2.49	n/a	28.3±1.94	30.8±3.76	31.8±1.98	19.2±2.08	32.8±2.83	18.5±2.04	26.1±3.30	31.5±2.27	n/a
PO*	50.2±15.0	77.0±36.9	n/a	60.1±18.8	126±29.5	67.4±8.72	31.8±7.95	n/a	55.1±15.3	39.9±5.46	116±21.0	n/a
	n/a	78.4±20.1	n/a	67.8±21.7	94.6±32.4	67.2±11.7	34.6±6.12	42.6±8.49	36.3±10.2	41.3±5.70	115±23.0	n/a
PE*	59.8±13.9	99.5±36.6	n/a	71.8±19.2	155±28.2	89.6±8.07	59.0±9.27	n/a	86.2±15.2	55.1±6.75	144±21.3	n/a
	n/a	99.9±19.9	n/a	79.2±21.8	117±35.4	88.4±11.5	63.2±6.88	58.8±10.5	54.9±13.5	59.9±5.78	142±23.0	n/a
PSN*	29.7±3.58	26.6±3.68	n/a	26.5±4.04	17.1±3.65	25.7±2.21	20.7±2.77	n/a	17.2±3.03	31.7±4.13	17.4±2.34	n/a
	n/a	26.1±3.26	n/a	25.0±4.01	21.7±5.89	25.9±2.63	20.5±2.46	36.4±1.26	24.1±4.31	32.5±3.39	17.6±2.62	n/a
PON*	49.3±6.24	50.5±5.96	n/a	54.7±5.46	66.8±5.61	53.8±3.04	34.7±4.46	n/a	49.8±6.49	53.6±4.33	63.0±4.40	n/a
	n/a	51.3±5.15	n/a	57.1±5.82	61.1±7.75	53.8±3.62	36.6±3.57	47.0±3.73	45.5±6.07	51.3±4.13	62.6±5.11	n/a
PEN*	59.2±3.83	66.1±3.91	n/a	65.7±4.47	82.8±2.04	71.8±1.56	65.0±3.44	n/a	79.1±2.57	74.2±3.54	77.9±2.75	n/a
	n/a	65.8±3.31	n/a	67.1±4.50	76.4±6.89	71.1±2.43	67.3±2.33	64.9±3.76	69.1±7.88	74.4±2.64	77.4±3.35	n/a
AMP*	9.15±0.59	14.0±1.22	n/a	4.34±0.39	10.4±0.43	10.5±0.86	7.31±1.12	3.22±0.13	7.18±0.87	5.57±1.86	4.56±0.33	11.5±1.53
	8.78±0.43	14.0±0.89	n/a	4.51±0.38	8.59±1.24	11.9±1.06	6.59±0.79	3.08±0.14	8.03±1.56	7.11±1.60	4.75±0.49	12.5±1.33
Trial #2												
HR	74.7±4.68	34.2±4.90	n/a	42.1±7.70	27.1±4.60	37.8±3.98	47.0±7.99	73.7±6.86	47.4±5.57	73.3±7.24	30.8±3.08	66.0±10.3
	74.6±4.67	38.1±8.22	n/a	47.1±11.1	25.0±5.28	39.7±4.74	46.4±7.79	73.9±6.89	52.9±9.77	77.8±7.62	39.7±4.19	62.2±7.74
PPSN*	224±15.3	200±14.8	n/a	339±25.3	116±16.8	159±13.8	215±29.1	176±34.1	347±26.3	309±50.3	318±29.7	219±11.8
	223±9.14	227±17.9	n/a	331±25.7	126±16.2	156±20.8	201±24.5	175±13.2	348±44.0	311±53.4	315±33.3	217±12.0
NPSN*	241±13.1	187±16.4	n/a	214±25.8	278±19.7	237±12.5	307±38.8	283±33.0	322±26.9	406±119	213±23.3	201±10.7
	248±7.77	167±20.7	n/a	216±27.8	274±26.5	253±21.3	299±36.5	275±37.9	370±48.4	500±163	209±27.0	208±14.1
PHN*	2.21±0.18	8.50±3.89	n/a	13.8±2.88	16.4±4.30	11.2±3.67	21.8±11.6	9.95±2.86	21.1±6.73	8.29±4.73	36.9±7.77	1.96±0.64
	2.67±0.79	5.87±3.17	n/a	11.5±3.11	18.8±5.41	11.4±4.25	22.0±11.8	10.8±5.43	21.7±9.16	10.2±6.60	19.8±6.21	2.25±1.13
PW*	23.2±1.44	86.2±29.0	n/a	76.5±30.4	157±37.5	83.4±14.7	75.0±19.3	41.7±10.7	73.5±14.4	35.9±6.33	123±19.8	24.4±6.54
	26.4±2.78	71.4±37.9	n/a	64.8±43.3	178±45.8	80.0±16.8	76.1±19.8	42.1±11.8	70.5±19.4	38.5±6.49	77.3±15.9	29.2±12.8
PWN*	24.6±1.22	46.8±6.70	n/a	50.1±8.44	68.3±5.06	51.5±3.88	55.8±5.74	37.7±5.53	56.8±4.27	40.4±5.09	62.0±4.16	20.3±4.20
	27.3±2.06	39.5±9.83	n/a	44.0±11.7	70.5±5.89	51.5±4.34	55.8±6.17	37.9±6.74	58.3±8.56	47.4±5.80	50.0±5.34	22.9±6.68
PRSN*	20.9±2.27	14.5±4.01	n/a	41.9±6.54	12.9±1.78	18.5±3.06	51.8±9.25	59.2±46.6	46.2±7.08	47.5±18.5	50.6±7.09	16.7±3.99
	21.0±4.58	14.7±2.74	n/a	45.8±15.4	12.7±1.31	20.1±3.91	50.9±8.94	72.3±55.9	54.6±10.3	61.1±32.1	51.2±11.5	16.9±5.42
PFSN*	211±25.7	113±12.0	n/a	115±21.4	274±17.1	238±16.7	201±22.5	185±31.4	143±18.7	126±36.8	128±23.2	32.2±7.93
	219±24.3	66.5±23.0	n/a	107±20.9	275±22.0	255±23.1	211±25.0	167±52.2	161±37.7	185±74.4	88.6±22.1	37.3±10.0
PSR*	9.98±1.23	13.4±6.86	n/a	37.8±9.39	4.73±0.73	7.78±1.24	26.1±5.35	39.5±51.4	32.9±6.82	39.1±14.2	40.8±10.9	55.1±19.5
	9.91±3.48	25.3±12.4	n/a	44.7±19.1	4.65±0.65	7.97±2.54	24.4±4.77	64.2±77.6	36.7±15.9	33.5±11.3	61.2±21.6	47.4±17.1
PS*	30.7±1.23	40.6±2.73	n/a	29.7±2.26	34.2±3.81	35.2±2.17	24.5±2.17	34.9±3.00	23.6±1.75	27.6±3.67	31.6±4.51	44.4±2.78
	30.7±0.62	41.0±2.82	n/a	30.3±2.45	34.2±3.21	34.0±2.27	24.3±2.01	33.3±3.61	20.7±2.11	22.3±4.71	31.0±2.86	45.1±2.88

*PO**	41.4±1.69	102±31.0	n/a	93.9±30.4	163±38.1	95.3±15.2	65.0±18.8	57.7±7.33	69.5±14.6	45.2±6.13	125±19.2	60.8±7.09
	43.6±2.72	95.2±38.5	n/a	83.9±43.1	185±45.5	90.9±17.8	65.5±18.6	52.7±7.30	61.2±16.2	39.3±7.03	82.7±15.4	66.6±13.2
*PE**	53.9±2.02	127±29.4	n/a	106±30.5	191±37.5	119±14.6	99.4±19.7	76.6±10.0	97.1±14.5	63.5±6.51	154±20.1	68.8±6.84
	57.2±2.90	112±37.7	n/a	95.1±43.5	212±44.8	114±17.2	100±19.9	75.4±11.2	91.2±18.9	60.8±7.38	108±16.0	74.3±13.4
*PSN**	32.6±1.01	23.0±3.40	n/a	20.8±3.97	15.4±3.07	22.1±2.33	18.9±3.07	33.0±7.78	18.7±2.43	31.3±3.98	16.2±2.33	37.4±2.79
	31.9±1.06	24.9±4.78	n/a	23.4±5.34	14.4±3.63	22.4±2.58	18.5±3.12	30.7±4.30	17.9±3.77	27.5±5.03	20.4±2.66	36.9±3.67
*PON**	43.9±1.03	55.8±6.51	n/a	62.3±6.06	71.0±4.55	58.9±3.38	48.1±6.20	53.6±8.95	53.6±4.50	51.0±4.28	63.0±3.23	50.9±3.06
	45.1±1.52	53.8±7.38	n/a	58.9±7.87	73.2±5.23	58.6±4.38	47.9±6.09	48.3±5.43	50.7±5.69	48.2±5.05	53.6±4.48	53.6±4.06
*PEN**	57.2±1.12	69.8±3.92	n/a	70.9±4.75	83.8±2.25	73.6±1.86	74.7±3.28	70.7±5.26	75.5±2.23	71.8±2.75	78.2±2.40	57.7±2.16
	59.2±1.29	64.4±5.64	n/a	67.4±6.67	84.8±2.38	73.9±2.19	74.3±3.62	68.7±3.15	76.3±5.17	75.0±3.52	70.5±3.38	59.8±3.49
*AMP**	9.78±0.52	14.0±0.86	n/a	4.48±0.39	9.23±0.48	10.7±0.78	8.02±0.66	3.35±0.20	8.29±0.53	6.26±1.38	4.68±0.41	14.8±0.88
	10.3±0.51	14.2±1.07	n/a	4.59±0.39	9.27±0.45	10.4±0.87	8.32±0.82	3.56±0.20	8.21±0.82	5.50±1.82	4.55±0.46	14.7±0.79
Trial #3												
HR	72.8±6.56	31.8±6.45	29.4±7.85	38.6±7.49	28.5±4.46	33.0±7.92	45.4±9.15	70.1±7.63	42.5±7.34	68.4±8.91	28.9±2.99	52.6±7.35
	83.1±7.96	31.6±6.44	30.1±7.58	38.1±7.07	24.4±3.25	34.3±5.29	49.8±8.85	75.0±7.63	47.9±13.7	64.7±8.21	29.4±2.77	53.0±6.58
*PPSN**	219±33.6	181±21.1	203±38.8	340±24.0	121±31.1	154±31.8	210±42.4	185±18.1	331±29.9	231±38.5	292±23.0	184±13.3
	268±43.6	183±19.4	215±46.5	336±23.4	114±13.2	154±15.0	252±35.6	170±25.6	367±60.6	229±32.2	297±24.0	191±12.8
*NPSN**	218±20.4	192±21.6	272±57.1	219±26.7	273±32.2	254±37.1	289±43.8	255±20.2	301±35.5	402±102	136±13.9	196±10.2
	194±62.9	192±20.1	280±54.4	212±22.9	267±15.5	245±24.3	312±43.2	228±41.0	311±53.8	372±80.6	147±15.7	195±10.9
*PHN**	6.08±4.55	10.5±5.95	34.4±11.2	17.9±4.30	15.7±4.66	15.0±7.89	28.1±15.7	9.70±5.10	23.1±9.66	6.56±6.04	14.3±4.25	3.69±2.11
	10.9±12.4	10.8±5.72	34.8±10.9	16.9±3.19	18.0±3.75	12.0±5.24	26.7±14.2	11.3±5.52	24.0±11.7	6.63±4.08	16.3±4.96	2.93±1.64
*PW**	47.9±29.7	107±43.4	161±39.2	91.4±38.2	146±36.0	122±51.7	82.1±29.3	47.1±7.58	87.5±23.4	43.8±13.3	122±20.2	36.6±11.2
	96.9±102	109±44.0	156±39.5	91.0±34.2	180±34.2	103±31.0	71.6±20.9	80.9±105	82.2±28.4	43.2±9.10	120±20.3	33.0±9.01
*PWN**	35.9±10.3	52.2±7.57	73.6±3.90	54.3±8.44	66.8±6.96	60.2±6.38	57.1±7.95	38.5±4.22	58.9±6.89	44.3±6.25	57.7±4.53	26.3±4.63
	45.9±14.1	52.6±7.31	73.6±4.13	54.0±7.86	71.4±3.56	56.1±5.34	55.5±6.80	41.7±16.5	57.4±10.9	42.9±5.86	58.1±4.32	24.5±4.71
*PRSN**	20.7±5.56	13.9±2.23	29.9±11.3	37.6±6.78	14.2±3.13	15.8±2.45	61.2±12.1	37.2±22.5	41.7±6.71	32.5±16.2	42.4±5.31	15.7±3.90
	34.5±26.3	14.0±2.46	30.6±9.43	36.9±6.26	12.2±1.10	15.5±1.98	71.0±14.2	42.0±23.3	53.9±27.8	30.7±12.7	42.9±5.43	15.2±3.82
*PFSN**	177±50.4	124±16.5	280±47.7	125±22.8	241±44.1	228±23.3	219±35.9	150±22.2	132±30.9	168±47.2	60.8±13.9	84.1±15.4
	110±69.6	122±14.3	285±43.4	122±20.9	255±15.3	223±16.7	203±25.0	135±39.7	126±40.1	168±34.2	66.5±15.7	68.7±19.7
*PSR**	13.6±8.47	12.0±10.6	10.5±3.19	31.1±8.05	6.85±6.09	6.98±1.17	28.7±7.43	24.8±12.7	35.0±18.5	20.1±13.8	75.4±30.1	19.7±7.62
	57.6±60.2	12.1±8.55	10.7±2.91	31.2±7.94	4.82±0.55	6.99±0.88	35.5±8.21	32.9±16.6	60.2±87.6	18.4±6.74	68.8±21.5	26.2±17.6
*PS**	32.7±2.50	39.9±3.03	17.6±3.93	29.5±2.18	32.9±5.93	35.1±2.71	22.8±2.34	36.3±3.07	25.2±2.13	28.3±4.93	34.5±2.69	48.7±3.64
	30.5±4.46	40.1±3.36	17.1±3.44	29.9±2.14	33.0±2.93	36.0±2.68	22.4±2.03	38.6±7.47	24.1±3.16	29.9±4.70	33.8±2.50	47.8±3.18
*PO**	65.8±30.0	117±45.2	143±42.1	106±37.9	148±34.4	131±54.6	68.4±26.4	63.6±6.46	83.4±22.6	48.6±13.6	133±18.7	71.8±12.2
	89.7±38.9	119±46.1	138±41.7	107±34.2	181±34.8	113±32.4	59.3±18.8	100±102	78.2±25.5	51.3±8.98	130±18.9	68.1±9.33
*PE**	80.6±29.9	147±43.6	178±40.3	121±38.2	179±34.9	157±52.5	105±29.4	83.4±6.83	113±23.4	72.2±14.1	157±20.0	85.3±12.3
	127±104	150±44.4	173±40.2	121±34.5	213±34.2	139±30.9	94.0±20.9	119±102	106±28.9	73.1±9.35	154±20.3	80.8±9.60
*PSN**	27.3±5.09	20.9±4.03	8.47±2.35	18.9±3.95	15.7±4.26	18.8±4.11	16.9±3.51	29.9±3.13	17.8±3.39	29.2±4.64	16.5±2.11	35.8±2.95
	21.2±8.84	20.7±3.98	8.47±2.26	19.0±3.58	13.4±2.09	20.6±3.38	18.1±3.16	29.0±9.33	18.1±4.25	29.9±4.41	16.6±1.94	36.2±2.91
*PON**	50.8±7.66	56.8±7.58	64.7±7.20	63.7±6.32	67.8±4.59	64.3±6.92	47.4±7.00	52.1±2.50	56.2±5.91	49.1±4.58	63.2±2.93	52.1±3.00
	54.2±14.4	57.4±7.19	64.4±7.28	63.9±5.85	71.9±3.60	61.9±5.09	45.8±5.98	56.3±11.8	55.0±7.21	50.8±3.97	62.8±2.93	51.1±2.90
*PEN**	63.2±5.44	73.0±3.85	82.1±2.24	73.2±4.68	82.5±3.10	79.0±2.57	74.1±4.86	68.4±1.88	76.7±3.87	73.5±3.72	74.3±2.86	62.1±2.35
	67.1±6.57	73.3±3.62	82.1±2.50	73.0±4.50	84.8±1.68	76.7±2.13	73.7±4.26	70.7±8.02	75.6±7.16	72.8±2.73	74.7±2.81	60.7±2.57
*AMP**	9.91±0.89	13.4±0.98	6.18±0.98	4.31±0.36	8.44±0.68	11.5±0.95	6.96±0.94	3.89±0.21	8.21±0.64	9.13±2.43	5.79±0.44	14.8±0.69
	9.50±0.86	13.4±0.90	5.79±0.88	4.43±0.36	8.52±0.29	11.6±0.49	7.40±1.13	4.07±0.25	8.21±1.08	9.57±2.21	5.59±0.46	14.8±0.69

*parameter values in the table are multiplied by 100

Table A.6. Mean and SD values of crayfish cardiac activity parameters before (1st row) and during (2nd row) the treatment with chloramine-T in concentration 10 mg/L.

Crayfish #	1	2	3	4	5	6	7	8	9	10	11	12
Trial #1												
HR	n/a	n/a	n/a	54.7±11.3	39.9±4.81	36.4±7.75	n/a	n/a	n/a	62.7±8.37	33.0±5.51	48.4±6.51
	n/a	n/a	n/a	51.5±8.91	90.7±21.7	51.9±15.5	n/a	n/a	n/a	63.6±8.33	37.0±7.29	47.8±5.89
*PPSN**	n/a	n/a	n/a	345±33.3	153±13.1	112±17.8	n/a	n/a	n/a	244±22.0	310±24.5	182±13.7
	n/a	n/a	n/a	350±25.6	309±86.5	189±36.1	n/a	n/a	n/a	229±27.9	315±27.9	202±15.7
*NPSN**	n/a	n/a	n/a	240±35.7	259±11.5	284±35.9	n/a	n/a	n/a	357±61.3	180±16.7	218±12.6
	n/a	n/a	n/a	236±29.9	297±34.4	276±30.4	n/a	n/a	n/a	368±79.1	190±20.3	209±10.4
*PHN**	n/a	n/a	n/a	12.1±4.40	11.2±3.78	13.9±6.45	n/a	n/a	n/a	6.96±4.35	18.8±5.51	4.17±3.84
	n/a	n/a	n/a	12.4±2.76	10±8.41	13.9±9.87	n/a	n/a	n/a	6.72±3.94	18.7±6.19	4.25±2.67
*PW**	n/a	n/a	n/a	51.7±26.7	81.2±19.2	107±38.1	n/a	n/a	n/a	42.1±14.6	113±24.7	41.0±21.6
	n/a	n/a	n/a	53.5±24.2	48.9±45.4	73.6±48.8	n/a	n/a	n/a	42.3±8.77	97.5±25.3	38.0±15.5
*PWN**	n/a	n/a	n/a	41.5±9.85	52.4±5.14	60.2±5.97	n/a	n/a	n/a	40.6±5.52	59.3±6.14	29.3±7.91
	n/a	n/a	n/a	42.7±8.37	34.9±18.7	49.2±11.1	n/a	n/a	n/a	41.5±5.07	55.5±7.35	28.1±6.77
*PRSN**	n/a	n/a	n/a	52.5±13.2	19.5±2.66	17.8±2.06	n/a	n/a	n/a	29.2±6.96	46.4±7.52	15.0±3.84
	n/a	n/a	n/a	52.5±9.48	51.6±32.7	27.3±5.69	n/a	n/a	n/a	28.8±9.46	50.1±9.31	17.2±4.17
*PFSN**	n/a	n/a	n/a	122±29.3	259±15.3	284±25.6	n/a	n/a	n/a	156±25.8	82.1±19.5	129±16.0
	n/a	n/a	n/a	120±22.8	146±55.1	287±38.8	n/a	n/a	n/a	184±41.9	84.3±22.0	103±24.7
*PSR**	n/a	n/a	n/a	45.9±20.0	7.55±1.07	6.34±1.07	n/a	n/a	n/a	18.8±4.23	62.2±31.1	12.0±3.99
	n/a	n/a	n/a	45.3±12.1	52.6±55.0	9.70±2.59	n/a	n/a	n/a	15.8±4.09	67.9±41.8	18.8±11.5
*PS**	n/a	n/a	n/a	28.7±2.98	33.1±2.64	31.2±2.97	n/a	n/a	n/a	31.7±2.79	32.4±2.80	46.8±2.97
	n/a	n/a	n/a	28.6±2.15	30.5±5.38	29.1±3.03	n/a	n/a	n/a	31.0±3.26	31.9±3.10	45.4±2.57
*PO**	n/a	n/a	n/a	69.5±26.5	90.8±19.4	110±39.9	n/a	n/a	n/a	55.2±14.9	123±22.9	73.7±22.3
	n/a	n/a	n/a	71.4±24.2	67.6±43.9	82.6±49.6	n/a	n/a	n/a	53.9±9.43	107±23.5	71.6±15.8
*PE**	n/a	n/a	n/a	80.4±26.8	114±19.1	138±38.5	n/a	n/a	n/a	73.9±14.7	146±24.7	87.7±22.0
	n/a	n/a	n/a	82.1±24.4	79.4±48.4	103±50.2	n/a	n/a	n/a	73.3±9.18	129±25.5	83.4±15.7
*PSN**	n/a	n/a	n/a	25.0±4.77	22.0±3.10	18.7±3.60	n/a	n/a	n/a	31.4±3.69	17.4±2.75	35.7±4.66
	n/a	n/a	n/a	24.3±3.99	30.7±8.87	22.6±5.19	n/a	n/a	n/a	30.9±3.56	18.8±3.12	35.1±3.88
*PON**	n/a	n/a	n/a	57.0±6.62	58.8±4.29	61.8±6.81	n/a	n/a	n/a	53.4±4.24	64.5±4.20	54.2±5.03
	n/a	n/a	n/a	57.8±5.87	55.0±11.0	56.1±9.68	n/a	n/a	n/a	53.0±3.90	61.3±5.25	54.1±4.15
*PEN**	n/a	n/a	n/a	66.5±5.50	74.4±2.31	78.9±2.94	n/a	n/a	n/a	72.0±2.77	76.7±3.71	65.0±3.59
	n/a	n/a	n/a	66.9±4.69	65.5±10.9	71.8±6.18	n/a	n/a	n/a	72.4±2.56	74.4±4.70	63.2±3.34
*AMP**	n/a	n/a	n/a	4.22±0.47	9.37±0.31	11.0±0.47	n/a	n/a	n/a	9.80±1.27	5.44±0.38	16.5±0.59
	n/a	n/a	n/a	4.17±0.35	8.61±0.68	10.0±1.12	n/a	n/a	n/a	10.1±2.20	5.30±0.48	16.3±0.65
Trial #2												
HR	n/a	n/a	n/a	46.4±10.8	27.1±2.92	27.3±7.03	n/a	n/a	n/a	51.0±6.28	30.3±4.53	42.5±6.89
	n/a	n/a	n/a	46.1±8.05	38.3±15.2	46.7±17.0	n/a	n/a	n/a	51.1±9.00	27.8±3.68	51.2±13.7
*PPSN**	n/a	n/a	n/a	342±25.1	178±15.6	149±22.4	n/a	n/a	n/a	230±20.2	295±29.0	175±10.3
	n/a	n/a	n/a	340±27.9	193±28.9	161±54.1	n/a	n/a	n/a	239±23.7	297±24.2	195±13.6
*NPSN**	n/a	n/a	n/a	233±29.4	239±11.4	260±42.3	n/a	n/a	n/a	298±39.8	161±17.0	205±11.5
	n/a	n/a	n/a	226±25.6	237±22.8	279±38.5	n/a	n/a	n/a	294±43.4	162±16.0	205±13.8
*PHN**	n/a	n/a	n/a	15.3±4.04	18.2±3.40	23.2±8.63	n/a	n/a	n/a	8.65±4.13	18.1±5.13	6.53±5.20
	n/a	n/a	n/a	13.5±3.76	15.5±7.07	15.6±9.34	n/a	n/a	n/a	10.2±5.67	19.9±3.75	4.95±3.09
*PW**	n/a	n/a	n/a	69.1±38.0	146±22.2	162±53.5	n/a	n/a	n/a	53.1±12.4	123±26.5	52.5±23.2
	n/a	n/a	n/a	66.4±23.9	114±47.3	94.3±42.6	n/a	n/a	n/a	56.2±17.2	137±26.2	44.5±23.2
*PWN**	n/a	n/a	n/a	47.4±10.4	65.1±3.75	67.7±6.71	n/a	n/a	n/a	43.4±5.61	59.1±6.01	33.3±8.53
	n/a	n/a	n/a	47.5±8.44	56.0±12.5	56.0±10.1	n/a	n/a	n/a	44.2±6.78	62.0±5.21	30.2±8.11
*PRSN**	n/a	n/a	n/a	44.5±11.6	14.9±1.53	17.4±1.95	n/a	n/a	n/a	24.6±4.74	41.0±6.15	16.2±4.21
	n/a	n/a	n/a	45.7±10.1	18.4±6.25	24.3±23.4	n/a	n/a	n/a	26.0±6.41	38.0±6.12	17.0±4.24
*PFSN**	n/a	n/a	n/a	133±25.7	236±13.4	287±24.3	n/a	n/a	n/a	166±20.1	72.0±17.7	124±11.6
	n/a	n/a	n/a	118±24.7	199±54.7	261±42.3	n/a	n/a	n/a	159±23.4	75.8±12.4	86.9±16.4
*PSR**	n/a	n/a	n/a	34.9±12.8	6.35±0.71	6.12±0.79	n/a	n/a	n/a	15.0±3.10	62.4±28.3	13.2±3.89
	n/a	n/a	n/a	40.9±14.4	11.7±11.7	9.87±14.2	n/a	n/a	n/a	16.7±5.03	51.6±12.9	20.5±7.58

PS*	n/a	n/a	n/a	29.2±2.22	35.3±3.70	32.8±2.73	n/a	n/a	n/a	33.7±2.97	34.1±3.58	49.9±2.79
	n/a	n/a	n/a	29.5±2.93	37.3±4.57	30.2±4.61	n/a	n/a	n/a	33.9±3.44	33.8±2.59	47.5±2.88
PO*	n/a	n/a	n/a	85.6±35.7	158±22.1	169±55.1	n/a	n/a	n/a	67.9±12.7	132±24.5	87.2±23.9
	n/a	n/a	n/a	84.3±23.8	130±44.5	99.8±45.4	n/a	n/a	n/a	71.9±18.2	144±25.4	78.9±23.7
PE*	n/a	n/a	n/a	98.3±38.1	182±21.9	195±53.6	n/a	n/a	n/a	86.7±12.6	157±26.1	102±23.6
	n/a	n/a	n/a	95.9±24.3	151±46.8	125±44.8	n/a	n/a	n/a	90.0±17.8	171±25.7	92.0±23.7
PSN*	n/a	n/a	n/a	22.2±4.86	16.0±2.35	14.9±3.97	n/a	n/a	n/a	28.2±3.57	16.9±2.87	33.7±4.97
	n/a	n/a	n/a	22.3±4.06	20.6±6.15	19.8±4.90	n/a	n/a	n/a	27.7±4.28	15.7±2.71	34.5±4.59
PON*	n/a	n/a	n/a	60.2±7.25	70.3±2.87	70.5±6.58	n/a	n/a	n/a	55.8±4.24	63.6±4.27	56.8±5.44
	n/a	n/a	n/a	61.0±5.50	65.3±8.10	59.1±10.1	n/a	n/a	n/a	57.0±5.47	65.3±4.20	55.1±5.07
PEN*	n/a	n/a	n/a	69.6±5.82	81.1±1.71	82.6±2.86	n/a	n/a	n/a	71.7±2.79	76.0±3.72	67.0±3.75
	n/a	n/a	n/a	69.7±4.69	76.6±6.66	75.9±5.78	n/a	n/a	n/a	72.0±3.25	77.7±2.79	64.7±3.82
AMP*	n/a	n/a	n/a	4.24±0.38	9.66±0.29	9.70±0.69	n/a	n/a	n/a	11.2±1.05	5.40±0.47	15.8±0.80
	n/a	n/a	n/a	4.16±0.36	8.83±0.57	10.1±1.23	n/a	n/a	n/a	11.7±1.49	5.39±0.34	15.4±1.21
Trial #3												
HR	n/a	n/a	n/a	40.9±5.56	34.0±5.51	29.6±5.54	n/a	n/a	n/a	41.5±6.80	31.2±3.17	36.4±6.61
	n/a	n/a	n/a	48.6±11.0	38.8±18.3	58.8±26.8	n/a	n/a	n/a	39.1±6.98	30.5±4.23	40.2±10.1
PPSN*	n/a	n/a	n/a	331±23.5	175±20.2	144±23.6	n/a	n/a	n/a	247±20.0	316±22.6	162±15.3
	n/a	n/a	n/a	320±29.4	151±35.1	135±48.6	n/a	n/a	n/a	243±23.8	306±27.7	172±12.0
NPSN*	n/a	n/a	n/a	200±18.4	238±12.3	267±28.2	n/a	n/a	n/a	257±28.7	179±13.1	192±11.8
	n/a	n/a	n/a	222±35.6	260±21.3	261±53.5	n/a	n/a	n/a	258±30.3	168±18.0	192±11.8
PHN*	n/a	n/a	n/a	13.4±2.41	14.0±4.95	17.8±6.54	n/a	n/a	n/a	14.2±5.55	25.9±3.30	8.44±5.43
	n/a	n/a	n/a	12.7±5.83	19.2±6.90	11.3±7.42	n/a	n/a	n/a	15.6±6.17	22.0±7.18	7.66±4.87
PW*	n/a	n/a	n/a	73.7±21.4	104±29.7	142±46.2	n/a	n/a	n/a	74.5±20.7	118±19.4	68.9±27.8
	n/a	n/a	n/a	59.9±39.8	142±52.3	104±52.5	n/a	n/a	n/a	83.4±26.3	122±27.6	62.0±25.0
PWN*	n/a	n/a	n/a	48.4±6.50	56.3±7.51	65.8±5.08	n/a	n/a	n/a	49.0±6.40	60.1±4.10	37.8±9.26
	n/a	n/a	n/a	42.0±11.3	63.9±12.3	56.0±10.4	n/a	n/a	n/a	51.4±7.04	60.0±6.20	35.8±8.57
PRSN*	n/a	n/a	n/a	38.3±6.40	17.9±3.72	15.9±1.67	n/a	n/a	n/a	25.1±3.93	51.4±6.42	15.4±3.61
	n/a	n/a	n/a	46.5±14.7	17.9±4.52	17.0±11.3	n/a	n/a	n/a	24.5±4.09	48.2±8.05	15.8±3.78
PFSN*	n/a	n/a	n/a	111±18.5	218±26.4	260±15.2	n/a	n/a	n/a	150±16.7	90.6±10.3	118±19.9
	n/a	n/a	n/a	125±35.9	249±44.6	223±47.5	n/a	n/a	n/a	152±18.3	79.0±20.8	108±14.9
PSR*	n/a	n/a	n/a	35.6±8.47	8.44±2.90	6.14±0.75	n/a	n/a	n/a	17.0±3.14	57.5±9.70	13.6±4.67
	n/a	n/a	n/a	40.3±19.4	7.82±4.44	12.1±30.8	n/a	n/a	n/a	16.3±3.22	68.2±34.4	15.2±6.25
PS*	n/a	n/a	n/a	30.4±2.20	35.1±3.73	32.3±2.60	n/a	n/a	n/a	35.5±2.68	31.8±2.24	54.2±3.53
	n/a	n/a	n/a	30.4±2.91	32.9±3.44	34.0±5.30	n/a	n/a	n/a	36.3±3.13	32.8±2.88	52.7±3.41
PO*	n/a	n/a	n/a	91.8±21.3	115±27.6	147±48.4	n/a	n/a	n/a	92.1±21.5	121±18.0	106±27.9
	n/a	n/a	n/a	77.6±39.2	148±47.5	109±55.5	n/a	n/a	n/a	101±26.6	127±24.9	99.2±25.3
PE*	n/a	n/a	n/a	104±21.4	139±28.7	174±46.5	n/a	n/a	n/a	110±21.1	149±19.6	123±27.9
	n/a	n/a	n/a	90.3±40.1	175±50.9	138±53.8	n/a	n/a	n/a	120±26.2	155±27.5	115±25.4
PSN*	n/a	n/a	n/a	20.7±3.10	19.9±4.23	15.9±3.01	n/a	n/a	n/a	24.3±3.76	16.5±1.92	31.7±5.45
	n/a	n/a	n/a	24.0±5.09	16.6±5.86	20.6±5.54	n/a	n/a	n/a	23.5±4.22	16.6±2.69	32.3±4.98
PON*	n/a	n/a	n/a	60.7±4.62	62.7±5.04	67.9±5.46	n/a	n/a	n/a	61.0±4.91	61.8±3.01	59.7±5.60
	n/a	n/a	n/a	56.1±8.31	67.4±7.85	58.8±9.84	n/a	n/a	n/a	62.8±5.27	62.8±3.97	58.6±5.25
PEN*	n/a	n/a	n/a	69.1±3.67	76.2±3.59	81.6±2.21	n/a	n/a	n/a	73.3±3.00	76.6±2.50	69.5±4.17
	n/a	n/a	n/a	66.1±6.66	80.5±6.65	76.5±5.82	n/a	n/a	n/a	74.9±3.27	76.6±3.94	68.0±3.84
AMP*	n/a	n/a	n/a	4.36±0.30	8.73±0.39	11.2±0.61	n/a	n/a	n/a	12.6±1.25	4.51±0.25	15.4±0.81
	n/a	n/a	n/a	4.69±0.53	9.19±0.57	10.7±1.96	n/a	n/a	n/a	12.5±1.23	5.04±0.55	15.7±1.24

*parameter values in the table are multiplied by 100

Table A.7. Mean and SD values of crayfish cardiac activity parameters before (1st row) and during (2nd row) the treatment with ammonia in concentration 1 mg/L.

Crayfish #	1	2	3	4	5	6	7	8	9	10	11	12
Trial #1												
HR	66.8±7.20	36.3±8.99	35.2±12.8	n/a	n/a	n/a	41.7±5.94	80.1±7.23	37.7±6.68	n/a	n/a	n/a
	66.5±6.35	37.9±10.7	34.1±8.87	n/a	n/a	n/a	46.1±8.98	87.9±9.62	38.0±6.37	n/a	n/a	n/a
*PPSN**	121±19.0	199±30.7	207±62.5	n/a	n/a	n/a	263±29.2	239±24.1	437±34.1	n/a	n/a	n/a
	109±18.6	207±40.4	191±77.8	n/a	n/a	n/a	268±35.7	274±51.0	439±38.2	n/a	n/a	n/a
*NPSN**	266±20.4	200±30.0	294±47.2	n/a	n/a	n/a	287±29.1	283±14.3	299±44.2	n/a	n/a	n/a
	268±20.4	187±42.4	310±54.9	n/a	n/a	n/a	298±30.5	296±31.0	313±50.0	n/a	n/a	n/a
*PHN**	3.76±3.48	9.49±5.65	32.3±13.6	n/a	n/a	n/a	31.8±10.7	6.65±2.27	31.8±6.03	n/a	n/a	n/a
	3.48±3.97	8.33±4.44	29.8±13.0	n/a	n/a	n/a	27.8±13.3	5.97±1.83	34.3±7.73	n/a	n/a	n/a
*PW**	47.7±45.3	93.6±51.6	151±64.5	n/a	n/a	n/a	87.1±21.4	25.4±6.08	114±31.3	n/a	n/a	n/a
	41.9±15.0	94.6±44.0	139±51.0	n/a	n/a	n/a	77.9±27.4	21.1±3.07	112±27.2	n/a	n/a	n/a
*PWN**	38.6±8.06	48.2±8.87	72.3±7.18	n/a	n/a	n/a	58.5±4.82	27.1±3.99	68.3±4.76	n/a	n/a	n/a
	37.7±5.01	47.5±9.32	72.0±5.47	n/a	n/a	n/a	55.9±8.21	26.3±3.44	68.2±4.65	n/a	n/a	n/a
*PRSN**	14.9±2.97	14.5±2.34	29.2±8.18	n/a	n/a	n/a	57.3±6.95	77.3±37.8	57.6±11.2	n/a	n/a	n/a
	14.7±3.34	13.9±2.50	29.0±6.13	n/a	n/a	n/a	59.6±12.8	103±43.0	57.8±10.7	n/a	n/a	n/a
*PFSN**	324±37.7	119±17.7	301±32.8	n/a	n/a	n/a	214±19.2	133±23.9	133±22.4	n/a	n/a	n/a
	337±24.7	97.9±32.3	300±34.9	n/a	n/a	n/a	210±25.1	123±31.9	142±27.9	n/a	n/a	n/a
*PSR**	4.73±1.53	12.6±3.66	9.72±2.88	n/a	n/a	n/a	27.0±4.08	62.7±41.8	43.7±8.31	n/a	n/a	n/a
	4.39±1.23	16.3±9.20	9.69±2.32	n/a	n/a	n/a	28.8±7.59	91.0±44.1	42.0±13.8	n/a	n/a	n/a
*PS**	30.4±3.54	40.2±3.47	17.6±3.29	n/a	n/a	n/a	24.9±2.05	33.6±3.64	23.0±1.74	n/a	n/a	n/a
	30.1±2.95	41.2±3.35	17.3±3.55	n/a	n/a	n/a	24.0±1.89	28.7±6.19	23.0±2.51	n/a	n/a	n/a
*PO**	58.7±46.0	110±54.8	136±65.6	n/a	n/a	n/a	80.9±21.8	44.2±7.89	113±31.2	n/a	n/a	n/a
	52.4±15.7	114±48.7	124±53.5	n/a	n/a	n/a	71.6±25.6	35.2±8.05	111±27.0	n/a	n/a	n/a
*PE**	78.0±45.3	134±52.8	169±64.3	n/a	n/a	n/a	112±21.9	59.0±8.47	137±31.3	n/a	n/a	n/a
	72.1±15.7	136±45.1	156±51.4	n/a	n/a	n/a	102±27.7	49.7±8.03	135±27.2	n/a	n/a	n/a
*PSN**	27.5±4.43	23.0±4.60	9.48±3.16	n/a	n/a	n/a	17.2±2.56	36.3±2.45	14.4±2.72	n/a	n/a	n/a
	27.8±3.02	22.9±4.84	9.73±2.74	n/a	n/a	n/a	18.3±3.34	35.2±3.96	14.5±2.72	n/a	n/a	n/a
*PON**	48.4±6.64	56.9±8.71	64.3±9.40	n/a	n/a	n/a	54.2±5.07	47.2±3.77	67.7±4.54	n/a	n/a	n/a
	47.4±4.33	57.9±9.34	63.0±8.89	n/a	n/a	n/a	51.3±7.28	43.2±5.03	67.5±4.32	n/a	n/a	n/a
*PEN**	66.1±4.08	71.3±4.68	81.8±4.42	n/a	n/a	n/a	75.8±2.66	63.3±2.77	82.7±2.29	n/a	n/a	n/a
	65.6±2.68	70.4±5.00	81.8±3.24	n/a	n/a	n/a	74.2±5.19	61.5±2.42	82.8±2.19	n/a	n/a	n/a
*AMP**	11.0±0.66	13.7±1.25	5.05±1.73	n/a	n/a	n/a	7.72±0.45	4.50±0.21	7.98±1.09	n/a	n/a	n/a
	10.9±0.69	14.2±1.59	4.74±1.15	n/a	n/a	n/a	7.66±0.55	4.52±0.31	7.68±1.06	n/a	n/a	n/a
Trial #2												
HR	67.5±7.37	26.6±4.91	24.5±7.98	n/a	n/a	n/a	50.9±12.4	84.8±9.89	31.4±4.99	n/a	n/a	n/a
	67.5±6.60	26.9±4.31	26.8±8.19	n/a	n/a	n/a	41.7±10.4	71.6±5.94	34.5±7.91	n/a	n/a	n/a
*PPSN**	120±22.9	202±15.4	336±60.2	n/a	n/a	n/a	257±46.9	268±38.1	458±25.3	n/a	n/a	n/a
	149±19.0	191±12.9	325±37.7	n/a	n/a	n/a	218±30.7	233±35.4	447±34.9	n/a	n/a	n/a
*NPSN**	256±20.0	174±14.2	206±29.4	n/a	n/a	n/a	275±35.5	342±52.6	379±42.0	n/a	n/a	n/a
	250±14.6	177±14.5	214±25.4	n/a	n/a	n/a	301±45.2	273±30.3	361±46.1	n/a	n/a	n/a
*PHN**	3.12±1.66	14.1±4.84	35.4±12.5	n/a	n/a	n/a	26.2±16.7	5.29±1.38	53.1±10.3	n/a	n/a	n/a
	3.11±1.63	13.2±4.60	33.1±12.5	n/a	n/a	n/a	33.0±15.7	8.18±3.39	46.0±12.7	n/a	n/a	n/a
*PW**	41.3±10.7	135±48.4	195±67.6	n/a	n/a	n/a	74.0±28.5	20.6±3.90	148±28.3	n/a	n/a	n/a
	36.9±7.38	132±47.4	175±64.9	n/a	n/a	n/a	97.7±28.1	32.2±11.2	133±35.0	n/a	n/a	n/a
*PWN**	36.5±4.60	56.3±7.19	71.3±7.59	n/a	n/a	n/a	53.4±9.27	26.0±2.87	75.3±3.29	n/a	n/a	n/a
	34.2±3.94	56.0±6.24	69.9±7.63	n/a	n/a	n/a	61.7±7.60	31.4±6.12	72.0±6.83	n/a	n/a	n/a
*PRSN**	15.4±4.07	13.8±1.14	23.2±5.11	n/a	n/a	n/a	62.2±15.2	107±35.8	50.6±6.07	n/a	n/a	n/a
	16.9±3.46	13.3±1.24	25.3±6.43	n/a	n/a	n/a	55.2±11.1	84.2±63.0	53.3±12.4	n/a	n/a	n/a
*PFSN**	302±23.8	109±11.2	188±25.4	n/a	n/a	n/a	193±28.2	140±38.7	186±22.8	n/a	n/a	n/a
	289±17.3	115±8.70	193±26.1	n/a	n/a	n/a	207±25.6	113±22.6	167±30.6	n/a	n/a	n/a
*PSR**	5.16±1.56	12.8±1.90	12.6±3.68	n/a	n/a	n/a	33.0±9.83	86.8±72.6	27.6±4.58	n/a	n/a	n/a
	5.85±1.18	11.6±2.04	13.4±4.04	n/a	n/a	n/a	27.3±8.18	87.0±85.0	34.3±17.9	n/a	n/a	n/a

*PS**	31.0±2.58	41.3±2.43	22.4±2.51	n/a	n/a	n/a	23.9±2.05	29.9±4.55	21.2±1.42	n/a	n/a	n/a
	30.7±2.27	41.9±2.58	21.2±2.09	n/a	n/a	n/a	23.9±2.44	34.5±5.54	21.9±1.73	n/a	n/a	n/a
*PO**	51.7±11.5	148±49.1	184±67.2	n/a	n/a	n/a	64.9±25.2	35.9±6.74	137±28.4	n/a	n/a	n/a
	49.0±7.11	145±48.3	163±64.8	n/a	n/a	n/a	82.8±25.8	50.2±14.3	125±32.7	n/a	n/a	n/a
*PE**	72.3±11.7	177±48.2	217±66.8	n/a	n/a	n/a	97.9±28.5	50.4±7.26	169±28.3	n/a	n/a	n/a
	67.6±7.06	174±47.2	196±64.7	n/a	n/a	n/a	122±28.1	66.7±15.2	155±34.7	n/a	n/a	n/a
*PSN**	28.0±2.53	18.3±3.54	9.20±3.38	n/a	n/a	n/a	18.5±3.79	38.0±4.20	11.1±1.91	n/a	n/a	n/a
	28.7±2.68	18.8±3.31	9.49±3.12	n/a	n/a	n/a	16.0±3.83	34.6±3.24	12.6±3.30	n/a	n/a	n/a
*PON**	45.9±4.14	62.0±6.18	67.0±8.56	n/a	n/a	n/a	46.7±7.59	45.6±6.04	69.7±3.95	n/a	n/a	n/a
	45.4±2.87	61.7±5.52	64.5±9.01	n/a	n/a	n/a	52.0±7.25	49.2±6.36	67.8±5.61	n/a	n/a	n/a
*PEN**	64.5±2.65	74.6±3.81	80.5±4.35	n/a	n/a	n/a	71.9±5.81	64.0±3.66	86.4±1.70	n/a	n/a	n/a
	62.9±1.78	74.8±3.07	79.4±4.65	n/a	n/a	n/a	77.7±4.36	66.0±4.60	84.7±3.72	n/a	n/a	n/a
*AMP**	11.8±0.88	13.1±0.50	3.50±0.36	n/a	n/a	n/a	8.05±1.33	5.01±0.35	6.33±0.53	n/a	n/a	n/a
	11.2±0.90	13.3±0.46	3.53±0.22	n/a	n/a	n/a	7.46±1.00	4.78±0.24	6.63±0.64	n/a	n/a	n/a
Trial #3												
HR	60.9±5.54	22.0±7.11	21.8±4.66	n/a	n/a	n/a	44.1±10.3	75.7±6.71	47.4±10.7	n/a	n/a	n/a
	62.0±7.71	23.3±7.85	21.7±4.62	n/a	n/a	n/a	35.6±4.95	82.8±7.15	31.4±5.12	n/a	n/a	n/a
*PPSN**	67.9±15.9	188±18.3	366±43.2	n/a	n/a	n/a	313±33.2	246±20.3	416±34.2	n/a	n/a	n/a
	95.8±38.8	190±20.5	292±42.8	n/a	n/a	n/a	312±24.2	277±37.1	420±33.7	n/a	n/a	n/a
*NPSN**	252±16.0	142±21.0	203±21.4	n/a	n/a	n/a	222±27.8	308±26.1	275±42.0	n/a	n/a	n/a
	249±21.0	151±24.5	216±27.2	n/a	n/a	n/a	236±22.9	348±24.8	272±32.7	n/a	n/a	n/a
*PHN**	2.64±1.90	13.4±6.01	39.8±9.15	n/a	n/a	n/a	21.9±12.2	6.42±3.55	22.6±10.2	n/a	n/a	n/a
	3.71±3.57	13.4±5.71	37.3±8.98	n/a	n/a	n/a	35.6±8.96	5.59±1.82	36.5±8.59	n/a	n/a	n/a
*PW**	47.2±11.1	190±104	216±53.3	n/a	n/a	n/a	75.4±28.7	28.7±9.47	77.0±29.7	n/a	n/a	n/a
	48.9±14.8	190±150	219±48.5	n/a	n/a	n/a	101±24.3	21.5±3.88	140±30.7	n/a	n/a	n/a
*PWN**	40.0±4.94	58.0±11.7	74.5±5.08	n/a	n/a	n/a	49.1±9.01	29.9±5.99	55.6±9.74	n/a	n/a	n/a
	40.3±5.60	58.0±11.8	75.7±4.86	n/a	n/a	n/a	58.2±5.02	26.7±2.99	70.7±4.72	n/a	n/a	n/a
*PRSN**	12.2±2.81	10.8±1.43	22.7±4.74	n/a	n/a	n/a	50.9±10.5	59.7±35.5	70.4±25.3	n/a	n/a	n/a
	13.8±4.07	11.3±1.59	20.7±3.71	n/a	n/a	n/a	53.4±6.06	99.1±39.3	46.0±6.53	n/a	n/a	n/a
*PFSN**	316±17.6	101±18.7	200±31.7	n/a	n/a	n/a	125±21.5	122±20.0	103±34.8	n/a	n/a	n/a
	300±33.7	107±18.1	224±27.9	n/a	n/a	n/a	149±16.5	145±20.1	128±23.9	n/a	n/a	n/a
*PSR**	3.86±0.88	11.0±2.25	11.7±3.30	n/a	n/a	n/a	42.2±13.4	53.0±45.7	87.6±80.3	n/a	n/a	n/a
	4.78±2.09	11.0±3.85	9.36±2.15	n/a	n/a	n/a	36.2±5.48	70.0±35.8	37.8±12.2	n/a	n/a	n/a
*PS**	29.9±3.50	47.6±3.40	20.9±2.71	n/a	n/a	n/a	28.4±2.47	34.1±2.64	23.9±2.03	n/a	n/a	n/a
	30.5±3.78	45.9±4.24	20.7±2.95	n/a	n/a	n/a	28.5±1.77	30.5±3.68	23.9±1.98	n/a	n/a	n/a
*PO**	51.8±10.2	213±108	205±52.6	n/a	n/a	n/a	75.6±27.6	47.6±9.79	78.7±26.7	n/a	n/a	n/a
	56.2±15.0	209±143	205±47.9	n/a	n/a	n/a	97.8±24.1	37.2±6.05	135±29.4	n/a	n/a	n/a
*PE**	77.0±10.3	238±104	237±53.1	n/a	n/a	n/a	104±29.1	62.8±10.1	101±29.5	n/a	n/a	n/a
	79.5±14.7	236±149	240±47.9	n/a	n/a	n/a	130±24.9	52.0±6.35	164±30.2	n/a	n/a	n/a
*PSN**	25.7±3.47	17.3±5.46	7.60±2.04	n/a	n/a	n/a	19.8±3.85	36.5±3.54	18.8±4.41	n/a	n/a	n/a
	25.8±3.69	17.5±5.67	7.51±2.22	n/a	n/a	n/a	16.8±2.18	38.0±2.51	12.5±2.49	n/a	n/a	n/a
*PON**	44.0±3.80	66.1±9.84	70.5±5.22	n/a	n/a	n/a	49.3±7.91	50.1±4.45	57.4±6.68	n/a	n/a	n/a
	46.4±4.77	65.1±10.0	70.6±5.31	n/a	n/a	n/a	56.1±4.53	46.1±3.61	68.1±4.16	n/a	n/a	n/a
*PEN**	65.7±2.37	75.3±6.38	82.1±3.26	n/a	n/a	n/a	68.9±5.67	66.4±3.13	74.4±5.62	n/a	n/a	n/a
	66.2±2.75	75.5±6.32	83.2±2.85	n/a	n/a	n/a	75.0±3.12	64.7±2.53	83.2±2.48	n/a	n/a	n/a
*AMP**	12.1±0.71	13.7±1.04	3.27±0.27	n/a	n/a	n/a	7.51±0.98	5.03±0.26	8.12±0.85	n/a	n/a	n/a
	11.9±0.99	13.0±0.86	3.70±0.27	n/a	n/a	n/a	7.74±0.68	5.08±0.35	7.65±0.71	n/a	n/a	n/a

*parameter values in the table are multiplied by 100

Appendix B:

Noninvasive crayfish cardiac activity monitoring system

LIMNOLOGY
and
OCEANOGRAPHY: METHODS

Limnol. Oceanogr.: Methods 12, 2014, 670–679

Noninvasive crayfish cardiac activity monitoring system

Aliaksandr Pautsina[1], Iryna Kuklina[2], Dalibor Štys[1], Petr Císař[1], and Pavel Kozák[2]*
[1]Institute of complex systems, CENAKVA, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Zámek 136, 373 33 Nové Hrady, Czech Republic
[2]Research Institute of Fish Culture and Hydrobiology, CENAKVA, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budéjovice, Zátiší 728/II, 389 25 Vodňany, Czech Republic

Abstract

Crayfish cardiac activity monitoring and analysis are widely used during water pollution and ethological studies. A noninvasive crayfish cardiac activity monitoring (NICCAM) system discussed in the current study permits long-term, continuous monitoring of several crayfish simultaneously. The advantages of the system are low price, low number of required components and the possibility of cardiac signal shape monitoring. Calculation and analysis of parameters characterizing the shape of the double peak cardiac activity allows not only reducing the number of incorrect peak detections improving the system accuracy but also can provide additional information on crayfish state. The discussed preliminary experiments on the effect of food odor and chloramine-T on crayfish showed promising potential of signal shape analysis for studying of crayfish cardiac reaction to changes in the aquatic environment.

High sensitivity to changes in the aquatic environment, especially to pollution, as well as the ease with which it can be used for experimentation, make crayfish one of the most promising animals for biomonitoring of water quality (Kholodkevich et al. 2008; Kuklina et al. 2013). Also, relative simplicity of its nervous system as compared with vertebrates make a crayfish model useful for studying the mechanisms underlying behavior (Li et al. 2000).

Cardiac activity provides a general indication of crayfish metabolic status (Depledge and Galloway 2005). It can signal the integrated impact of natural and anthropogenic stressors and may reflect the availability of energy required for crayfish normal life, growth, and reproduction (Depledge and Galloway 2005).

Previous studies have shown the response of the crayfish cardiac system to selected chemical agents in water, e.g., potassium nitrate and ammonium phosphate (Kholodkevich et al. 2008), sodium chloride (Styrishave et al. 1995; Kozák et al. 2009), ammonia (Bloxham et al. 1999), chlorides, and nitrite together (Kozák et al. 2011). Several authors reported the results of their studies on cardiac activity at different physiological states (Fedotov et al. 2006; Cooper et al. 2011).

Several approaches have been developed for monitoring of crayfish cardiac activity. Invasive methods based on electrocardiogram recording require drilling the crayfish dorsal carapace directly over the heart and implanting two metal electrodes (Pollard and Larimer 1977; Li et al. 2000; Schapker et al. 2002). The drawbacks of this approach include the possibility of injuring the crayfish and altering its behavior by using implanted electrodes, and the complexity of connecting the sensor device to crayfish that require precise surgery.

The noninvasive methods are based on measuring of the amount of scattered light from crayfish heart modulated by changes of the heart volume while contracting. The CAPMON system proposed by Depledge and Andersen (1990) utilizes a transducer consisting of an infrared (IR) light-emitting diode (LED) and a phototransistor fixed on the animal's dorsal carapace and connected to the external amplification and filtering circuit via thin flexible electrical wires. Improved and renovated CAPMON system for intertidal animals was recently developed by Burnett et al. (2013). Another photoplethysmograph system proposed by Fedotov et al. (2000) is based on the same operating principle as the CAPMON system but utilizes optical fibers as light guides between the crayfish and an externally placed IR semiconductor laser and photo receiver.

The main drawback of the noninvasive systems described above is that they are focused on measuring and analyzing

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*Corresponding author: E-mail: pautsina@frov.jcu.cz

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heart rate (*HR*), whereas other chronotropic and inotropic parameters describing the shape of the cardiac signal are not considered. Because each crayfish can have a unique heartbeat pattern (Burnett et al. 2013) depending on its size, age, physiology, and species, analysis of the shape of the cardiac signal can provide additional information about the crayfish state. Another drawback of the systems is the complexity of their hardware: both the original (Depledge and Andersen 1990; Aagaard et al. 1991) and the improved CAPMON (Burnett et al. 2013) systems require a conditioning circuit for filtering and amplification of the signal, whereas a photoplethysmograph (Fedotov et al. 2000) additionally requires precise optic components. Recent advances in electronics and optoelectronics eliminate the need in hardware amplification circuit, whereas the increased computational power of modern personal computers allows real-time digital filtering of the signal in software. Processing the signal in software permits easier and more flexible control over the data processing parameters and allows excluding hardware signal conditioning circuit, thus making the whole system cheaper and easier to manufacture.

The noninvasive crayfish cardiac activity monitoring (NICCAM) system described here permits long-term continuous noninvasive monitoring of cardiac activity of up to 16 crayfish, simultaneously. It allows measuring and recording of chronotropic and inotropic parameters of crayfish cardiac activity together with raw cardiac data for further manual or semiautomatic analysis. The apparatus was originally developed for crayfish but can be also used with other benthic hard-shell invertebrates. Possible applications of the system include pollution and ethological studies. It was tested in studies of chloramine-T and food odor effects on crayfish cardiac activity.

Materials and procedures

Overview of the system

The NICCAM system is comprised of a set of 16 IR optical sensors for crayfish, a multichannel 14 bit analog-to-digital converter (ADC) with USB interface, and a personal computer (PC) with software for data processing. It allows obtaining raw cardiac activity of up to 16 crayfish simultaneously with a sampling rate of 500 samples/s, processing the data to obtain *HR* and other 14 cardiac signal inotropic/chronotropic parameters, and saving the calculated parameters to local hard drive. A schematic representation of the system is shown in Fig. 1A.

The software's graphical user interface is capable of displaying raw cardiac activity signals of all monitored crayfish and their main parameters in real-time. It allows choosing which cardiac activity parameters from the set will be calculated and recorded. Optionally, raw cardiac activity can be recorded for further manual or semiautomatic analysis.

IR optical sensors

The IR optical sensor is comprised of an IR LED axially coupled with a phototransistor (Fig. 1). It is placed in the waterproof package and can be fixed for several weeks on the dorsal side of crayfish carapace above the heart with nontoxic epoxy

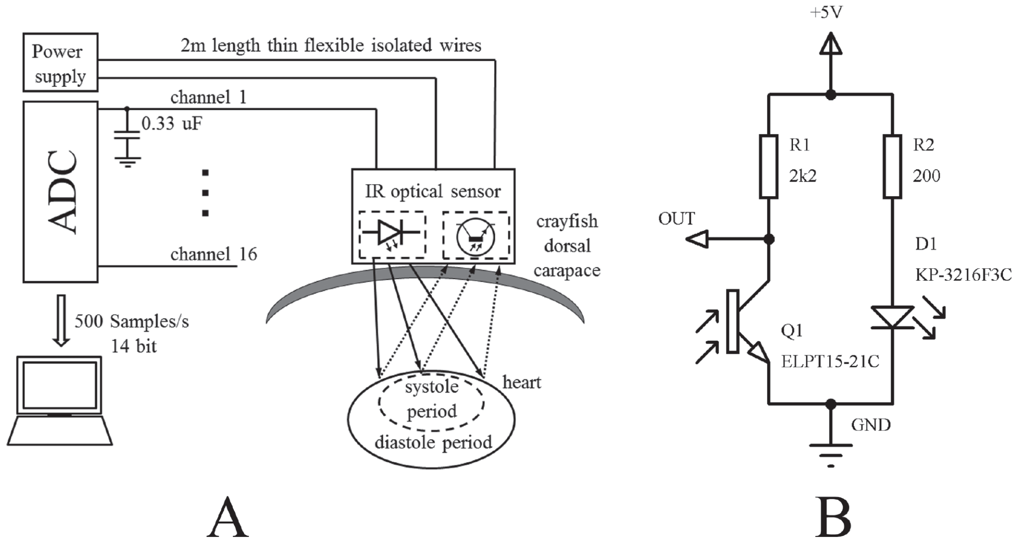

Fig. 1. NICCAM system. (A) Overview of the system. (B) Circuit diagram of IR optical sensor.

glue. The connection of sensors to the ADC is done with thin flexible wires of 2 m length.

The sensor's operation is based on the photoplethysmography principle described previously (Depledge and Andersen 1990; Fedotov et al. 2000), and the sensor is a reflective optocoupler. The electrical signal on the phototransistor output depends on the ambient illumination level and the amount of light emitted by the LED and then reflected by the objects in the phototransistor's field of view. When the sensor is placed on the crayfish carapace, the output sensor's signal is modulated by the amount of hemolymph filling the heart that effectively scatters the incident light from the LED. The described approach allows noninvasive monitoring of cardiac activity as a function of size and shape of the crayfish heart.

Except for a capacitor suppressing high frequency noise on the input to ADC the sensor's output, the signal does not require any specific amplification and/or filtering circuits, unlike the CAPMON system (Depledge and Andersen 1990) or the improved CAPMON system (Burnett et al. 2013). Increased efficiency of LEDs in the last decade allows obtaining considerably more optical power from a single device at the same electrical power consumption. Currently, a typical commercially available IR LED has total optical output power of ~ 15 mW, assuming a driving current of 20 mA and an efficiency of 60%. The increase in optical output power leads to increase of the optical power reflecting toward the phototransistor, thus amplifying its electrical output signal and eliminating the need for an external amplification circuit. Installing several small size surface mount LEDs (e.g., in 0402 packages) in one sensor can amplify the electrical output signal even more without considerable increase in overall sensor size.

It should be noted that only a small part of the total not focused optical irradiation emitted by the LED reaches the crayfish heart, and it neither harms the animal nor affects its behavior. Most of the light is radiated elsewhere because of the wide 120° viewing angle of the LED, and is scattered on boundaries among several optically different layers, e.g., the waterproof sensor's covering, the thin water layer between crayfish and sensor, and the crayfish carapace.

The benefits of not using external hardware amplification and filtering circuits include preserving the original signal shape for further software processing, minimizing signal distortion caused by improper hardware adjustments, eliminating of clipping problems (Burnett et al. 2013), and the greater simplicity and lower cost of hardware.

With the current version of the sensor hardware, typical obtained heartbeat and noise amplitudes are approximately 50 mV and 2 mV, respectively. The noise level can be reduced later in software. The signal's fluctuations caused by changes in ambient illumination fall in the range from 2 to 3 V.

Data processing

Examples of typical crayfish cardiac activity are shown in Fig. 2. The amplitude and the shape of heartbeat pattern slightly changes over time. The unique heartbeat pattern of individual crayfish varies with size, age, physiology, and species, as noted by Burnett et al. (2013). Our observations show that in general cardiac activity can be considered as having two peaks: a primary peak that defines the HR and a sec-

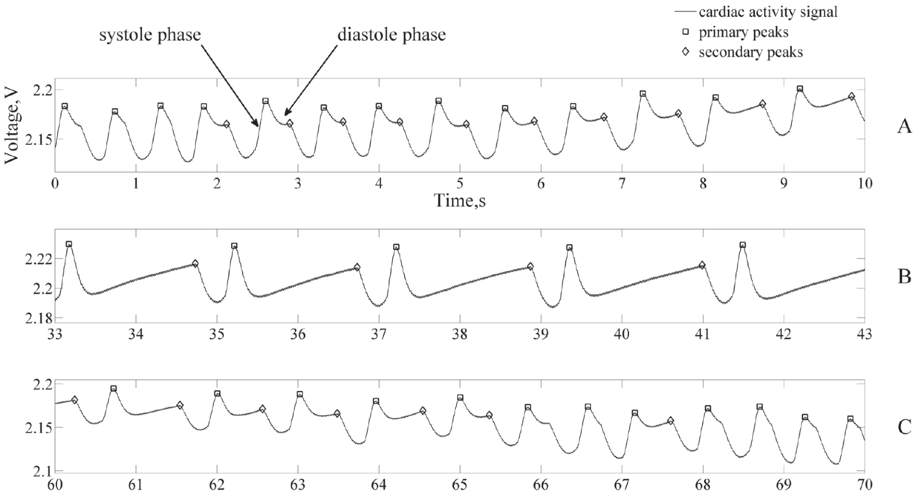

Fig. 2. Examples of cardiac activity signal recorded from one crayfish during 70 s interval. (A) Appearance of secondary peak. (B) Large amplitude secondary peak. (C) Disappearance of secondary peak.

ondary peak that sometimes (more often at low HR) appears in the diastolic phase between primary peaks.

The appearance of primary and secondary peaks in the cardiac signal is the basis of the data processing sequence implemented in the NICCAM software. Generally, it can be divided into three steps: preprocessing, detection of signal local extrema and classification of primary and secondary peaks (Fig. 3), and calculating chronotropic and inotropic parameters of cardiac signal.

At the preprocessing step, the high frequency noise of the signal is reduced by applying software implementation of a RC low-pass filter. The filter parameters can be adjusted manually to keep the noise level visually lower than the peak detection threshold, without considerable distortion to signal shape. Software implementation of an RC filter generally allows tuning the filter parameters more easily and in a wider range in comparison to typical hardware implementations that are usually designed without any tuning, largely to lower the cost.

Detection of signal local extrema at the second data-processing step is done using an algorithm to calculate the difference between the tested data point and its neighbor points. The tested data point is marked as extremum, in case the difference exceeds the specified voltage and time thresholds. This peak detection algorithm is similar to the one implemented in function “findpeaks” in Mathworks Matlab.

Classification of primary and secondary peaks is done by analyzing the peak’s shape. Visual analysis of raw crayfish cardiac data shows that the shape of the secondary peaks generally differs from the shape of the primary peaks (Fig. 2). The rising slope of the secondary peak is gentler, and height is smaller. These peculiar features of secondary peaks allow distinguishing them in software by first calculating two peak shape parameters: normalized peak height (*PHN*) and normalized peak rising slope (*PRSN*) using formulae in Table 1, and then assessing empirically obtained classification criterion *K*:

$$\frac{1}{0.9125\left(PHN+0.2336\right)}-PRSN-0.1001=K \qquad (1)$$

If the value of criterion *K* is positive, the peak is classified as secondary. Otherwise, it is classified as a primary peak.

At the final step, chronotropic and inotropic parameters of the cardiac activity are calculated separately for the primary and secondary peaks. The set of parameters is presented in Table 1.

Because of the need for combined analysis of the data obtained from different crayfish generally having different amplitudes of cardiac signal oscillations, normalization was introduced for the parameters *PPSN, NPSN, PHN, PRSN, PFSN* characterizing height and slopes of the peaks.

If the secondary peak was not present between subsequent primary peaks, only the *HR* parameter was calculated, parameters *PHN*, *PW*, *PWN* were set to zero and calculation of the rest of the parameters was skipped.

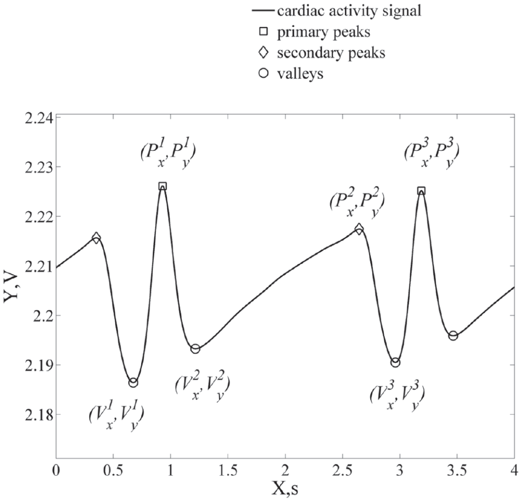

Fig. 3. Local extremum points of the crayfish cardiac activity signal used in data processing sequence.

Assessment

Operating principle

Previous reports evaluated the use of photoplethysmography for monitoring of selected invertebrates cardiac activity. Depledge and Andersen (1990) found that heart beats observed directly coincide with peaks on the photoplethysmogram for benthic decapods. Coincidence of electrocardiogram and photoplethysmogram peaks was observed by Fedotov et al. (2009) for the crayfish *Pontastacus leptodactylus*.

The presence of the secondary peaks in the crayfish cardiac signal was noticed earlier (Depledge and Andersen 1990; Fedotov et al. 2000; Burnett et al. 2013). The amplitude of the secondary peak sometimes can increase considerably and become comparable with the primary peak’s amplitude. Previous experimental results showed that it can reach 54.5% and 68.7% of primary peak amplitude in water and in air, respectively (Fedotov et al. 2000).

The origin of the secondary peaks can be connected with nonsynchronous work of two pacemaker groups of cardioneurons at crayfish cardiac ganglion that provide work of the crayfish heart. Particularly, when activity of small neurons (intrinsic cardiac pacemakers) dominates over the work rhythm of large cells (anterior group connected with cardiac muscle) (Fedotov et al. 2009).

Experimental dataset

The experimental data for the assessment of the system and software algorithms was obtained from signal crayfish *Pacifastacus leniusculus*, that were caught in baited traps in early June 2013 in Vysočina Region, Czech Republic. The experimental

Table 1. Calculation of chronotropic and inotropic parameters of crayfish cardiac activity. P_x^1, P_y^1, P_x^2, P_y^2, P_x^3, P_y^3, V_y^1, V_x^2, V_y^2, V_x^3, V_y^3 – coordinates of the local extrema on the crayfish cardiac activity curve according to Fig. 3.

Parameter	Description	Formula
HR	heart rate	$60 \ / \ \left(P_x^3 - P_x^1\right)$
PPSN	normalized previous peak slope	$-\left(\left(V_y^2 - P_y^1\right) \ / \ \left(P_y^1 - \min\left\{V_y^1, V_y^2\right\}\right)\right) \ / \ \left(V_x^2 - P_x^1\right)$
NPSN	normalized next peak slope	$\left((P_y^3 - V_y^3) \ / \ \left(P_y^1 - \min\left\{V_y^1, V_y^2\right\}\right)\right) \ / \ (P_x^3 - V_x^3)$
PHN	normalized peak height	$\left(P_y^2 - \min\left\{V_y^2, V_y^3\right\}\right) \ / \ \left(P_y^1 - \min\left\{V_y^1, V_y^2\right\}\right)$
PW	peak width	$V_x^3 - V_x^2$
PWN	normalized peak width	$\left(V_x^3 - V_x^2\right) \ / \ \left(P_x^3 - P_x^1\right)$
PRSN	normalized peak's rising slope	$\left(\left(P_y^2 - V_y^2\right) \ / \ \left(P_y^1 - \min\left\{V_y^1, V_y^2\right\}\right)\right) \ / \ \left(P_x^2 - V_x^2\right)$
PFSN	normalized peak's falling slope	$-\left(\left(V_y^3 - P_y^2\right) \ / \ \left(P_y^1 - \min\left\{V_y^1, V_y^2\right\}\right)\right) \ / \ \left(V_x^3 - P_x^2\right)$
PSR	peak slopes ratio	$PRSN \ / \ PFSN$
PS	peak start	$V_x^2 - P_x^1$
PO	peak offset	$P_x^2 - P_x^1$
PE	peak end	$V_x^3 - P_x^1$
PSN	normalized peak start	$PS \ / \ \left(P_x^3 - P_x^1\right)$
PON	normalized peak offset	$PO \ / \ \left(P_x^3 - P_x^1\right)$
PEN	normalized peak end	$PE \ / \ \left(P_x^3 - P_x^1\right)$

group consisted of 10 adult individuals (half males, half females; mean total length, 108 ± 4 mm; mean carapace length, 54 ± 2 mm; mean weight, 56 ± 6.1 g).

The experiments were conducted in July–August 2013. For acclimatization, crayfish were maintained for 1 week in individual 10 L (water volume 7 L) glass nontransparent aquaria, each equipped with a shelter. Before manipulations, selected crayfish were marked with individual numbers by permanent water-resistant marker. A week before recording data, sensors were attached to the dorsal side of the crayfish carapace directly over the heart using two-component, rapid epoxy adhesive. Throughout the experimental period, water temperature was 19°–21°C, pH was 7.2–7.4, dissolved oxygen level was greater than 6.5 mg/L, and the illumination regime was set and automatically maintained at 12 h light and 12 h dark. Crayfish were fed twice a week on *Chironomidae* larvae, and the remaining food and crayfish life byproducts were removed during the water exchange. No mortality occurred during the experimental period.

The first part of the dataset containing 25 h raw cardiac activity data were recorded in 1 h intervals at different dates and time during experimental period. The data were obtained from an approximately equal mix of male and female crayfish.

The second part of the dataset containing 9 h raw cardiac activity data were recorded during two experiments. In the first, "food odor" (mashed *Chironomidae*) was added after 24 hours of starvation. In the second, 10 mg/L chloramine-T was added to the aquaria with crayfish. Both were first mixed into 50 mL tap water. During exposure periods, animals were not fed. To initiate the exposure, the 50 mL treatment volume was pumped into aquaria with a Watson-Marlow peristaltic pump at a flow rate of 50 mL/min. Six crayfish were exposed to food odor, and three crayfish to chloramine-T. The recording of raw cardiac activity of each crayfish started 30 min before the exposure and finished 30 min after exposure. After recording was finished, the water in aquaria was changed.

All 34 hours of raw cardiac data described above were processed by specially developed software to find local extrema of the cardiac signal and roughly classify between primary and secondary peaks.

The resulting ~ 191000 of classified peaks were manually checked and found peak classification errors were corrected.

Classification of primary and secondary peaks

Large amplitude secondary peaks of the crayfish cardiac signal can be mistakenly considered as primary peaks that will result in errors during *HR* calculation (Depledge and Andersen 1990; Burnett et al. 2013). In previous studies, the error rate was minimized by carefully adjusting the hardware peak counting threshold according to particular cardiac signal shape/amplitude parameters and/or inverting the cardiac sensor output signal (Depledge and Andersen 1990). Unfortunately, no quantitative information on the accuracy of peak counting and classification algorithms was presented in the previous studies (Depledge and Andersen 1990; Fedotov et al. 2000; Burnett et al. 2013). We propose a classification algorithm based on analysis of the peak's shape and will address its accuracy.

The manually chosen parameters *PHN* and *PRSN* were calculated using formulae in Table 1 for the complete dataset described above. The obtained parameter data and manually verified classification data were used to optimize classification criteria, and for the assessment of peak classification accuracy.

The scatter plot of *PHN* and *PRSN* parameters calculated from the dataset is presented in Fig. 4. It can be noticed that primary and secondary peaks are relatively well separated into two clusters in the plot. The chosen analytical criterion for classification of primary and secondary peaks was in the form of the following parametric equation:

$$\frac{1}{a\left(PHN+b\right)}+PRSN+c=0 \qquad (2)$$

in which *PHN* and *PRSN* are the classified peak's parameters as defined in Table 1; *a*, *b*, and *c* – optimization parameters.

The values of optimization parameters minimizing the number of classification errors in the dataset were determined using a Nelder-Mead simplex algorithm implemented in Matlab function "fminsearch." The resulted values of 0.9125, 0.2336, and –0.1001 for optimization parameters *a*, *b*, and *c*, respectively, give a classification error of less than 0.5% on the complete dataset (see Eq. 1).

A single peak classification error (assuming neighbor peaks are classified correctly) can result in approximately doubled or halved *HR* at a given point in time. This, in turn, can considerably affect the mean and variance of *HR*, especially if the period used for calculation of statistics is short.

Correlation between pairs of cardiac activity parameters

A secondary peak appears in the crayfish cardiac activity signal more often at periods of low *HR*, whereas at high *HR*, it disappears. To examine the relationship between *HR* and amplitude of the secondary peak, as well as the relationship between other pairs of cardiac activity parameters, a set of inotropic and chronotropic parameters was calculated using formulae in Table 1 from the complete dataset described above.

The resulted Pearson product-moment and Spearman's rank correlation coefficients between all possible pairs from the whole set of chronotropic and inotropic parameters of cardiac activity are shown in Fig. 5. Calculation of the high number of correlations can be influenced by the Type I error. Therefore, the correlation significance test was performed. Testing of the hypotheses of no correlations showed correlation significantly different from zero for all the parameter pairs except for {*HR*:*PPSN*}, {*NPSN*:*PPSN*}, {*PS*:*PHN*}, {*PSN*:*NPSN*}, {*PSN*:*PRSN*} pairs for Pearson correlation and {*PSN*:*NPSN*}, {*PSN*:*PSR*} pairs for Spearman correlation. The used *p* value threshold maintaining 95% confidence in the set of totally 105 correlation tests was set to 0.00048 after applying Bonferroni correction. Scatter plots of HR and each of the parameters of cardiac activity are shown in Fig. 6.

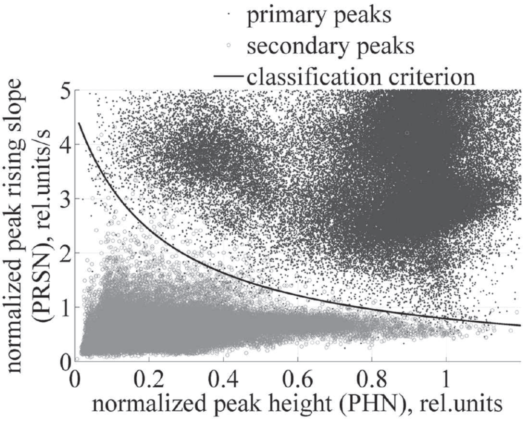

Fig. 4. Classification of primary and secondary peaks of the crayfish cardiac activity data based on peak's shape parameters: normalized peak height (*PHN*) and normalized peak rising slope (*PRSN*). Calculation of *PHN* and *PRSN* was done using formulae in Table 1.

As it is seen from Figs. 5 and 6, *HR* is highly correlated with *PW, PO, PE* parameters. Both Pearson product-moment and Spearman's rank correlation coefficients measuring linear correlation and monotonic relation between pairs of variables, respectively, give absolute values greater than 0.85. Both Pearson and Spearman pairwise correlation coefficients between *PW, PO, PE* parameters are even higher, giving the values greater than 0.96. Normalized chronotropic parameters *PWN, PON, PEN* are also highly correlated with *HR* and among each other.

Parameters describing the slopes of primary and secondary peaks (*PPSN, NPSN, PRSN, PFSN, PSR*), height (*PHN*) and starting time (*PS*, *PSN*) of secondary peaks are less correlated with *HR* and among themselves.

Effect of food odor and chloramine-T on crayfish cardiac activity parameters

It was shown above that inotropic and chronotropic parameters of crayfish cardiac activity change over time. However, it is an open question whether these parameters carry additional information on crayfish state independent of heart rate, or if they change randomly. It is also an open question as to whether monitoring of these parameters can be used for assessment of water quality. We answer these questions by analyzing the results of two experiments, adding food odor and chloramine-T.

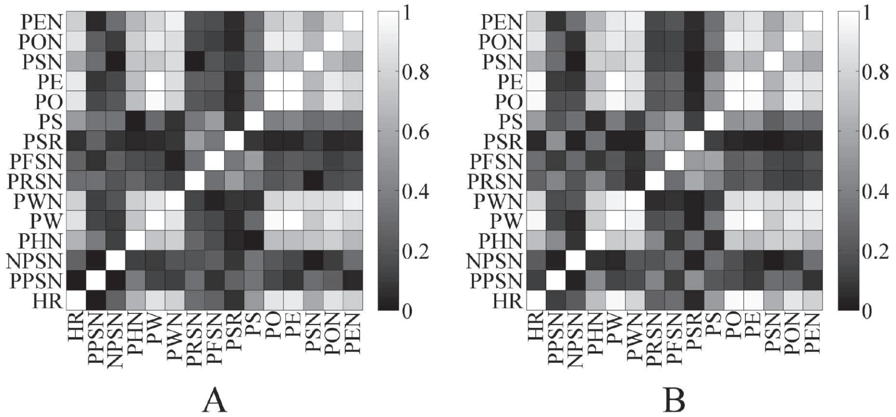

Fig. 5. Absolute values of correlation coefficients between pairs of crayfish cardiac activity parameters defined in Table 1 that can carry additional independent information on the crayfish state: (A) Pearson product-moment, (B) Spearman's rank.

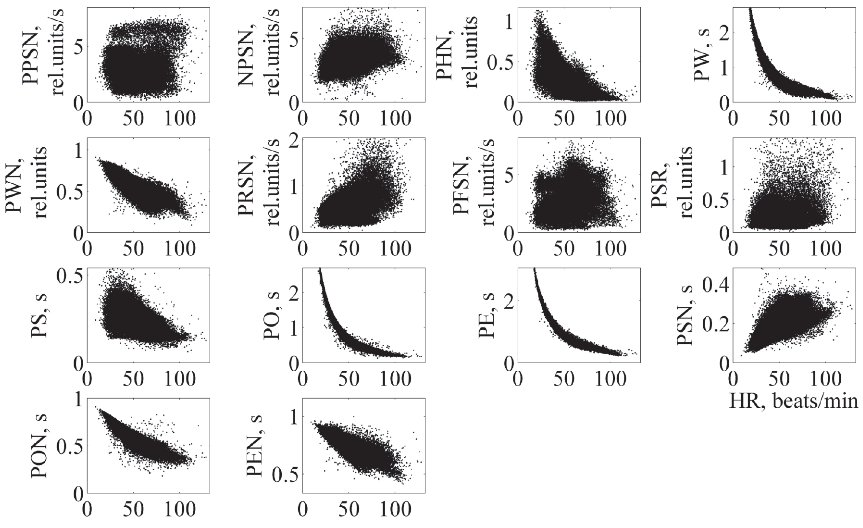

Fig. 6. Correlation between HR and other inotropic and chronotropic parameters of crayfish cardiac activity defined in Table 1 that can carry additional information on crayfish state other than what is provided by HR data.

We separately processed data on crayfish cardiac activity recordings before and after the exposure to food odor and chloramine-T. For each crayfish the mean and standard deviation (SD) values of 15 cardiac activity parameters (Table 1) before and after the impact were calculated. The mean and SD of parameters for each crayfish were then averaged over the group of crayfish that were used in each of the two experiments using Eq. 3 and Eq. 4:

$$\overline{P^j} = \frac{\sum_{i=1}^{N} \overline{P_i^j}}{N} \tag{3}$$

$$std\left(P^j\right) = \frac{\sum_{i=1}^{N} std\left(P_i^j\right) \;/\; \overline{P_i^j}}{N} \overline{P^j} \tag{4}$$

in which $\overline{P^j}$, $std\left(P^j\right)$, mean and SD values of *j*-th parameter over the group of crayfish, respectively; *N*, number of crayfish used in the experiment; P_i^j, $std\left(P_i^j\right)$, mean and SD value of *j*-th parameter for *i*-th crayfish, respectively.

The changes in mean and SD of cardiac activity parameters are presented in Fig. 7. Each parameter's mean and SD values were normalized for better visualization of all the parameters on a single graph. The normalization was done by dividing mean and SD value of each parameter by the maximum parameter mean value before or after the impact.

The results of the experiments show that relative change of parameters *PHN*, *PW*, and *PWN* describing secondary peak height and width was 1.5–2 times bigger after the impact in comparison to *HR*. Comparison of both experiments show that parameters *PS*, *PO*, *PE* decreased after both experimental impacts, meaning that the secondary peak shifted left, whereas *HR* increased in experiment with food odor and decreased in experiment with chloramine-T. However, additional experiments with a bigger number of crayfish are required to prove that described effects are statistically significant.

Discussion

The NICCAM system described here is cheap and easy to manufacture (Depledge and Andersen 1990; Fedotov et al. 2000; Burnett et al. 2013). Except for ADC and PC, it requires

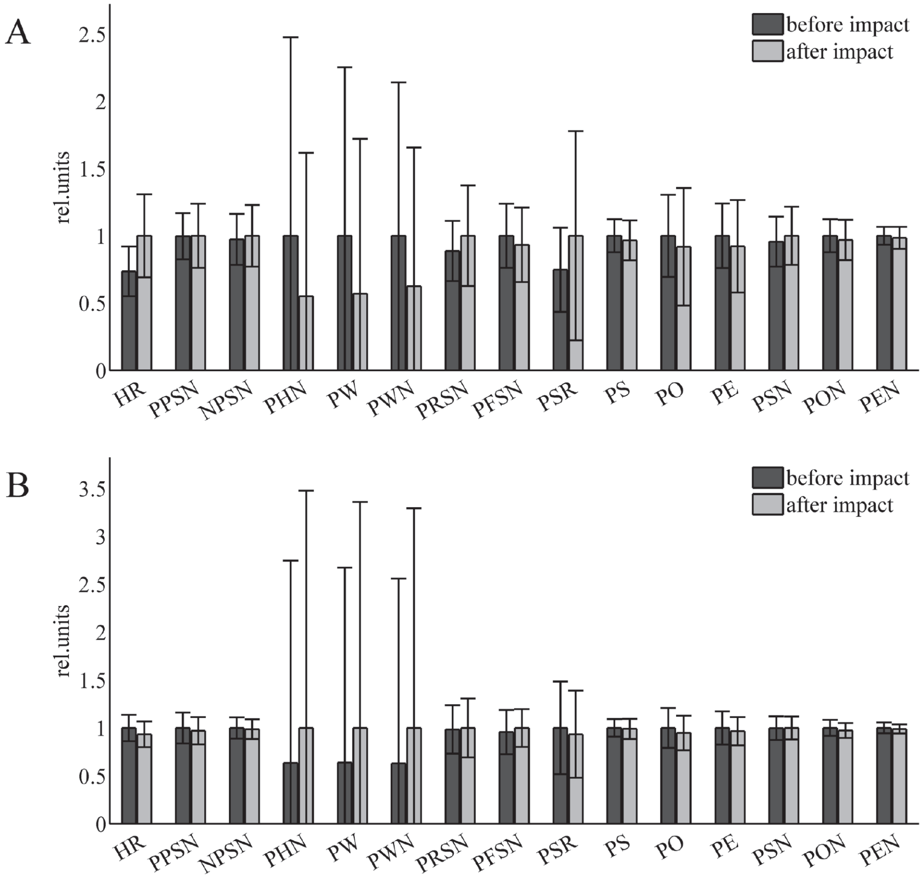

Fig. 7. Changes in mean and SD of crayfish cardiac activity parameters (see Table 1) in response to odor of food averaged over six crayfish (A) and chloramine in concentration 10 mg/L averaged over three crayfish (B). The mean and SD values are normalized by the maximum parameter's mean value before or after the impact.

only a few hardware components. The total price of the components and materials required for manufacturing of one sensor is 1-2 EUR depending on the ordering quantity. The IR optical sensors that can be connected to the crayfish within a few minutes do not affect the crayfish behavior and do not restrict its movements. Up to 16 crayfish can be monitored simultaneously 24 h/day. Also, the system allows recording and storing raw cardiac activity data for further manual or semiautomatic analysis.

Implementing most of the signal preprocessing and filtering in software not only simplifies the hardware design but also minimizes the signal distortion that can be caused by improperly adjusted hardware parameters. Also, it grants the operator more flexibility and easier control over the data processing sequence.

The complexity of the crayfish cardiac activity signal leads to errors in calculating heart rate and other cardiac activity parameters. The algorithm proposed here, based on analysis of peak shape, simplifies the classification of peaks and improves the accuracy of data processing. It can be also implemented in modifications of the previously developed systems (Depledge and Andersen 1990; Fedotov et al. 2000; Burnett et al. 2013) for improving their accuracy.

One of the benefits of the proposed system is its ability to analyze the shape of the cardiac activity signal. Previously developed systems were focused on calculating the *HR* from the cardiac activity, whereas shape analysis of the signal can provide additional useful information on crayfish state. This information can be used in ethological studies as well as in studies using aquatic organisms as biomarker of water pollution (Depledge and Galloway 2005).

Analysis of correlation between *HR* and other chronotropic and inotropic parameters of crayfish cardiac activity (Figs. 5 and 6) show that the slopes of the peaks, the amplitude and starting time of the secondary peak can potentially carry additional information beyond that provided by heart rate. Whether these parameters describe some internal variables of the crayfish cardiac system or they change randomly are to be studied additionally.

The results of the experiments on the effect of food odor and chloramine on chronotropic and inotropic parameters of cardiac activity (Fig. 7) showed that the monitoring of some of these parameters can be promising for analysis of crayfish state during eco-ethological studies. Particularly, parameters that change more considerably than *HR* allow easier detection of changes in crayfish state and parameters that change in opposite directions after different types of impacts can potentially allow qualitatively distinguishing crayfish states. However additional experiments proving the obtained results and conclusions are needed.

Comments and recommendations

One of the factors affecting the accuracy of cardiac activity parameter calculation is crayfish movement. It can result in varying of the signal offset or even short-time spikes in the signal during rapid crayfish movement. Such signal distortions can lead to incorrect cardiac activity parameter calculation by software. One of the possible ways to solve the problem can be installing of a camera monitoring system that can track the crayfish and mark the periods of its movement using software motion detection algorithms. One camera can be used for monitoring of several aquaria at the same time. The data from the camera system can be used for excluding the periods of high crayfish locomotive activity from consideration during cardiac activity parameters calculation and also as an independent source of information on crayfish state.

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Appendix C:

NICCBAM system - Czech Republic national patent application

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(72) Původce:
doc. Ing. Pavel Kozák, Ph.D., Protivín, CZ
Iryna Kuklina, MSc., Vodňany, CZ
Aliaksandr Pautsina, MSc., České Budějovice, CZ
Ing. Petr Císař, Ph.D., České Velenice, CZ
Ing. Antonín Kouba, Ph.D., České Budějovice, CZ

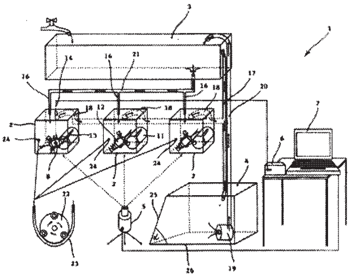
(74) Zástupce:
PatentCentrum Sedlák a Partners s.r.o., Husova 5, 370 01 České Budějovice

(54) Název vynálezu:

Způsob etologického sledování korýšů a/nebo měkkýšů a etologický systém pro sledování chování korýšů a/nebo měkkýšů

(57) Anotace:

Způsob etologického sledování korýšů a/nebo měkkýšů (12), při kterém se snímá alespoň jedna fyziologická funkce korýše a/nebo měkkýše (12) neinvazivním čidlem (8) upevněným ke korýši a/nebo měkkýši (12) v reálném čase a přenáší se do počítače (7) ve formě digitálního signálu, který je zpracován softwarem pro vyhodnocování reakcí korýše a/nebo měkkýše (12) na přirozené a/nebo uměle vyvolané environmentální změny. Podstata řešení spočívá v tom, že současně s fyziologickou funkcí se kontinuálně v reálném čase sleduje i přirozené chování korýše a/nebo měkkýše (12) pomocí kamery (5), přičemž se současně sledují alespoň tři korýši a/nebo měkkýši (12). Každý korýš a/nebo měkkýš (12) je umístěn ve vlastním akváriu a obrazový záznam přirozeného chování pořízený kamerou (5) se ve formě digitálního signálu přenáší do počítače (7), kde se porovnává a vyhodnocuje současně s digitálním signálem z neinvazivního čidla (8) pro sledování fyziologické funkce pomocí společného vyhodnocovacího softwaru. Předmětem řešení také je etologický systém pro provádění výše popsaného způsobu.



Způsob etologického sledování korýšů a/nebo měkkýšů a etologický systém pro sledování chování korýšů a/nebo měkkýšů

Oblast techniky

Vynález se týká oblasti způsobu etologického sledování korýšů a/nebo měkkýšů a etologického systému pro sledování chování korýšů a/nebo měkkýšů, kde se snímá alespoň jedna fyziologická funkce korýše a/nebo měkkýše neinvazivním čidlem.

Dosavadní stav techniky

Využití živých organismů jako biologických indikátorů kvality prostředí, které obývají, patří v současné době mezi hlavní směry eko–biologických výzkumů. Živočich je umístěn do svého běžného prostředí, kde je neustále sledován. Vzorce jeho chování jsou neustále zaznamenávány a následně vyhodnocovány. Lze tak zaznamenat případné reakce sledovaného živočicha na znečištění jeho životního prostředí. Pro účely takového sledování se jako nejvhodnější jeví skupina korýšů, obzvlášť dobrých výsledků bylo dosaženo pozorováním raků. Jejich výhodou je velká biodiverzita, takže pro každé prostředí lze najít odpovídající druh. Rak má jednoduchý kardiovaskulární systém, snadno sledovatelné přenosy v nervové soustavě a exoskeleton. Dobře přežívá v laboratorních podmínkách.

Pro sledování pohybu raka v jeho běžném prostředí, např. v akváriu nebo jiném zásobníku s vodou, se běžně používá kamera. Nevýhodou prostého sledování račího pohybu kamerou je skutečnost, že rak je aktivní v noci, což snižuje kvalitu kamerového záznamu. Pro překonání této nevýhody byly rakům lepeny na krunýř diody vydávající červené světlo, popř. raci byli natáčeni kamerou, na jejímž krytu byla umístěna polarizovaná, částečně lineární světla. Umělá světla ale raky znepokojovala, takže jejich pohyb a tep nebyl přirozený a výsledky pozorování poskytovaly zkreslené údaje.

Kamery se umisťují nad nebo pod akvárium, které sledovaný rak obývá. Pro přesnější zachycení pohybových vzorců sledovaného raka bylo vytvořeno zařízení tvořené pojízdným stolem s rotační deskou s upevněným ramenem, na jehož konci je upevněna kamera. Toto zařízení umožňuje kopírování pohybu raka kamerou a tím i lepší a přesnější záznam.

Nicméně pouhé sledování pohybu raků není dostatečným indikátorem změny prostředí v důsledku znečištění, protože umožňuje pouze vizuální kontrolu chování raka, což může být velmi nepřesné.

Pozorování raků a jiných korýšů se běžně provádí způsobem nazvaným invazivní biomonitoring, kdy se sledují a vyhodnocují životní funkce raka. Mezi tyto způsoby sledování patří např. impedanční životní funkce raka. Mezi tyto způsoby sledování patří např. impedanční pneumografie (IPG), sledující dýchací pohyby. Pomocí impedančního pneumografu se nízký oscilační proud (2 μA) přenáší mezi malými drátky, které slouží jako elektrody. Jeden z takových způsobů invazivního měření byl testování na *Palaemon elegans.* Stříbrná elektroda byla implantována do perikardu krevety přes malinkou dírku vyvrtanou do jejího krunýře přímo nad srdcem. Elektroda byla zafixována kyanoakrylátovým lepidlem. Druhá elektroda byla upevněna na první elektrodě, ihned nad otvorem v krunýři, v němž je vložena první elektroda. Takové uspořádání udržuje rozmístění elektrod, což je ale nevýhodné v tom, že elektrody neumožňují zachytit pohyb krevety.

Elektrokardiografie (ECG) je dalším způsobem invazivního biomonitoringu. Dva izolované nerezové drátky byly umístěny pod dorzálním krunýřem přímo nad srdcem. Drátky byly uloženy do dírek vyvrtaných v krunýři a zacementovány vteřinovým kyanoakrylátovým lepidlem.

kardiální projevy byly zachyceny impedančním detektorem, který měřil dynamický odpor mezi drátky z nerezové oceli, a zaznamenány do počítače.

Jako vhodnější se jeví neinvazivní způsoby sledování životních funkcí živočichů, např. raků. Takovým způsobem je např. pletysmografie, založená na průchodu světelných paprsků tkání vyšetřované oblasti. Pulzní pletysmografické čidlo je tvořeno dvěma žárovkami s nízkou intenzitou záření a fototranzistorem. Pro měření srdeční aktivity korýšů, musí být čidlo připevněno z vnějšku na krunýř nad oblastí srdce pomocí voděodolného ekologického lepidla. Světlo s nízkou intenzitou záření vydávané žárovkami prochází krunýřem do korýšova perikardu. Při kontrakci srdečního svalu perikard odrazí část světla, které dopadne na fototranzistor, který přemění světelnou energii na proporční změnu napětí, kterou předá zpracovacímu zařízení. Nevýhoda tohoto zařízení spočívá v jeho složitém nastavení a následném vyhodnocování údajů.

Pletysmografii (PPG) využívá systém počítačového sledování fyziologických funkcí (CAPMON), který umožňuje neustále a dlouhodobé zaznamenávání srdeční aktivity bezobratlých. Používají se stejné prvky a postupy jako u pletysmografie, ovšem s několika úpravami převodníku. Zařízení sestává z diody vydávající téměř infračervené záření a detektoru fototranzistoru. Prvky jsou zapojeny paralelně a směřují stejným směrem. Fototranzistor snímá změny v intenzitě infra–červeného světla a vyrábí proud, který závisí na odraženém světle, a který je dále filtrován, zesílen a převeden do počítačového rozhraní. Převodník je na počítačové rozhraní napojen tenkým pružným drátkem, takže sledovaný živočich není nijak omezován při svém pohybu po akváriu.

Navíc sledování tlukotu srdce metodou elektrokardiografie potvrdilo, že jednotlivé tepy souhlasí s vrcholy získanými pletysmografií, takže srdeční aktivita může být sledovaná u několika jedinců současně 24 h denně po dlouhé časové intervaly.

Automatické měření interpulzní doby (AIDA) umožňuje přesnější analýzu variability srdečního tepu a odhalení vzruchů. Přesná analýza odhalila intervaly pravidelné a nepravidelné srdeční aktivity, které jsou zahrnuty do vyhodnocení.

Na způsob měření CAPMON navázal způsob zaznamenávání a analýzy srdeční aktivity bentických bezobratlých. Princip zaznamenávání zůstal stejný, měří se výkyvy rozptýleného a odraženého infra–červeného světla, ale zdroj světla byl nahrazen polovodičovým laserem a drátky byly nahrazeny optickými kabely. Paprsek infra–červeného záření vytvořený v laserovém optickém fotopletysmografu se přenese na živočicha tenkým optickým vláknem a malé čidlo spojené s optickým vláknem a upevněné na krunýři živočicha osvětluje oblast srdce rozptýleným světlem. Optický signál je vytvořen stahem srdečního svalu. Po odpovídajícím zvětšení a odfiltrování se analogový signál konvertuje do digitální formy a je předán počítači.

Nevýhodou stávajících systémů a způsobů sledování chování a fyziologických funkcí korýšů a/nebo měkkýšů je jejich jednostrannost, která neumožňuje komplexní vyhodnocení příčin a důsledků změn v chování korýšů a/nebo měkkýšů.

Úkolem vynálezu je vytvoření etologického způsobu sledování korýšů a/nebo měkkýšů, který by umožňoval současné snímání chování korýše a/nebo měkkýše a snímání jeho fyziologických funkcí, např. tepu srdce, kdy získané záznamy se porovnávají v reálném čase a vyhodnocuje se podle nich stav životního prostředí korýšů a/nebo měkkýšů.

Podstata vynálezu

Tento úkol je vyřešen vytvořením způsobu etologického sledování korýšů a/nebo měkkýšů, při kterém se snímá alespoň jedna fyziologická funkce korýše a/nebo měkkýše neinvazivním čidlem upevněným ke korýši a/nebo měkkýši v reálném čase a přenáší se do počítače ve formě digitálního signálu, který je zpracován softwarem pro vyhodnocování reakcí korýše a/nebo měkkýše na přirozené a/nebo uměle vyvolané environmentální změny. Podstata vynálezu spočívá v tom, že současně s fyziologickou funkcí se kontinuálně v reálném čase sleduje i přirozené chování korýše a/nebo měkkýše pomocí kamery, přičemž se současně sledují alespoň tři korýši a/nebo měkkýši, aby výsledky sledování byly objektivní a nedocházelo ke zkreslení na základě např. zvýšeného tepu nebo neobvyklého chování jednoho ze sledovaných živočichů. Každý korýš a/nebo měkkýš je umístěn ve vlastním akváriu, aby nedocházelo k vzájemnému ovlivňování živočichů, čímž by se jim mohl např. zvýšit tep nebo frekvence dechu, což by poskytnulo nepřesný záznam. Obrazový záznam přirozeného chování pořízený kamerou se ve formě digitálního signálu přenáší do počítače, kde se porovnává a vyhodnocuje současně s digitálním signálem z neinvazivního čidla pro sledování fyziologické funkce pomocí společného vyhodnocovacího softwaru. Díky porovnání obou záznamů se získá velmi přesný údaj o stavu živočicha a o stavu jeho prostředí, např. čistotě vody. Na některé polutanty totiž korýš může reagovat tak, že se stáhne do úkrytu, na jiné zvýšeným tepem, popř. obojím.

Environmentální změny jsou uměle vyvolané, přičemž do všech sledovaných akvárií se současně dávkují stejné toxiny a/nebo nepřirozené látky pro korýše a/nebo měkkýše. Lze tak přesně stanovit a vyhodnotit, jak sledování živočichové environmentální změnu zachytili a jak na ni reagují. Vyhodnocuje se fyziologická odpověď korýše a/nebo měkkýše snímaná neinvazivním čidlem a změny v přirozeném chování korýše a/nebo měkkýše snímané kamerou v závislosti na množství, druhu a dávkování toxinů a/nebo nepřirozených látek. Kromě změny ve fyziologických funkcích, např. zvýšení tepu srdce, lze zaznamenat i změnu v chování sledovaného živočicha, což poskytuje přesnější vyhodnocení toho, jak živočich environmentální změnu vnímá.

Předměty v každém akváriu se přesně lokalizují a jejich pozice se zanesou do softwaru. Vyhodnocování obrazových záznamů z kamery se zpracovává ve vztahu k lokalizovaným předmětům. To je důležité právě pro vyhodnocení změny v chování živočicha, především korýše, který na environmentální změny reaguje tendencí vlézt do úkrytu nebo naopak úkryt opustit.

Fyziologická funkce, která se pro účely tohoto způsobu sledování snímá, je tep srdce korýše a/nebo měkkýše. Tep srdce je velmi spolehlivý ukazatel, který lze snadno a spolehlivě sledovat a zaznamenávat.

Neinvazivní čidlo je opatřeno zdrojem infračerveného světla, které vysílá do těla korýše a/nebo měkkýše, a přijímacím zařízením, kterým se přijímá infračervené světlo odražené od srdce korýše a/nebo měkkýše. Na základě objemu krve v komoře srdce tak lze snadno zjistit frekvenci typu srdce sledovaného živočicha. Infračervené světlo, které čidlo do těla živočicha vysílá, živočich nevnímá, takže neovlivňuje a nezkresluje výsledky sledování.

Sledovaný živočich je s výhodou korýš patřící do třídy *Malacostraca*, konkrétně je korýš rak signální (*Pacifastacus leniusculus*) a neinvazivní čidlo se přilepí na krunýř raka. Výhody využití raků pro účely tohoto vynálezu spočívají v tom, že mají jednoduchý kardiovaskulární systém, takže je snadné sledovat a vyhodnocovat tep račího srdce. Raci také mají exoskelet, takže na jejich krunýř lze velmi snadno upevnit neinvazivní čidlo, které se na krunýř přilepí netoxickým ekologickým lepidlem.

Předmětem vynálezu také je etologický systém pro sledování chování korýšů a/nebo měkkýšů výše popsaným způsobem. Podstata etologického systému spočívá v tom, že sestává z alespoň tří akvárií, kde v každém akváriu je jeden korýš a/nebo měkkýš, aby se živočichové, zejména korýši, nemohli vzájemně ovlivňovat, a jejich chování a fyziologické reakce na environmentální změny byly co nejobjektivnější. Každý korýš a/nebo měkkýš je opatřen neinvazivním čidlem pro snímání tepu oběhové soustavy korýše a/nebo měkkýš, kde neinvazivní čidlo je opatřeno zdrojem infračerveného záření pro vysílání infračerveného záření do těla korýše a/nebo měkkýše a přijímacím zařízením pro příjem odraženého infračerveného záření z těla korýše a/nebo měkkýše. Protože čidlo je neinvazivní, nevadí sledovanému živočichovi, a poskytuje objektivní záznam z živočichovy oběhové soustavy. Systém dále sestává z kamery pro současné snímání přirozeného chování každého korýše a/nebo měkkýše, protože záznam z kamery upřesňuje fyziologické reakce zachycené neinvazivním čidlem. Systém dále sestává z jednoho počítače opatřeného softwarem pro současné vyhodnocení záznamů z kamery a z neinvazivního čidla předávaných do počítače současně, kontinuálně, a v reálném čase. Systém dále obsahuje dávkovací zařízení pro dávkování testovacích látek do akvárií, čímž se provádí environmentální změny pro sledování fyziologických změn, alespoň jednu nádrž pro průtočné napájení akvárií a alespoň jednu retenční nádrž pro sběr odtékající vody z akvárií.

Každé čidlo je opatřeno kabelem pro přenášení záznamů do počítače, přičemž neinvazivní čidlo je opatřeno přepážkou pro oddělení zdroje infračerveného záření od přijímacího zařízení infračerveného světla. Kabelový přenos umožňuje jednodušší konstrukci neinvazivního čidla, které pak je méně náchylné k poškození. Oddělení vysílacího zařízení od přijímacího zařízení zabraňuje vzájemnému rušení obou členů čidla, takže signál, který přijímací zařízení vysílá dále do převodníku, není zkreslený a nepřesný.

Každé akvárium je opatřeno úkrytem pro korýše s polohou zanesenou v softwaru počítače přes analogově–digitální převodník. Lze tak sledovat pohyb korýše po akváriu a na základě toho odhadovat dopad environmentálních změn na korýše. Každý úkryt je opatřen vybráním pro pohyb kabelu čidla upevněného na těle korýše, takže korýš může zalézt do úkrytu bez toho, aby mu kabel neinvazivního čidla bránil v pohybu.

Etologický systém je ve výhodném provedení uspořádán v horizontálních rovinách, kde nádrž pro průtočné napájení akvária je uspořádána v horní rovině, pod ní je uspořádána horizontální řada alespoň tří akvárií a pod řadou akvárií jsou uspořádány dvě retenční nádrže. Voda z nádrže pro průtočné napájení může samospádem vtékat do akvárií a přebytečná voda přepadem vtéká do spodní retenční nádrže. V prostoru mezi retenčními nádržemi je uložena kamera, která má ve svém zorném poli všechna akvária, která snímá současně v jediném okamžiku.

Retenční nádrž má tvar nepravidelného čtyřúhelníku, jehož vertikální stěna sousedící s kamerou svírá s podstavou ostrý úhel α. Skosení vnitřních stěn retenční nádrže rozšiřuje snímací úhel kamery, která díky tomu zabírá všechna akvária v jediném okamžiku.

Korýš patří do třídy *Malocostrata*, konkrétně se jedná o raka signálního. Výhoda využití třídy *Malocostrata* spočívá v tom, že její zástupci dobře přežívají v laboratorních podmínkách, navíc má tato třída velkou biodiverzitu, takže pro různá prostředí lze najít jejich přirozené obyvatele, čímž se docílí vysoká přesnost prováděných měření.

Výhody vynálezu spočívají v tom, že sledováním korýšů a/nebo měkkýšů způsobem podle vynálezu lze získat velmi přesný přehled o reakcích sledovaných živočichů na různou škálu environmentálních změn a lze tak spolehlivě vyhodnotit škodlivost a celkový vliv těchto změn na přirozené obyvatele vodního prostředí.

Objasnění výkresů

Vynález bude blíže objasněn pomocí obrázků na výkresech, na nichž obr. 1 znázorňuje schéma etologického systému pro sledování korýšů a/nebo měkkýšů a obr. 2 je schéma neinvazivního čidla.

Příklady uskutečnění vynálezu

Rozumí se, že dále popsané a zobrazené konkrétní případy uskutečnění vynálezu jsou představovány pro ilustraci, nikoliv jako omezení příkladů vynálezu na uvedené příklady. Odborníci znalí stavu techniky najdou nebo budou schopni zajistit za použití rutinního experimentování větší či menší počet ekvivalentů ke specifickým uskutečněním vynálezu, která jsou zde popsána. I tyto ekvivalenty budou zahrnuty v rozsahu následujících patentových nároků.

Etologický systém 1 podle vynálezu je tvořen horizontální řadou alespoň tří akvárií 2, kde každé akvárium 2 je obývané korýšem a/nebo měkkýšem 12, v tomto příkladu uskutečnění rakem signálním (*Pacifastacus leniusculus*). Nad řadou akvárií 2 je upevněna nádrž 3 pro průtočné napájení akvárií 2, odkud je voda přiváděna společným vedením 21, z něhož ústí do každého akvária 2 zvláštní přívod 16 vody z nádrže 3. V tomto příkladu uskutečnění je pod akvárií 2 uložena retenční nádrž 4, která sbírá jednak přepadem 17 vodu z nádrže 3 pro průtočné napájení akvárií 2. Do přepadu 17 je také zaústěn výstup 18 použité vody z akvárií 2. V retenční nádrži 4 je uloženo čerpadlo 19, které v případě potřeby přečerpává vodu z retenční nádrže 4 zpět do nádrže 3 pro průtočné napájení akvárií 2, kam je voda dopravována potrubím 20.

Systém 1 je dále opatřen peristaltickou pumpou 22, pomocí které jsou do akvárií 2 dávkovány toxiny a jiné látky nepřirozené pro raky 12 obývající jednotlivá akvária 2 systému 1. Toxiny a jiné látky jsou do peristaltické pumpy 22 přiváděny vedením 23 ze zásobníků, které na obr. 1 nejsou znázorněny. Toxiny a jiné látky, které nejsou pro druh raka 12 obývajícího jednotlivá akvária 2 přirozené, se do akvárií dávkují vstupy 24, kde každé akvárium 2 má svůj vlastní vstup 24. Pro účely tohoto vynálezu jsou dávky do každého akvária 2 stejné, protože se sledují a porovnávají fyziologické odpovědi raků 12 a změny v jejich přirozeném chování, na jejichž podkladě se vyhodnocuje škodlivost látek přidaných do akvárií 2.

Pod řadou akvárií 2 vedle retenční nádrže 4 je uspořádána kamera 5, podle tohoto příkladu uskutečnění je použita síťová IP kamera, ale je možné použít i web kameru, analogovou kameru, aj. Retenční nádrž 4 má tvar nepravidelného čtyřúhelníku, jehož stěna 25 sousedící s kamerou 5 je skosená a se základnou 26 retenční nádrže 4 svírá ostrý úhel α. Díky této úpravě retenční nádrže 4 má kamera 5 dostatečný zorný úhel, aby pokryla všechna tři akvária 2 v řadě.

V jiném příkladu uskutečnění neznázorněném na výkresech mohou být pod řadou akvárií 2 uspořádány dvě retenční nádrže 4, za podmínky, že obě mají skosenou stěnu 25 sousedící s kamerou 5, aby byl zachován zorný úhel kamery 5.

Obrazový záznam z kamery 5 se v případě síťové IP kamery 5 přenáší přímo do počítače 7 napojeného na systém 1. V případě analogového signálu z kamery 5 by bylo nutné jej převádět v převodníku 6, který také tvoří součást systému, a z převodníku 6 jej převádět do počítače 7. Počítač 7 je vybaven speciálním softwarem, který vyhodnocuje záznam z kamery 5 ze všech tří akvárií 2 současně a v reálném čase.

Na krunýř každého raka 12 v každém akváriu 2 se ekologickým lepidlem přilepí neinvazivní čidlo 8. Neinvazivní čidlo 8 je opatřeno kabelem 14 pro přenášení elektrického signálu, která se v převodníku 6 převádí na analogově digitální signál a dále se přenáší do počítače 7, kde se softwarem vyhodnocuje současně se záznamem z kamery 5.

Neinvazivní čidlo 8 je tvořeno zdrojem 9 infračerveného záření, který do těla raka 12 vysílá infračervené záření. V tomto příkladu uskutečnění se jako fyziologická funkce raka 12 měří jeho srdeční tep. Infračervené záření se odráží od krve raka 12 shromážděné v srdeční komoře. Odražené světlo přijímá přijímací zařízení 10, které infračervené světlo přijímá a vysílá je v podobě elektrického signálu do převodníku 6. V každém akváriu 2 je jeden úkryt 11 pro raka 12. Každý úkryt 11 se přesně lokalizuje a jeho pozice se zanese do vyhodnocovacího softwaru v počítači 7, takže software obrazový záznam z kamery 5 vyhodnocuje ve vztahu k lokalizovaným úkrytům 11 v každém akváriu 2. Každý úkryt 11 má ve své horní části vytvořené vybrání 13 pro kabel 14 neinvazivního čidla 8, aby rak 12 mohl do úkrytu 11 bez překážky zalézat, aniž by mu kabel 14 neinvazivního čidla 8 bránil v pohybu.

Průmyslová využitelnost

Způsob etologického sledování korýšů a/nebo měkkýšů a etologický systém podle vynálezu lze využít pro laboratorní nebo průmyslové sledování znečištění vody tvořící jejich přirozené prostředí.

PATENTOVÉ NÁROKY

1. Způsob etologického sledování korýšů a/nebo měkkýšů (12), při kterém se snímá alespoň jedna fyziologická funkce korýše a/nebo měkkýše (12) neinvazivním čidlem (8) upevněným ke korýši a/nebo měkkýši (12) v reálném čase a přenáší se do počítače (7) ve formě digitálního signálu, který je zpracován softwarem pro vyhodnocování reakcí korýše a/nebo měkkýše (12) na přirozené a/nebo uměle vyvolané environmentální změny, **vyznačující se tím**, že současně s fyziologickou funkcí se kontinuálně v reálném čase sleduje i přirozené chování korýše a/nebo měkkýše (12) pomocí kamery (5), přičemž se současně sledují alespoň tři korýši a/nebo měkkýši (12), kde každý korýš a/nebo měkkýš (12) je umístěn ve vlastním akváriu a obrazový záznam přirozeného chování pořízený kamerou (5) se ve formě digitálního signálu přenáší do počítače (7), kde se porovnává a vyhodnocuje současně s digitálním signálem z neinvazivního čidla (8) pro sledování fyziologické funkce pomocí společného vyhodnocovacího softwaru.

2. Způsob podle nároku 1, **vyznačující se tím**, že environmentální změny jsou uměle vyvolané, přičemž do všech sledovaných akvárií (2) se současně dávkují stejné toxiny a/nebo nepřirozené látky pro korýše a/nebo měkkýše (12) a vyhodnocuje se fyziologická odpověď korýše a/nebo měkkýše (12) snímaná neinvazivním čidlem (8) a změny v přirozeném chování korýše a/nebo měkkýše (12) snímané kamerou (5) v závislosti na množství, druh a dávkování toxinů a/nebo nepřirozených látek.

3. Způsob podle nároků 1 a 2, **vyznačující se tím**, že předměty v každém akváriu (2) se přesně lokalizují a jejich pozice se zanesou do softwaru, přičemž vyhodnocování obrazových záznamů z kamery (5) se zpracovává ve vztahu k lokalizovaným předmětům.

4. Způsob podle alespoň jednoho z nároků 1 až 3, **vyznačující se tím**, že jako fyziologická funkce se snímá tep srdce korýše a/nebo měkkýše (12).

5. Způsob podle alespoň jednoho z nároků 1 až 4, **vyznačující se tím**, že neinvazivní čidlo (8) je opatřeno zdrojem infračerveného světla (9), které vysílá infračervené světlo do těla korýše a/nebo měkkýše (12), a přijímacím zařízením (10), kterým se přijímá infračervené světlo odražené od srdce korýše a/nebo měkkýše (12).

6. Způsob podle alespoň jednoho z nároků 1 až 5, **vyznačující se tím**, že korýš (12) patří do třídy *Malacostraca*, konkrétně je korýš (12) rak signální a neinvazivní čidlo (8) se přilepí na krunýř raka.

7. Etologický systém (1) pro sledování chování korýšů a/nebo měkkýšů způsobem podle nároku 1, **vyznačující se tím**, že sestává z alespoň tří akvárií (2), kde v každém akváriu (2) je jeden korýš a/nebo měkkýš (12), kde každý korýš a/nebo měkkýš (12) je opatřen neinvazivním čidlem (8) pro snímání tepu oběhové soustavy korýše a/nebo měkkýše (12), kde neinvazivní čidlo (8) je opatřeno zdrojem (9) infračerveného záření pro vysílání infračerveného záření do těla korýše a/nebo měkkýše (12) a přijímacím zařízením (10) pro příjem odraženého infračerveného záření z těla korýše a/nebo měkkýše (12), systém (1) dále sestává z kamery (5) pro současné snímání přirozeného chování každého korýše a/nebo měkkýše (12), z jednoho počítače (7) opatřeného softwarem pro současné vyhodnocení záznamů z kamery (5) a z neinvazivního čidla (8) předávaných do počítače (7) současně, kontinuálně, a v reálném čase, a systém (1) dále obsahuje dávkovací zařízení (12) pro dávkování testovacích látek do akvárií (2), alespoň jednu nádrž (3) pro průtočné napájení akvárií (2) a alespoň jednu retenční nádrž (4) pro sběr odtékající vody z akvárií (2).

8. Etologický systém podle nároku 7, **vyznačující se tím**, že každé neinvazivní čidlo (8) je opatřeno kabelem (14) pro přenášení záznamů do počítače (7), přičemž neinvazivní čidlo (8) je opatřeno přepážkou (15) pro oddělení zdroje (9) infračerveného záření od přijímacího zařízení (10) infračerveného světla.

9. Etologický systém podle nároků 7 a 8, **vyznačující se tím**, že každé akvárium (2) je opatřeno úkrytem (11) pro korýše (12) s polohou zanesenou v softwaru počítače (7) přes analogově–digitální převodník (6), přičemž každý úkryt (11) je opatřen vybráním (13) pro pohyb kabelu (14) čidla (8) upevněného na těle korýše (12).

10. Etologický systém podle alespoň jednoho z nároků 7 až 9, **vyznačující se tím**, že je uspořádán v horizontálních rovinách, kde nádrž (3) pro průtočné napájení akvária (2) je uspořádána v horní rovině, pod ní je uspořádána horizontální řada alespoň tří akvárií (2) a pod řadou akvárií (2) jsou uspořádány dvě retenční nádrže (4), přičemž v prostoru mezi retenčními nádržemi (4) je uložena kamera (5).

11. Etologický systém podle alespoň jednoho z nároků 7 až 10, **vyznačující se tím**, že retenční nádrž (4) má tvar nepravidelného čtyřúhelníku, jehož vertikální stěna (25) sousedící s kamerou (5) svírá s podstavou (26) ostrý úhel (α).

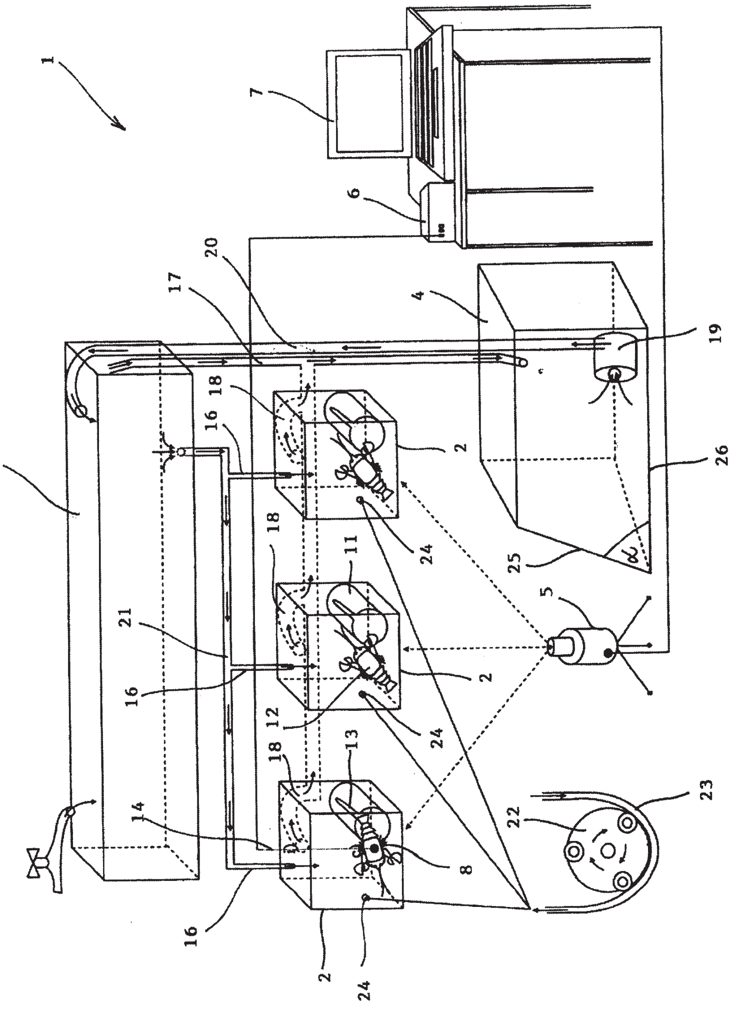
12. Etologický systém podle alespoň jednoho z nároků 7 až 10, **vyznačující se tím**, že korýš (12) patří do třídy *Malacostrata*, konkrétně se jedná o raka signálního.

2 výkresy

Přehled vztahových značek:

1	etologický systém
2	akvárium
3	nádrž pro průtočné napájení akvária
4	retenční nádrž
5	kamera
6	převodník
7	počítač
8	neinvazivní čidlo
9	zdroj infračerveného světla
10	přijímací zařízení infračerveného světla
11	úkryt
12	korýš a/nebo měkkýš
13	vybrání v úkrytu pro pohyb kabelu čidla
14	kabel čidla
15	přepážka

16	přívod vody do akvárií
17	přepad
18	výstup vody z akvárií
19	čerpadlo
20	potrubí
21	vedení vody z nádrže pro průtočné napájení akvárií
22	peristaltická pumpa
23	vedení toxinů a jiných látek ze zásobníků
24	vstup toxinů a jiných látek do akvárií
25	skosená stěna retenční nádrže
26	základna retenční nádrže.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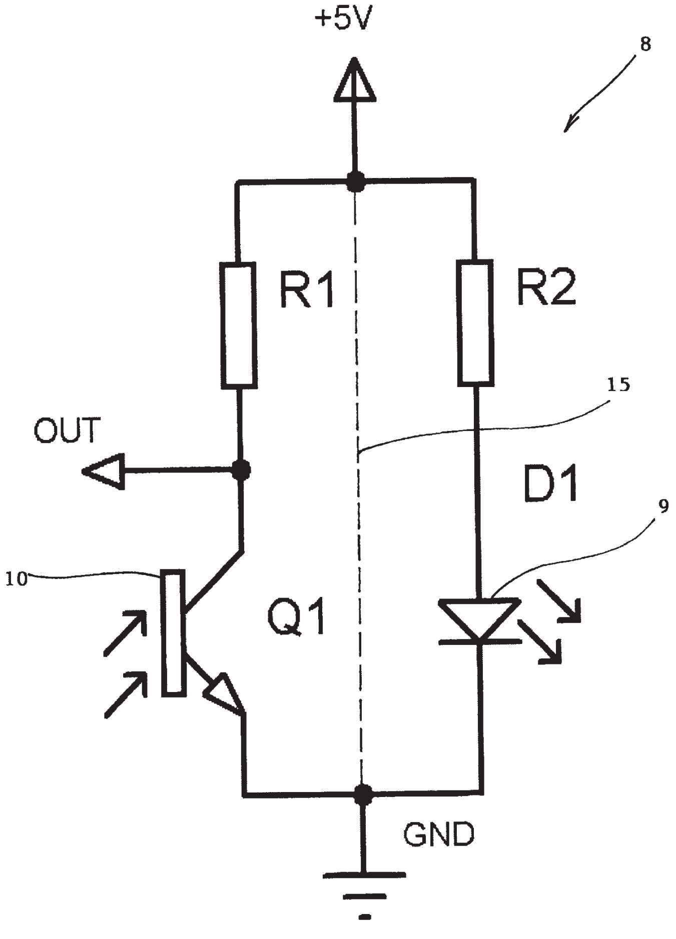

OBR.2

Konec dokumentu

Appendix D: NIR optical sensor - Czech Republic national utility model application

UŽITNÝ VZOR

(11) Číslo dokumentu:

27 114

(13) Druh dokumentu: **U1**

(51) Int. Cl.:

A61B 5/02 (2006.01)

(19)
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VLASTNICTVÍ

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Jihočeská univerzita v Českých Budějovicích,
Fakulta rybářství a ochrany vod, Vodňany, CZ

(72) Původce:
Aliaksandr Pautsina, MSc., České Budějovice, CZ
Ing. Petr Císař, Ph.D., České Velenice, CZ
Iryna Kuklina, MSc., Vodňany, CZ
Ing. Antonín Kouba, Ph.D., České Budějovice, CZ
doc. Ing. Pavel Kozák, Ph.D., Protivín, CZ

(74) Zástupce:
PatentCentrum Sedlák a Partners s.r.o., Husova 5,
370 01 České Budějovice

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(54) Název užitného vzoru:
Neinvazivní čidlo

CZ 27114 U1

Úřad průmyslového vlastnictví v zápisném řízení nezjišťuje, zda předmět užitného vzoru splňuje podmínky způsobilosti k ochraně podle § 1 zák. č. 478/1992 Sb.

Neinvazivní čidlo

Oblast techniky

Technické řešení se týká neinvazivního čidla pro sledování tepu srdce raka a zařízení zahrnující toto čidlo pro sledování tepu srdce raka.

Dosavadní stav techniky

Snímání srdečního tepu u vodních živočichů umožňuje sledovat změny v prostředí, které obývají, především z hlediska znečištění a zvyšování teploty.

Pro neinvazivní sledování srdečního tepu korýšů a měkkýšů se používá systém pro sledování fyziologických funkcí na bázi počítačového zpracování (CAPMON). Průměrná tepová frekvence sledovaného živočicha se automaticky přepočítává pomocí speciálně vytvořeného hardwaru připojeného k počítači.

Zásadní součástí systému CAPMON je infračervené čidlo zahrnující vysílač infračerveného světla a přijímač infračerveného světla (IR). Čidlo se upevní, např. přilepením na krunýř živočicha tak, aby skrz krunýř mohlo procházet IR světlo a mohlo osvětlit srdce a na něj napojené cévy. Změny tvaru a objemu oběhových struktur během srdečních kontrakcí způsobují změny v množství IR světla odraženého z těla živočicha do přijímače IR. Tyto změny v odraženém IR světle jsou převedeny na změny v elektrickém napětí, jsou elektricky zesíleny, filtrovány a dále zpracovány softwarem.

Provedení čidla IR světla je popsáno v dokumentu Burnett N. P. et al: An improved noninvasive method for measuring heartbeat of intertidal animals. Pro výrobu čidla byla použita dvouvrstvá deska plošných spojů. Jako zdroj byla použita baterie o napětí 6 V, napojená na regulátor napětí LM7805, který napájí elektrický obvod napětím 5 V. Pro zlepšení stability napětí byly do obvodu zapojeny dva blokovací kondenzátory (1 μF a 22 μF) a LED dioda pro indikaci napájení obvodu. Čidlo je napojeno na tenký pružný drát pro maximální snížení pohybového a jiného omezení sledovaného živočicha.

Obvod pro úpravu signálu je vytvořen kolem jediného čipu, který obsahuje dva identické zesilovače napájené ze stejného zdroje. Na vstup napětí do čipu je napojen blokovací kondenzátor pro snížení šumu ze zdroje. Obě neměnící zesilovací fáze vydávají maximálně 78 dB a jsou opatřeny filtry pro snížení zesílení nechtěných vysokých frekvencí elektrického šumu z ostatního vybavení a vedení napětí.

Dvě diody sledují mřížkové předpětí na zesilovacích fázích. Třetí dioda umístěná na výstupu z druhého zesilovače bliká při příjmu a zesílení pulzů z obvodu. Zesílený signál je dále vyhodnocován v počítači nebo jiném zařízení.

Nevýhody tohoto provedení čidla pro snímání tepu srdce raka spočívají v jeho složité konstrukci, která způsobuje, že čidlo je poruchové, je třeba ho častěji měnit, čímž se narušuje průběh sledování. Další nevýhodou je úprava signálu získaného z tepu srdce sledovaného živočicha přímo v obvodu čidla, protože v počítači se zobrazí pouze srdeční frekvence v Hz, což umožňuje pouze omezené vyhodnocení srdečního tepu. Z těchto hodnot nelze vyvodit další fyziologické informace ohledně sledovaného živočicha. Další nevýhodou je vzájemná blízkost světelného zdroje a optoelektrického členu, protože dochází k vzájemnému rušení infračerveného světla vyslaného ze světelného zdroje do srdce raka a infračerveného světla odraženého ze srdce raka do optoelektrického členu a v důsledku toho k nepřesnému vyhodnocení získaných údajů vyhodnocovacím softwarem.

Úkolem technického řešení je vytvoření neinvazivního čidla s maximálním zjednodušením a omezením počtu elektrických prvků, které by bylo minimálně poruchové a které by umožňovalo co nejpřesnější sledování tepu račího srdce.

Podstata technického řešení

Tento úkol je vyřešen vytvořením neinvazivního čidla podle tohoto technického řešení, které je upevnitelné na těle raka pro snímání tepu račího srdce a je tvořené obvodem uspořádaným na desce plošných spojů. Obvod zahrnuje alespoň jeden světelný zdroj pro vyzařování infračerveného světla směrem k srdci raka a alespoň jeden optoelektrický člen pro zachycení infračerveného světla odraženého od srdce raka a pro převod reflexních změn odraženého světla na elektrický signál. Podstata technického řešení spočívá v tom, že deska plošných spojů je opatřena přepážkou oddělující světelný zdroj od optoelektrického členu. Nedochází tak k vzájemnému rušení infračerveného světla vyslaného ze světelného zdroje do račího srdce a infračerveného světla odraženého z račího srdce do optoelektrického členu. Údaje o pulzu račího srdce jsou tak maximálně přesná a lze je lépe a s větší přesností interpretovat. Přepážka je ve výhodném provedení vytvořena z antistatického plastu a je upevněna na desce plošných spojů nebo ve voděodolném obalu. Lze ji na desku plošných spojů upevnit pouze vmáčknutím do mezery mezi ostatní elektrické součástky, především optoelektrický člen a světelný zdroj, nebo je možné ji k desce plošných spojů přilepit vteřinovým lepidlem.

Světelný zdroj je LED dioda o výkonu alespoň 15 mW a účinnosti 60 %. Výhoda použití LED diody spočívá v tom, že má zvýšený optický výkon při stejné spotřebě elektrické energie. Zvýšení optického výkonu LED diody vede ke zvýšení optického výkonu, který se odráží do fototranzistoru. Proto není nutný externí zesilovací obvod.

Optoelektrický člen je fototransistor, který citlivě reaguje na infračervené světlo odražené z hemolymfy vyplňující komoru račího srdce.

Aby nedošlo k poškození součástek, je neinvazivní čidlo uloženo ve voděodolném obalu z dvousložkového nízkoviskozního silikonového dielektrickýho gelu pro zalévání elektronických sestav.

Předmětem vynálezu také je zařízení pro sledování tepu srdce raka zahrnující neinvazivní čidlo pro snímání tepu srdce raka, tvořené obvodem uspořádaným na desce plošných spojů a zahrnujícím alespoň jeden světelný zdroj pro vyzařování infračerveného světla směrem k srdci raka a alespoň jeden optoelektrický člen pro zachycení infračerveného světla odraženého od srdce raka a pro převod reflexních změn odraženého světla na elektrický signál. Zařízení také zahrnuje analogově-digitální převodník, do kterého je přiveden signál z neinvazivního čidla a počítač, na jehož vstup je analogově-digitální převodník napojen. Podstata zařízení spočívá v tom, že deska plošných spojů je opatřena přepážkou oddělující světelný zdroj od optoelektrického členu, a že na vstupu do analogově digitálního převodníku je uspořádán kapacitor pro potlačení vysokofrekvenčního šumu výstupního signálu z neinvazivního čidla a počítač je opatřen softwarem pro zpracování nefiltrovaného signálu z neinvazivního čidla v digitalizované formě. Přepážka je ve výhodném provedení vytvořena z antistatického plastu a je upevněna na desce plošných spojů nebo ve voděodolném obalu. Lze ji na desku plošných spojů upevnit pouze vmáčknutím do mezery mezi ostatní elektrické součástky, především optoelektrický člen a světelný zdroj, nebo je možné ji k desce plošných spojů přilepit vteřinovým lepidlem. Světelný zdroj je LED dioda o výkonu alespoň 15 mW a účinnosti 60 % a optoelektrický člen je fototransistor. Neinvazivní čidlo je uloženo ve voděodolném obalu.

Výhody neinvazivního čidla podle technického řešení spočívají v zjednodušení elektrického obvodu, čímž se snížila poruchovost čidla a celého zařízení. Neinvazivní čidlo a zařízení také poskytují přesné a nefiltrované údaje pro vyhodnocení v počítači.

Objasnění výkresů

Technické řešení bude blíže objasněno na přiložených výkresech, kde znázorňují obr. 1 schéma elektrického obvodu, obr. 2 neinvazivní čidlo a obr. 3 zařízení pro sledování tepu srdce raka.

Příklady uskutečnění technického řešení

Rozumí se, že dále popsané a zobrazené konkrétní příklady uskutečnění technického řešení jsou představovány pro ilustraci, nikoli jako omezení příkladů provedení technického řešení na uvedené případy. Odborníci znalí stavu techniky najdou nebo budou schopni zjistit za použití rutinního experimentování větší, či menší počet ekvivalentů ke specifickým uskutečněním technického řešení, která jsou zde speciálně popsána. I tyto ekvivalenty budou zahrnuty v rozsahu následujících nároků na ochranu.

Neinvazivní čidlo 1 pro snímání tepu srdce raka podle technického řešení je uloženo ve voděodolném obalu a lze je upevnit přímo na krunýř raka v jeho dorsální části v místě nad srdcem netoxickým epoxidovým lepidlem. Netoxické lepidlo je silikonový kaučuk pro elektroniku Sylgard 517 Dow Corning, vytvořený jako dvousložkový nízkoviskozní silikonový dielektrický gel pro zalévání elektronických sestav. Vytvrzuje se za normální či zvýšené teploty do formy samosezacelujícího gelu.

Obvod 4 neinvazivního čidla 1 byl uspořádán na speciálně vyvinuté jednostranné desce plošných spojů 8 a vyžaduje napájení 5 V. Obvod 4 neinvazivního čidla 1 je tvořen světelným zdrojem 2, který je v tomto příkladu uskutečnění tvořen jednou LED diodou typu KP-3216F3C. LED dioda je axiálně spojena s optoelektrickým členem 3, který je v tomto příkladu uskutečnění tvořen fototranzistorem typu ELPT15-21C. Pro zapojení LED diody byl do obvodu 4 umístěn rezistor R2 o hodnotě odporu 200 Ω. Pro zapojení fototransistoru byl do obvodu 4 umístěn rezistor R1 o hodnotě odporu 220 Ω.

Neinvazivní čidlo 1 funguje na principu pletysmografie a je vytvořeno jako reflektivní optočlen. Elektrický signál na výstupu fototranzistoru závisí na hladině okolního osvětlení a na množství světla vydávaného LED diodou a následně na světle odraženém z předmětů v dosahu příjmu fototransistoru. Když se čidlo 1 umístí na krunýř raka, je výstupní signál čidla 1 modulován množstvím hemolymfy, která vyplňuje srdce a rozptyluje dopadající světlo z LED diody. Tento přístup umožňuje využít neinvazivním způsobem srdeční činnost raka jako funkci závislou na velikosti a tvaru račího srdce.

Zvýšená účinnost LED umožňuje získat výrazně víc optického výkonu z jediného světelného zdroje 2 při stejné spotřebě elektrické energie. V současné době má běžně dostupná infračervená LED dioda optický výkon přibližně ~15 mW při proudu 20 mA a účinnosti 60 %. Zvýšení optického výkonu LED diody vede ke zvýšení optického výkonu, který se odráží do fototransistoru, čímž zvyšuje svůj elektrický výstupní signál. Díky tomu není nutný externí zesilovací obvod. Instalace více malých povrchových LED diod v jednom neinvazivním čidle 1 může ještě zesílit elektrický výstupní signál, aniž by se výrazně zvýšila celková velikost čidla 1.

Pouze malá část celkového nezacíleného optického záření vydaného LED diodou zasáhne srdce raka, přičemž živočichovi neškodí, ani neovlivňuje jeho chování. Většina světla se vyzáří jinam kvůli velkému širokému úhlu 120°LED rozptýleného na okrajích mezi několika opticky odlišnými vrstvami, např. voděodolným obalem čidla 1, tenkou vrstvou vody mezi rakem a vodou a krunýřem raka.

Aby nedocházelo k vzájemnému rušení vyzařovaného infračerveného světla ze světelného zdroje 2 odraženého infračerveného světla ze srdce raka, které zachytává optoelektrický člen 3, je na desce plošných spojů 8 upevněna přepážka 5. Přepážka 5 je vytvořena z původního krytu 3,5 palcové diskety, jehož plast je antistatický. Přepážka 5 je v čidle 1 upevněna vtlačením do mezery vytvořené mezi LED diodou a optoelektrickým členem 3, popř. je vklíněna ve voděodolném obalu 9. Lze ji také k desce plošných spojů 8 přilepit.

Předmětem technického řešení také je zařízení pro sledování tepu srdce raka, v tomto příkladu uskutečnění raka signálního (*Pacifastacus leniusculus*), které zahrnuje neinvazivní čidlo 1, analogově digitální převodník 6 a počítač 7. Nefiltrovaný signál z optoelektrického členu 3 čidla 1 je veden tenkými pružnými kabely 10 dlouhými 2 m, aby neomezovaly pohyb raka, do analogově-digitálního převodníku 6. Kromě kapacitoru na vstupu, který potlačuje vysokofrekvenční šum na vstupu do analogově-digitálního převodníku 6, nepotřebuje výstupní signál z neinvazivního čidla 1 žádné specifické zesílení a/nebo filtrační obvody, jako popisuje systém CAPMON.

Běžné amplitudy tlukotu račího srdce a šumu, které lze získat díky hardwaru čidla 1 jsou 50 mV a 2 mV. Hladinu šumu lze dodatečně v softwaru počítače 7 snížit. Fluktuace signálu způsobené změnami okolního osvětlení spadají do rozsahu od 2 do 3 V.

Výhody, které vyplývají z vynechání externího zesilovacího hardwaru a filtrovacích obvodů jsou: zachování původního tvaru signálu pro další zpracování pomocí softwaru, minimalizace zkreslení signálu nesprávným nastavením hardwaru, odstranění problémů s upevněním, levnější a jednodušší hardware.

Průmyslová využitelnost

Neinvazivní čidlo a zařízení pro sledování tepu srdce raka podle tohoto technického řešení lze využít pro laboratorní a průmyslové sledování znečištění vod.

NÁROKY NA OCHRANU

1. Neinvazivní čidlo (1) upevnitelné na těle raka pro snímání tepu račího srdce, tvořené obvodem (4) uspořádaným na desce plošných spojů (8) a zahrnujícím alespoň jeden světelný zdroj (2) pro vyzařování infračerveného světla směrem k srdci raka a alespoň jeden optoelektrický člen (3) pro zachycení infračerveného světla odraženého od srdce raka a pro převod reflexních změn odraženého světla na elektrický signál, **vyznačující se tím**, že deska plošných spojů (8) je opatřena přepážkou (5) oddělující světelný zdroj (2) od optoelektrického členu (3).

2. Neinvazivní čidlo podle nároku 1, **vyznačující se tím**, že přepážka (5) je vytvořena z antistatického plastu.

3. Neinvazivní čidlo podle nároku 1 nebo 2, **vyznačující se tím**, že světelný zdroj (2) je LED dioda o výkonu alespoň 15 mW a účinnosti alespoň 60 %.

4. Neinvazivní čidlo podle nároku 1 nebo 2, **vyznačující se tím**, že optoelektrický člen (3) je fototransistor.

5. Neinvazivní čidlo podle alespoň jednoho z nároků 1 až 4, **vyznačující se tím**, že je uloženo ve voděodolném obalu z dvousložkového nízkoviskozního silikonového dielektrického gelu pro zalévání elektronických sestav.

6. Zařízení pro sledování tepu srdce raka zahrnující neinvazivní čidlo (1) pro snímání tepu srdce raka, tvořené obvodem (4) uspořádaným na desce plošných spojů (8) a zahrnujícím alespoň jeden světelný zdroj (2) pro vyzařování infračerveného světla směrem k srdci raka a alespoň jeden optoelektrický člen (3) pro zachycení infračerveného světla odraženého od srdce raka a pro převod reflexních změn odraženého světla na elektrický signál, analogově-digitální převodník (6), do kterého je přiveden signál z neinvazivního čidla (1) a počítač (7), na jehož vstup je analogově-digitální převodník (6) napojen, **vyznačující se tím**, že deska plošných spojů (8) je opatřena přepážkou (5) oddělující světelný zdroj (2) od optoelektrického členu (3), a že na vstupu do analogově digitálního převodníku (6) je uspořádán kapacitor pro potlačení vysokofrekvenčního šumu výstupního signálu z neinvazivního čidla (1) a počítač (7) je opatřen softwarem pro zpracování nefiltrovaného signálu z neinvazivního čidla (1) v digitalizované formě.

7. Zařízení podle nároku 6, **vyznačující se tím**, že přepážka (5) je vytvořena z antistatického plastu.

8. Zařízení podle nároku 6 nebo 7, **vyznačující se tím**, že světelný zdroj (2) je LED dioda o výkonu alespoň 15 mW a účinnosti alespoň 60 %.

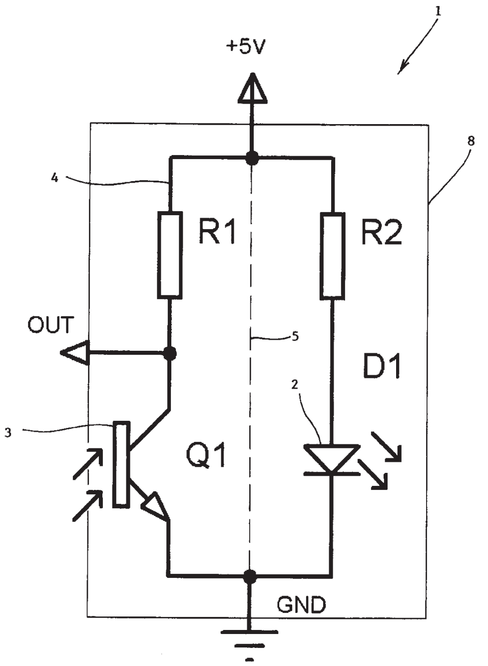
9. Zařízení podle nároků 6 až 8, **vyznačující se tím**, že optoelektrický člen (3) je fototransistor.

10. Zařízení podle alespoň jednoho z nároků 6 až 9, **vyznačující se tím**, že je uloženo ve voděodolném obalu.

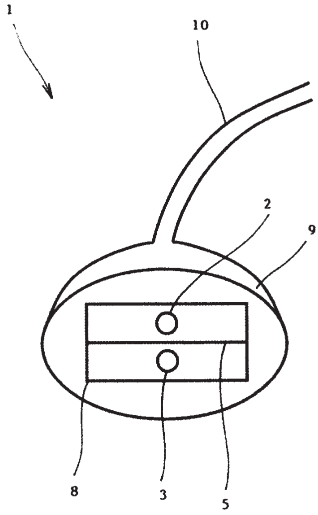
3 výkresy

Přehled vztahových značek:

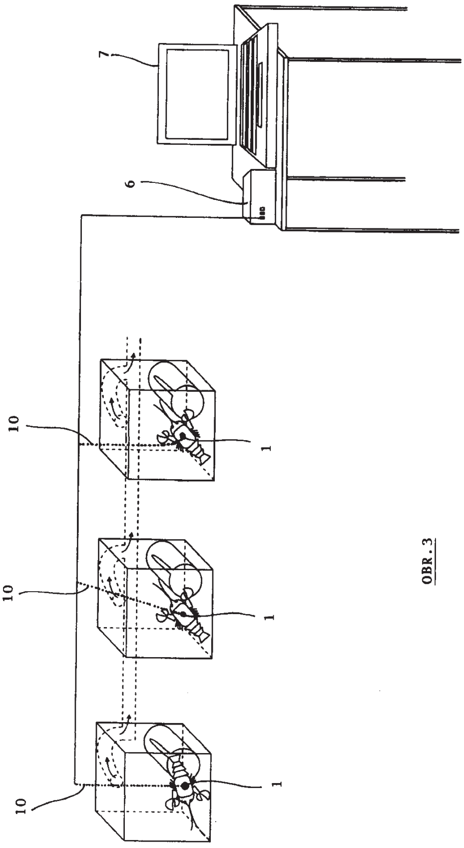
1	neinvazivní čidlo
2	světelný zdroj
3	optoelektrický člen
4	obvod neinvazivního čidla
5	přepážka
6	analogově digitální převodník
7	počítač
8	deska plošných spojů
9	voděodolný obal
10	kabel.



OBR.1



OBR.2



OBR. 3

Konec dokumentu

pautsina@frov.jcu.cz

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University of South Bohemia in České Budějovice
Faculty of Science
Branišovská 1760
CZ-37005 České Budějovice, Czech Republic

Phone: +420 387 776 201
www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.cz