University of South Bohemia in České Budějovice

Faculty of Fisheries and Protection of Waters

Institute of Aquaculture and Protection of Waters

DIPLOMA THESIS

Effect of hygienically treated water on crayfish heart rate and their subsequent mortality

Author: Mgr. Bc. Viktoriia Shchennikova

Supervisor: prof. Ing. Pavel Kozák, Ph.D.

Consultant: Ing. Filip Ložek

Study program: Master study program

Field of study: Agricultural specialization/Fisheries and Protection of Waters

Form of study: Full-time

Year of study: 2016-2018

České Budějovice, May 2018

Declaration

I declare that I have written my Diploma thesis on my own and that I have used only the sources listed in the bibliography. I declare that in compliance with Section 47b of the Act No. 111/1998 Coll., as amended, I agree with publishing of my Diploma thesis in its unabbreviated version or in an adapted version based on leaving out marked parts archived by the FFPW USB. The publishing is made through an electronic way in the part of the STAG database, which is accessible to public and managed by the University of South Bohemia in České Budějovice on its internet pages. When doing so, my copyright to the submitted text contained in this final thesis will be maintained. I also agree that supervisor's and reviewers' opinions as well as the record of the course and results of the final thesis defence are published through the same electronic way in compliance with the above- mentioned provisions of Act No. 111/1998 Coll.. I also agree that the text of my final thesis will be compared with the Theses.cz database which is administered by the National Registry of Theses and the system for plagiarism search.

_____ _____

Date Student's signature

Acknowledgement

I would first like to express my sincere appreciation and gratitude to my supervisor, Prof. Ing. Pavel Kozák, Ph.D., for the patient guidance, encouragement, remarks and advice he has provided through the learning process of this master thesis.

Furthermore, I would like to thank my consultant, Ing. Filip Ložek, for his useful, valuable comments and for paying great attention to my thesis. In addition, I would like to thank Ing. Petr Císař, Ph.D. for consulting, help and maintenance with technical aspects of the monitoring system. Also, I appreciate MSc. Iryna Kuklina, Ph.D. for her help and recommendations.

I am grateful to all members of my family for their love and constant support in everything. Most importantly, I wish to thank my loving husband, Oleksandr, who provides infinite inspiration and motivation for me at every important stage of my life.

JIHOČESKÁ UNIVERZITA V ČESKÝCH BUDĚJOVICÍCH
Fakulta rybářství a ochrany vod
Akademický rok: 2017/2018

ZADÁNÍ DIPLOMOVÉ PRÁCE

(PROJEKTU, UMĚLECKÉHO DÍLA, UMĚLECKÉHO VÝKONU)

Jméno a příjmení:	**Mgr. Bc. Viktoriia SHCHENNIKOVA**
Osobní číslo:	**V16N019P**
Studijní program:	**N4106 Zemědělská specializace**
Studijní obor:	**Rybářství a ochrana vod**
Název tématu:	**Effect of hygienically treated water on crayfish heart rate and their subsequent mortality**
Zadávající katedra:	**Výzkumný ústav rybářský a hydrobiologický**

Zásady pro vypracování:

The aim of this study will be to examine the effect of disinfectants used in real water treatment conditions on crayfish cardiac physiology. A patented system of monitoring crayfish heartbeat will be used. Crayfish will be continually exposed in water flow tanks directly after water disinfection, when their cardiac activity will be recorded. As practical part, student will evaluate recorded data and correlate them with measured concentration of disinfectants. As theoretical part, student will elaborate literary introduction focusing on the use of crayfish as bioindicators, their ethological and physiological response to environmental changes and the monitoring methods.

This work will be funded by projects GAČR (16-06498S) and CENAKVA II CZ.1.05/2.1.00/01.0024.

Rozsah grafických prací:	**dle potřeby (do 20 stran)**
Rozsah pracovní zprávy:	**50-70 stran**
Forma zpracování diplomové práce:	**tištěná**
Seznam odborné literatury:	**viz příloha**

Vedoucí diplomové práce:	**prof. Ing. Pavel Kozák, Ph.D.** Výzkumný ústav rybářský a hydrobiologický
Konzultant diplomové práce:	**Ing. Filip Ložek** Výzkumný ústav rybářský a hydrobiologický
Ostatní konzultanti:	**Ing. Petr Císař, Ph.D.** Ústav komplexních systémů

Datum zadání diplomové práce:	**10. ledna 2018**
Termín odevzdání diplomové práce:	**4. května 2018**

prof. Ing. Pavel Kozák, Ph.D.
děkan

L.S.

doc. Ing. Tomáš Randák, Ph.D.
ředitel

V Českých Budějovicích dne 19. února 2018

Příloha zadání diplomové práce

Seznam odborné literatury:

Holdich, D. M., Lowery, R. S., 1988. Freshwater Crayfish Biology, Management and Exploatation. Chapman & Hall, London & Sydney, Timber Press, Portland, Oregon, 498 s.

Holdich, D. M., 2002. Biology of Freshwater Crayfish. Blackwell Science Ltd., Oxford, London, 702 s.

Kozák, P., Ďuriš, Z., Petrusek, A., Buřič, M., Horká, I., Kouba, A., Kozubíková, E., Policar, T., 2013. Biologie a chov raků. Jihočeská univerzita v Českých Budějovicích, Fakulta rybářství a ochrany vod, Vodňany, 1. vydání, 418 s.

Kozák, P., Buřič, M., Policar, T., 2007. Metodika lovu raků. Edice Metodik (technologická řada), VÚRH JU Vodňany, č. 81, 24 s.

Kozák, P., Kuklina, I., 2015. Crayfish as Tools of Quality Monitoring. In: Kawai, T., Faulkes, Z., Scholtz, G. (Eds), Freshwater Crayfish: A Global Overview. CRC Press, Taylor & Francis Group, Boca Raton, USA, pp. 275-297.

Kozák, P., Císař, P., Kouba, A., Kuklina, I., Pautsina, A., 2015. Způsob etologického sledování korýšů a/nebo měkkýšů a etologický systém pro sledování chování korýšů a/nebo měkkýšů. Úřad průmyslového vlastnicí ČR, patent č. 305212.

Kuklina, I., Kouba, A., Buřič, A., Horká, I., Ďuriš, Z., Kozák, P., 2014. Accumulation of Heavy Metals in Crayfish and Fish from Selected Czech Reservoirs. BioMed Research International: 306103

Kuklina, I., Sladkova, S., Kouba, A., Kholodkevich, S., Kozák, P., 2014. Investigation of chloramine-T impact on crayfish *Astacus leptodactylus* (Esch., 1823) cardiac activity. Environmental Science and Pollution Research 21 (17): 10262-10269.

Kuklina, I., Kouba, A., Kozák, P., 2013. Real-time monitoring of water quality using fish and crayfish as bio-indicators: a review. Environmental Monitoring and Assessment 185 (6): 5043-5053.

Souty-Groset, C., Holdich, D.M., Noel, P.Y., Reynolds, J.D., Haffner, P., (EDS), 2006. Atlas of Crayfish in Europe. Museum national d'Histoire naturelle, Paris.

Contents

1 Introduction

This study focuses on the effect of hygienically treated water by chlorine dioxide on crayfish cardiac physiology. Crayfish are known for their high sensitivity to chemical agents in aquatic ecosystems. The reaction on contamination expressed through the changes in behavior, cardiac and locomotion activity, is used for indication of changes in quality of water. The data, obtained from monitoring of crayfish responses to different stimuli, help to understand the effect of pollution environment on living organisms.

There are a lot of studies about the influence of various substances on functional state of crayfish. However, only a few of them are considering the effect of disinfectants on animal's physiology. Signal crayfish, one of the most commonly used crayfish species for toxicological experiments, was chosen for this study because of its suitability as an experimental model.

The aim of this study is the evaluation of crayfish physiological reaction on water disinfected with ClO_2 during long-term exposure under the conditions of the brewery, which has water purification system. Also, an essential part of the present study is a literature overview, featuring crayfish etiological and physiological reaction on exposure of different stimuli and methods for the monitoring of cardiac activity and behavior.

2 Literature overview

2.1. Chemical contamination in aquatic ecosystems

Large numbers of industrial chemicals and heavy metals are entering the natural environment (e. g. Nriagu and Pacyna, 1988; Faria et al., 2010; Strickman and Mitchell, 2017). Increasing agricultural, industrial, and urban use of water bodies lead to elevated contamination of the aquatic environment (Coimbra et al., 1996; Faria et al., 2010). Degradation of aquatic ecosystem health, caused by anthropogenic activities and naturally occurring events, made water quality necessary to monitor (Loeb, 1994).

Pharmaceuticals, veterinary and illicit drugs and personal care products (food supplements, the ingredients in cosmetics) pass through sewage treatment plants into the aquatic environment because of their partial elimination during the cleaning process. These substances are more regularly infused into water bodies than industrial chemicals (Daughton and Ternes, 1999).

Increasing production of different anthropogenic chemicals has a negative effect on water conditions and organisms (Connon et al., 2012). Ecosystems are mainly polluted by atrazine, simazine, persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE) and other pesticides (Comber, 1999; Fernandes et al., 2002; Holmqvist et al., 2007).

Metal contaminants, such as copper, zink, cadmium, lead, chromium, mercury, are widespread pollutants in the surface water (Taylor et al., 1995; Soedarini et al., 2012;). Because of industrial human activity, concentrations of heavy metals in the aquatic ecosystems are elevated (Graedel et al., 2004). Also, biodiesel and crude oil in the environment of streams, rivers, oceans negatively affect water organisms (Bucas and Saliot, 2002; Khan et al., 2007).

For sensitive species, which are already damaged by sublethal effects, different additional stressors (supplemental pollution, increasing temperature) can lead to mortality (Triebskorn et al., 2002). Generally, the effects of the usual environmentally toxicant are depending on concentration levels, time exposure and species responses to contaminants: in one case affected animal has physiological destruction (Bollinger et al., 1997;

Nathaniel et al., 2012) and in another situation, pollute substances impact causes even mortality (Naqvi and Howell, 1993; Barbee et al., 2010).

Therefore, quality of water is deteriorating and has an influence on aquatic inhabitants (Bowen and Depledge, 2006). Directly or through the food chain these toxic substances accumulate in tissues of living aquatic organisms (McIntyre and Beauchamp, 2007). Pollutants influence on their ability of reproduction and grow and affect stress response (Waye and Trudeau, 2011). Many industrial and agricultural contaminants exert the impact on the immune system of aquatic organisms and elevate sensitivity to different diseases (Luebke et al., 1997). Chemical contaminants presented in an aquatic environment potentially could negatively affect neuroendocrine control mechanisms of hydrobionts (Gore, 2010). In addition, pollutants are dangerous because of their continuous entry into water bodies, which may cause chronic effects on living organisms (Daughton and Ternes, 1999).

2.2. Aquatic organisms as indicators of environmental changes

Due to permanent environmental pollution many methods for detection of water quality were developed (Hirsch et al., 1991). Using toxicity tests and chemical-specific sensors could not provide sufficient information about the toxicity in aquatic habitat (Van der Schalie et al., 2001). Living organisms may display the contamination history of a specific place, when they have been exposed to chemicals during a long period of their life (Kraak et al., 1991).

The most common, accurate and important approach to study environmental pollutants is the use of bioindicators (e. g. Füreder and Reynolds, 2003; Hodkinson and Jackson, 2005). Aquatic organisms at different trophic levels could indicate changes in the ecosystem (e. g. Kholodkevich et al, 2008; Rahmanpour et al., 2016). Often these changes affect population numbers and ecological distribution that allow them to be used as bioindicators (Luebke et al., 1997). Hydrobionts, that bioaccumulate contaminants from water bodies can transfer them to other (mainly higher) trophic levels of the food chain (Maeda et al., 1990).

Fish are often used as biological indicators of environment changes in the aquatic ecosystems (e. g. Fernandes et al., 2002; Birungi et al., 2007). They can demonstrate different levels of pollutant accumulation depended on various contamination degrees.

Analyzing fish tissues allow determining contaminant substances in investigated individuals (Aguilar-Betancourt et al., 2016). The most common species used in pollution research are: *Perca flavescens*, *Cyprinus carpio*, *Oreochromis niloticus* and other fish species (Parks et al., 1991; Rice et al., 1996; Fernandes et al., 2002; Berglund, 2003; Birungi et al., 2007).

Invertebrates, which represent 95v% of all animal abundance in the world (Depledge and Fossi, 1994), are performing as common indicators of organic and inorganic pollution through their reactions at different levels of organization. Demographic features, such as wide spreading in the environment, availability and abundance, make them suitable candidates for the role of an indicator (Schilderman et al., 1999; Hodkinson and Jackson, 2005).

Decapods, such as crayfish, are known to be sensitive to contamination in freshwater. Also, they demonstrate a high response to changes in aquatic ecosystems because of their sensitivity to water quality. Crayfish can reflect on water pollution during a long-term period. As polytrophic organisms, they could hold a predator position and at the same time be a prey for different levels of the freshwater food chain (Schilderman et al., 1999; Soedarini et al., 2012; Kuklina et al., 2014). They are characterized by high abilities to accumulate various pollutants in their tissues and display disorders in the body functions as reaction on environmental contaminants (Reddy et al., 1997; Pennuto et al., 2005; Alcorlo et al., 2006; Holmqvist et al., 2007).

In comparison with fish, crayfish have a relatively primitive circulatory and vascular system (Randall, 1970; Vogt, 2002). Such a simple organization makes the cardiac activity monitoring process clearer and easier (Bojsen et al., 1998; Pautsina et al., 2014). Crayfish heart rate and locomotor activity under natural living conditions are increasing during night time because of their nocturnal activity and this physiological feature was observed under the influence of various factors (Styrishave et al., 1995; Bojsen et al., 1998; Udalova et al., 2009). Also, several studies have described the response of the cardiac system on different chemical stimuli as ammonia, chlorine organic compound (e. g. Bloxham et al., 1999; Kholodkevich et al., 2008).

Different crayfish species are used with the aim of scientific research: *Astacus astacus*, *Pacifastacus leniusculus*, *Procambarus clarkii* and many other species of diverse genuses: *Austropotamobius*, *Cherax*, *Euastacus*, *Orconectes*, *Astacopsis* (Styrishave et

al., 1995; Bloxham et al., 1999; Listerman et al., 2000; Bergman and Moore, 2003; Goudkamp et al., 2004; Kozák and Kuklina, 2016).

2.3. The effects of pollutants on the crayfish organism

2.3.1. Heavy metals

The result of metal contaminant water exposure to crayfish is a significant bioaccumulation of these substances in animal tissues. The highest concentration of metals was foundeded in the crayfish hepatopancreas, then in exoskeleton and muscles (Alcorlo et al., 2006).

Copper is an essential element for functioning of all living organisms (Linder, 1991), but when its level exceeds the required norm of the physiological requirements, this metal becomes toxic (Taylor et al., 1995; Bini and Chelazzi, 2006; Lahman et al., 2015). Copper gets to crayfish haemocyanin through respiration and circulates with haemolymph to all organs (Soedarini et al., 2012). Its higher concentration decreases the cardiac and ventilatory rates (Bini and Chelazzi, 2006). Also, this metal reduces an animal's ability to detect a food odor source, restraining walking speeds toward it and impairing orientation pathways (Sherba et al., 2000; Lahman et al., 2015). Styrishave et al., 1995 found that because of the impact of copper the crayfish heart rate increased in day period and decreased at night. Taylor et al. (1995) observed the reaction of crayfish from both metal-contaminated and uncontaminated nature habitat on different copper levels. The group of animals, which already were exposed earlier to copper, did not exhibit any disturbances in the behavior during a short time period (24 hours) of copper contact, unlike the second group. But after long term exposure, the behavior of the first crayfish group changed and they could not withstand further impact of this metal. It may be caused by the breakdown of copper regulatory mechanisms. However, it was proved, that crayfish can survive when copper concentrations in their environments significantly exceed their physiological needs.

Mercury is also present in water ecosystems and the major step of its cycling is methylation (Gilmour and Henry, 1991). Methylmercury, formed both in biotic and abiotic processes, is accumulated and transported in food chains effectively (Simon and Boudou, 2001). It represents 88% of total mercury, accumulated in crayfish body (Pennuto et al., 2005). Mercury and methylmercury enters the crayfish from food or water

pathways (Simon and Boudou, 2001). The amount of mercury in the organism can be due to various factors such as habitat type or animal size. For example, crayfish from rivers or streams have more mercury then crayfish from natural ponds and the smaller individuals have less mercury then the largest individuals (Pennuto et al., 2005). Styrishave et al. (1995) described the effect of mercury on the crayfish heart rates, which increased in day time to levels characteristic of night time. It led to loss of circadian rhythmicity and the subsequent death.

Cadmium is a non-essential element, which may negatively influence on aquatic living organisms (Reddy et al., 1997). Because of high concentration exposure of this metal protein level and caloric content in crayfish gill tissue decreased. Also, it induces to decline of lipid and protein concentration in hepatopancreas and glucose levels and caloric content both in hepatopancreas and in muscle (Torreblanca et al., 1991). Cadmium chloride affects the level of glucose in the blood of crayfish, thus causing hyperglycemia (Reddy et al., 1994). Naqvi and Howell (1993) founded, that cadmium affect crayfish egg laying and hatching. While the control crayfish laid 203 eggs, treated animals laid only 48 eggs. Moreover, in the control group 95v% of the eggs hatched in opposite to exposed group, where it was only 17v% of the eggs.

Both cadmium and mercury restrain crayfish ovarian maturation. Particularly, these metals inhibit the stimulatory action of neurotransmitter 5-Hydroxytrypta-mine (5-HT), which induces ovarian maturation *in vivo*. In addition, in this case mercury had the highest toxic effect, then cadmium. Similar events in nature may cause significant negative effect on the crayfish reproduction (Reddy et al., 1997).

Uranium is a non-essential inorganic element, which as other trace metals has accumulative and toxic effects (WHO, 2001). The concentration of uranium in the crayfish gills is higher than in the hepatopancreas (Kaddissi et al., 2012). As several studies have shown, the impact of uranium does not induce crayfish mortality (Kaddissi et al., 2012; Simon et al., 2013). But after long period of influence this metal lead to oxidative stress and to decrease in antioxidant response in animal organism (Kaddissi et al., 2012).

Chromium compounds are entering to water bodies from metal and leather industries (Armienta et al., 1996). Chromium is accumulating in crayfish gills and haemolymph, but not in muscle and digestive glands. It does not cause animal mortality, but it impairs haemolymph cells of the crayfish (De la Sienra et al., 2003). This metal destructs the

tubules of the hepatopancreas and disrupts the epithelial cells of gills (Bollinger et al., 1997).

Investigation, carried out with lead, demonstrated decrease of heart rate of crayfish after 24 hours of exposure, but then, after 21 days of contact with lead, it was recovered (Ahern and Morris, 1999). Lead accumulated in all the crayfish tissues after 14 days of exposure. The order of tissues, where lead was presented, was the following: gill > exoskeleton > mid-gut gland > muscle > haemolymph (Morris et al., 2005). But this order can vary depending on the dose and time metal bioaccumulation (Anderson et al., 1997). And Naqvi and Howell (1993) researched lead toxicity to juvenile crayfish and found, that with lead addition in the water mortality elevated respectively and after 96 hours with peak of metal concentration mortality was 92 %.

Zink, as well as cadmium and copper, is an essential element necessary for biological activity, but it could affect negatively on water organisms in higher concentration (Brinkman and Johnston, 2012). Mirenda (1986) found, that this metal is accumulate in crayfish tissues in the next order: gill > hepatopancreas > abdominal muscle > carapace > intestine. Zink significantly influence crayfish oocytes development. The gonadosomatic index (GSI) decreased with elevated metal concentration (Martin-Diaz et al., 2005).

2.3.2. Pesticides and fuel

Herbicides, one of the groups of pesticides, are widely used in agricultural activities and they are entering aquatic organisms through inhalation, consumption or through epithelium (Helfrich et al., 1996).

Browne and Moore (2014) investigated the effect of 2,4-Dichlorophenoxyacetic Acid Herbicide (2,4-D) on crayfish behavior. It was observed, that treated animals need more time to find a food and their walking speed was increased. Also, they did not stay long in the labyrinth part with food, spending most of the time in the neutral part.

Atrazine is a synthetic herbicide, which commonly used by humans (Comber, 1999). Under atrazine exposure the crayfish lost an ability to locate the odor source, but simultaneously it does not affect walked velocity (Belanger et al., 2015).

Velisek et al. (2013) studied the effect of six triazine pesticides (atrazine, hexazinone, metribuzine, prometryne, simazine, terbutryne) on juvenile crayfish. Higher concentration of these substances induces difficulties in animal walking. They

demonstrated a rocking motion. Some crayfish tried to climb the walls, further individuals were in the middle of the chamber. Backward movement increased simultaneously with loss of walking legs and claws. Eventually, crayfish lost balance, overturned on the back and died.

Another herbicide, metolachlor, in higher concentration decrease animal aggression and aspiration to dominate (Cook and Moore, 2008). Also, crayfish showed a weakened reaction to the food odor. It was suggested that behavior changes could be due to odor masking by the chemical substance, physiological deterioration, which can affect the motor abilities of the animal or disturbance of external chemosensory receptors (Wolf and Moore, 2002).

Crayfish are the most sensitive freshwater species to insecticides, another group of pesticides (Paul and Simonin, 2006). However, its continuous influence does not lead to significant animal mortality (Barbee et al., 2010).

The presence of biodiesel and crude oil in water ecosystems also impairs physiological processes of crayfish. Both fuels negatively affect the chemosensory behavior. Exposed animals choose the right side of maze with food less successfully then control individuals (Jurcak et al., 2015).

2.3.3. Drugs

Different types of drugs are known to contribute to unconditional behavioral reactions in living organisms (Panksepp and Huber, 2004; Imeh-Nathaniel et al., 2014).

Cocaine has a significant influence on each locomotion sub-component. Crayfish exposed to this substance had increase in velocity, traveled distance, mobility and immobility (Nathaniel et al., 2012).

Panksepp and Huber (2004) described different behavior changes as responses to cocaine and amphetamine exposures. Intramuscular injections of cocaine induced abdomen flexion, quick backwards walking and claw waving. Similar injections of amphetamine led to crayfish positioning themselves in the corner of the aquarium and to subsequent exploration of walls by their antennae.

Morphine injections into the brain intensified a strong excitation followed by elevated locomotion and surround investigation. Then, when morphine exposures were terminated, exploratory a behavior was elevated (Imeh-Nathaniel et al., 2014).

2.3.4. Disinfectants

Kouba et al. (2012) investigated the effect of peracetic acid on crayfish. High tolerance was observed to this product in adult animals, exposed 96 hours, and total mortality in 9 months old juveniles exposure to peracetic acid concentrations from 250 to 350 $mg \cdot l^{-1}$. Also, mortality occurred after use of concentrations higher than 20 $mg \cdot l^{-1}$ for 10 days old juveniles.

The crayfish gills are the main organ affected by sub-acute peracetic acid toxicity. Crayfish, exposure to peracetic acid for 7 days, have disorganization of epithelial cell in gills, infiltration of hemocytes in antennal gland tissue (Chupani et al., 2015).

2.4. Physiology of crayfish heart

Decapoda which includes crayfish have open circulatory system (McMahon and Burnett, 1990; McMahon, 2012), containing the heart, arteries and sinuses (Vogt, 2002). The circulation fluid of the open system, haemolymph, consists of blood and lymph, which are often contiguous, while in closed system blood is always separated from lymph. In opposite to a closed system, where fluid returns via finite tubes connected to heart, tubular connection in an open system of the arthropods is absent and haemolymph goes directly through arteries to the open body cavity. Then blood circulates back through sinuses to the pericardium, where the heart is placed, and penetrates the heart via the three paired ostia (Fig. 1), which are located dorsally, laterally and ventrally (Vogt, 2002; McMahon, 2012). The haemolymph is transported by the heart to seven main arteries and then, through smaller vessels, it gets to all organs. The oxygen, nutrients and hormones are spread throughout the body via the circulatory system (Vogt, 2002).

The crayfish have a neurogenic heart (Maynard, 1960), which is situated on the dorsal side under the carapace in the pericardial cavity (Vogt, 2002). It has three levels of cardiac regulation, which is common for many crustaceans: cardioacceleratory and cardioinhibitory nerves, neurohormones from the pericardial organs and the cardiac ganglion (Maynard, 1960; Cooke, 2002). The cardiac ganglion is situated along a median line of the inner dorsal wall and constitutes of eight large cells of the neurons, which drive contractions of crustacean heart muscle (Kuramoto and Yamagishi, 1990).

Also, crayfish have a second organ, which serves as a pump, an auxiliary heart or, another designation, the cor frontale (Vogt, 2002). The heart and auxiliary heart are

connected by median artery. Three blood vessels are extending from auxiliary heart: one branch is directed to the brain and two branches go to the eyes (Ache and Sandeman, 1980; Sandeman and Sandeman, 1998).

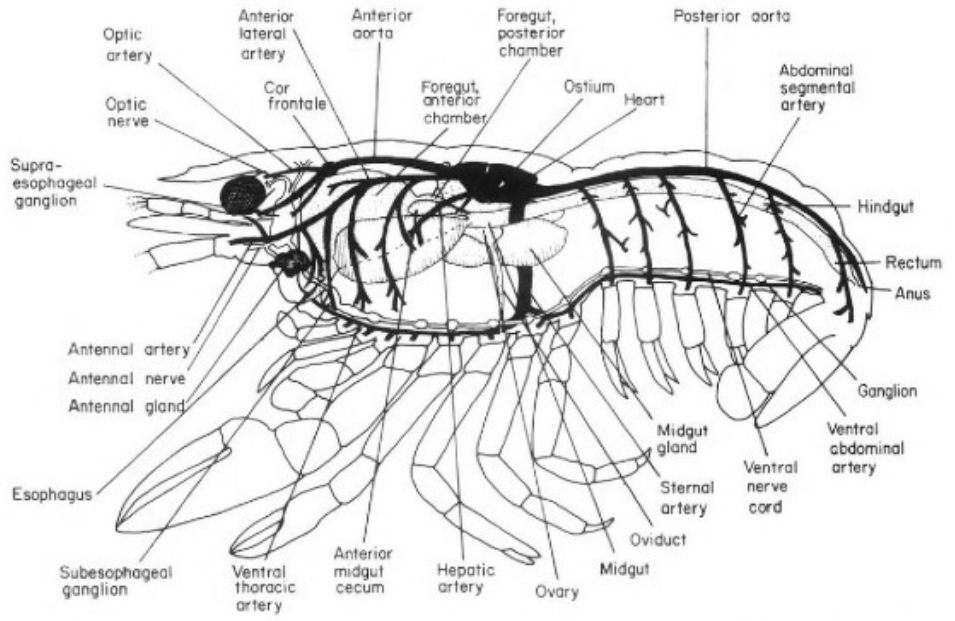


Figure 1. Diagrammatic astacidean crayfish with gills and musculature removed to show major organ system. Obtained from (McLaughlin, 1983).

The haemolymph, containing the haemocytes and the plasma, occupies about 27 % of a crayfish body volume. The most prevailing protein in the haemolymph is the haemocyanin, which contains copper and acts as oxygen carrier (Vogt, 2002; Lee et al., 2004; Hagner-Holler et al., 2005). This respiratory protein pertains to the most complicated molecules in the animal kingdom. The haemolymph has a bluish color because of the copper-oxygen molecular complex (Terwilliger and Ryan, 2001; Vogt, 2002).

Cardiac system passes a series of changes during crayfish growth and development. Heart rate declines until hatching, then it increases until a juvenile stage and, finally, when the animal mass grows, heart rate decreases simultaneously (Reiber, 1997). Early function of the heart presents commonly as insensitive to the environmental factors such as pollutants or temperature. However, adult crayfish are more sensitive toward these factors (Spicer, 2001).

2.5. Methods for observing crayfish behavior and measuring cardiac activity

Demonstration of crayfish circadian rhythms is an essential part of behavioral response to environmental changes (Styrishave et al., 1995). The cardiovascular system performs the important vital function for survival of an organism (Reiber, 1997; Harper and Reiber, 2006).

2.5.1. Invasive methods for cardiac activity recording

Invasive methods of crayfish physiological activity recording accompanied with damage of the exoskeleton cuticle due to injection or implantation. There are several invasive methods for measuring cardiac activity. All of them use special wires, which are connected directly by electrodes to the crayfish. The crayfish perform a kind of gauge during these processes (Kozák and Kuklina, 2016). Monitoring heart rate allow to obtain information about the animal's response to different stimuli, even if the external behavior does not change (Listerman et al., 2000).

In the study of Tsuchida et al. (2004) was used both dual and quad channel transmitters regarding recording of electromyogram and neural activity. A pair of Teflon coated wires connected with transmitters was used for recording electromyograms (EMGs). Registration of spike activities from the circumesophageal connective was more complicated. To place the electrode in this area it was necessary to make a small hole (5 mm × 5 mm) in cuticle on the dorsal side of the cephalothorax. Then, a visually monitored electrode was inserted into the body cavity. After the electrode was installed, the removed part of cephalothorax was placed back on the hole and fixed by adhesive. This technique was specially adapted for applying to crayfish. That is why the weight of transmitters was about 20 % of total animal body (9 and 6.2 g for each of two transmitters). The lithium battery of transmitter remains active for 4 hours. It is also possible to use a smaller battery to reduce the weight of the transmitter, but the operating time of battery will also decrease. The signal could be obtained at a 50cm distance from the transmitter.

Applications of this method allow animals to move freely. Thereby, recording was possible even from resting animals that could move.

It is also possible to obtain electromyograms (EMGs) of the four crayfish leg muscles during reflexively or spontaneously walking and simultaneously to video-record the leg motions. Each leg muscle is extracellular recorded by mounting a pair of silver wires

interposed against a relatively immobile area of the muscle. Two holes are drilled in the cuticle and after the fixing wires they are sealed with glue. Crayfish can initiate movements not only as response on the external changes, but also in the absence of any impacts. This method could provide electrical signals from activated and non-activated leg muscles of crayfish (Chikamoto et al., 2008; Kozák and Kuklina, 2016).

Another invasive method is obtaining electrocardiograms (ECGs). Stainless steel electrodes are placed under the dorsal carapace on either side of the heart. Small holes are punctured in the carapace. These procedures are done 3 days before the experiment, because all manipulations lead to animal stress and after this period the heart rate is stabilized. Then, after inserting of wires, holes are sealed with instant cyanoacrylate glue. Cardiac signals are taped with a computerized recording system. Determination of the heart rate is obtained by direct measurement with a window discriminator of an instantaneous measure with next converting to beats per minute. For recording the crayfish physiological responses under controlled condition heart rate could be determined both out of and in water (Listerman et al., 2000; Goudkamp et al., 2004).

Invasive methods traumatize the animals. Consequently, often it leads to annulment of measurement within 24 hours after implantation or injection (Depledge, 1984).

2.5.2. Non-invasive methods for cardiac activity recording

New opportunities for more realistic measurements of cardiac activity were detected after the appearance of non-invasive techniques, which allow to measure heart rate both in the laboratory and the field (Depledge et al., 1996; Bloxham et al., 1999). Non-invasive biomonitoring systems decrease animal disturbance and time manipulation. As a result, theoretically it provides to the investigation the most actual physiological data (Kozák and Kuklina, 2016). Numerous techniques are applying for monitoring cardiac activity in decapods crustaceans (Depledge, 1984).

Photoplethysmography (PPG) which allows monitoring heart rate both in air and water was described by Depledge (1984) as a cheap and available technique with potentially widespread use. This device consisted of two small bulbs and a highly sensitive photosensor, which is fixed to the carapace cardiac area by momentary and water-resistant glue. Each of the two bulbs transmitted low intensity light, which came through the carapace directly into the pericardium. When the heart muscle contracted certain amounts of light were reflected by the pericardium and fell on the photosensor,

which transformed this light into a proportional voltage change for the further work of the oscilloscope. After the recording was finished, the device could be easily separated, and this allowed minimizing disturbance to the animal.

The next stage in the development of non-invasive techniques was a computer-aided physiological monitoring system (CAPMON). The equipment described above has been improved by using a special transducer, which comprised phototransistor detector and infrared light emitting diode operating in the near range. These components were placed parallel and towards one side. The phototransistor identified changes in infrared light intensity and generated a current, which was reflected by light and then was amplified and filtered before computer registration. A plastic ring of the transducer was attached to the animal carapace over the heart using by quick-drying and non-toxic glue. A flexible wire, which connected transducer with computer interface, allowed the observing organism to move freely. Direct heart beat monitoring using by ECG showed that beating was equivalent to the peaks received by PPG, but it is necessary that transducers should be correctly installed to avoid its loose during operation. With using of CAPMON system, monitoring of circadian rhythmicity in heart rates of 8 individuals simultaneously, 24 hours per day for long periods became possible. Moreover, the system is versatile and could be used in researches of contamination (Depledge and Andersen, 1990). The data were continuously recorded on a personal computer in the form of beats per minute (Styrishave et al., 1995).

Although the CAPMON system was demonstrated as functional and adaptable (Styrishave et al., 1995; Bojsen et al., 1998), but it could not supply the data regarding means of rate functions (for example, mean heart rates). The beating may be regular in some time intervals and contrariwise it may become irregular in another time intervals, but obtaining mean heart rates may be the same for both these periods. An Automated Interpulse Duration Assessment system (AIDA), the next modified technique, allowed conducting more detailed measurement of variability and disturbances of heart rate. This system gave opportunity for continuous interpulse intervals recording (the time interval between heart beats), beat regularity estimation and determination reasons which cause irregularities. Animals used in the experiment were not overly disrupted during the transducer affixing. It leads only to short-lived changes in interpulse durations and the mean heart rate was not changed (Depledge et al., 1996).

In addition, the CAPMON system was modified and enabled monitoring of several physiological and behavioral parameters such as heart and ventilatory rate and locomotor activity (Aagaard, 1996; Bojsen et al., 1998; Bloxham et al., 1999).

Measurement of the scattered and reflected infrared light variation was conducted with several changes. Photoplethysmograph was improved by low-power semiconductor laser, which produced infrared light and fibre-optic cables, which transmitted this light to the animal heart region through the sensor affixed to the carapace, thus providing scattered laser light. The send back signal provide data about regular changes in the heart size and shape. During systole, the optical signal is modulated. The identical electric signal is transformed to digital after appropriate amplification and filtration. Then it is registered in a computer (Fedotov et al., 2000). Recorded cardiogram can be analyzed using by diverse mathematical and statistical methods (Fedotov et al., 2009; Kholodkevich et al., 2008).

Pautsina et al. (2014) used a crayfish cardiac activity monitoring (NICCAM) system, which allow continuous cardiac activity observing of 16 animals simultaneously during to a long period of time. This system includes 16 IR optical sensors for crayfish, a multichannel analog-to-digital converter (ADC) with USB interface, and a personal computer with information processing software. Sensors are connected to the ADC with flexible 2m wires. Sensors is functioned on the photoplethysmography principle described above (Depledge and Andersen, 1990). The NICCAM system permits measuring and recording of heart rate chronotropic and inotropic parameters together with raw cardiac data. Further obtained information could be manual or semiautomatic analyzed. This improved system allows to carry out investigation in pollution and ecological area. Also, it is a cheap system with low number of required components and opportunity to monitoring a cardiac signal shape.

Contactless crayfish cardiac activity monitoring (COLECCAM) system was introduced by Cisar et al. (2018). The system consists of near infra-red (NIR) illuminator, NIR camera, computer, the software for data processing and two NIR light reflecting markers fixed by glue to the carapace of crayfish. A simple setup allows repeating observations in any laboratory. The COLECCAM system made possible simultaneous monitoring of more animals in one aquarium.

Obviously non-invasive methods have more advantages in contrast to invasive techniques including its reliability of the recorded data and this leads to an increase in their use (Fedotov et al., 2009).

2.5.3. Methods of behavioral observation

A variety of information about aquatic ecological status and environmental changes can be obtained using by different methods of animal behavioral tracking (e. g. Schapker et al., 2002; Kane et al., 2004; Panksepp and Huber, 2004).

One of the ways used to study behavior of aquatic organisms is radio-telemetry. Single stage radio-transmitters, fitted with whip antennas, have a suitable size for large macroinvertebrates (Robinson et al., 2000) and its weight is not more than 5 % of the total animal body mass, for example, of adult crayfish (Lowe et al., 2010). Fixed to the crayfish carapace with instant epoxy adhesive, the radio-transmitter does not limit free animal movement. The locations are obtained by a radio receiver and a special monitor with wide range (Webb and Richardson, 2004; Lowe et al., 2010). Radio-telemetry makes available information about movement patterns in natural surroundings, daily activity, habitat selection as response to different environmental changes, but it cannot be use during long periods in natural conditions, because crayfish lose the radio-tags after moult (Robinson et al., 2000).

For detection crayfish locomotion, Bojsen et al. (1998) used two sensors with holders, fixed to the aquarium transparent floor so that every even slight movement was detected. The beam of infrared light was reflected from the crayfish to one of positioned sensors and thus the motion event was registered. This method allowed obtaining of a semiquantitative measure of locomotor activity.

Another method is acoustic behavior monitoring. It means that the acoustic signals are emitted by the crustacean and recorded with the aid of a calibrated hydrophone. Further a digital acquisition card, connected with device, is calibrated using pure tone sine waves with frequency and intensity variations, produced by a signal generator. At the same time, another hydrophone and two cameras produce the signals that are synchronized, digitized and saved. Then these two parts (audio-video system and a system with acoustic signals) are synchronized. Thus, it is possible to investigate the response, for example, to a predator, individual of the same species or another external factor. All acoustic signals have a relationship with movement or behavioral events. It is possible to

conduct a research in an experimental tank and natural environment (Buscaino et al., 2011; Buscaino et al., 2012).

Denissenko et al. (2007) described monitoring of crayfish olfactory system using Particle Image Velocimetry (PIV). The flow, produced by crayfish as reaction on odour stimulation, induces influx, that involves smells to the anterior chemoreceptors. A two-dimensional PIV system was applied, which consist of a double-frame video camera with high resolution and a double pulse laser and it allow measurements in horizontal and vertical positions. The fan organs, swimmerets and antennules were observed by a discrete video camera. In additional, the special treadmill, performed natural crayfish walking movements, was used to increase an animal stress.

Applying a separately video analysis procedure can also provide an animal reaction to different stress factors (Basil and Sandeman, 2000; Schapker et al., 2002). Also, this method allows to observe the various locomotion patterns, even a trajectory of the crayfish four pairs of legs (Jamon and Clarac, 1995).

Basil and Sandeman (2000) studied crayfish locomotor activity using a video camera, which was placed over the experimental tank, and a video recorder. The animal position was registered by the digitized paths and thus the distance, covered by crayfish, and its velocity were available. Obtained data were digitized and analyzed by a tracking device.

It is well known, that crayfish are active at night period and to do observation during this time it is possible to use a video camera, that are illuminated by white lights mounted on an underwater housing, where the camera is kept. But this method is accompanied by some difficulties in observation process. Crayfish often try to escape from the observed area because of camera light, which is recognized as a threat. It means that such interactions could not be used for analysis (Bergman and Moore, 2003).

Analyzing crayfish movement patterns in reaction to drugs, Panksepp and Huber (2004) used an aquarium with lighting system, placed under it, which improved the video tracking resolution. Additional traction for crayfish walking was applied by a sand blaster for floor roughening. An adjustable video camera was positioned above the aquarium. Analyzing of video tracking were performed using specially written Java-based software and a program allowed to extract the crayfish coordinates from a single video frame.

2.6. Chlorine dioxide as water disinfectant

Several compounds could be used for water purification. Nevertheless, the most effective oxidant is chlorine dioxide (Lalezary et al., 1986).

Chlorine dioxide is a powerful oxidant among chlorine compounds and it is applied in surface water disinfection. Chlorine dioxide use for the drinking water purification became possible after the commercial availability of sodium chlorite, from which all chlorine dioxide is currently produced for purification of drinking water. Generally, treatment levels of concentrated chlorine dioxide solution range from 0.1 to 5.0 $mg \cdot l^{-1}$. There are a few reactions, that produce chlorine dioxide, and one of them is the hydrochloric-acid-sodium-chlorite reaction (Aieta and Berg, 1986):

$$5NaC1O_2 + 4HC1 = 4C1O_2 + 5NaCl + 2H_2O$$

Chlorine dioxide acts as a manganese oxidant, although in the presence of organic substances and at low temperatures the reactions are partly slowed. Also, chlorine dioxide is used for iron oxidation. It could be applied even at 2 $^{\circ}C$ and pH 5.5 (Knocke et al., 1991). Moreover, chlorine dioxide shows efficient removal of tastes and odors (Lalezary et al., 1986).

3 Materials and methods

3.1. Experimental animals

Adult male and female of signal crayfish *Pacifastacus leniusculus* were obtained from ponds near Velké Meziříčí, Czech Republic. More than two weeks before beginning of experiment crayfish were acclimated in laboratory conditions. They were held individually in aquariums. Feeding and fresh water was provided two times per week. No mortality was observed during the acclimation period.

Each crayfish was measured before the experiment. Biometrical parameters were: Carapace length: 43.8±0.77 mm; Total weight: 33.68±2.03 g; Body length: 90.13±1.6 mm. In the experiment crayfish were held separately in 10 L aquariums under constant photoperiod 12:12 light-to-dark cycle and water temperature of 10 ºC. Each aquarium had an artificial shelter (a half of a flower pot) where individuals were able to hide. A longitudinal hole made on the superior surface of the shelter allowed to record cardiac activity even when crayfish with the attached sensor were inside the shelter. According to experimental design five crayfish were monitored simultaneously and in case of death an individual had to be replaced by new one. Thus, twenty-five animals were used in total. The near infrared (NIR) optical sensor had been attached using epoxide glue to carapace of each monitored individual (Appendix 1). Animals were fed on commercial pellets daily. Only crayfish with non-damaged appendages (e.g. antennae, chelae, walking legs) were used in the experiment.

3.2. Description of the monitoring system

The non-invasive crayfish cardiac and behavioral activities monitoring system (NICCBAM) described in Pautsina (2015) was used. The NICCBAM system is consisted of: a multichannel 14 bit analog-to-digital converter (ADC) with USB interface; crayfish motion detection module; personal computer with software for processing a raw obtaining data; near infrared (NIR) optical sensors.

The equipment allowed to monitor, record and analyze not only crayfish cardiac activity, but also animal locomotor activity and store the data to local hard drive. The software graphical user interface displayed raw cardiac activity signals of five crayfish

simultaneously and motion detection module indicated animal movements. Motion detection module included wide field of view NIR camera and external NIR illuminator. The system is represented schematically in Fig. 2.

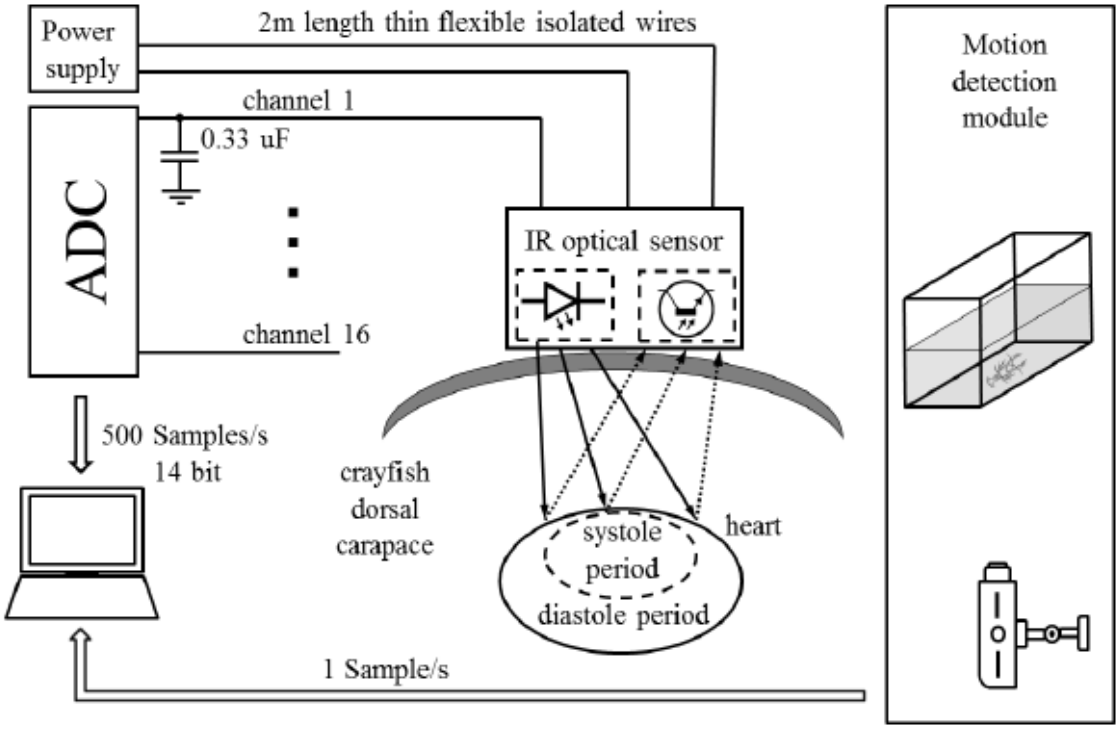


Figure 2. Overview of the NICCBAM system. Obtained from (Pautsina, 2015).

All cardiac activity signals of monitored crayfish were recorded and displayed on the software's graphical user interface in real-time. The NIR optical sensor included light-emitted diode (LED) coupled with a phototransistor and placed in the waterproof package. It was connected to ADC by flexible wire. The sensor was fixed with non-toxic two-component epoxy adhesive on the dorsal side of crayfish carapace above the heart. The sensor was attached to the carapace on the place where the clearest heart rate signal was found. Glue hardening time was about 10 minutes. Crayfish with the attached sensor could freely move around the aquarium.

3.3. Description of the monitoring process

Monitoring was conducted from February to August 2017 under conditions of private enterprise in Protivin, Czech Republic, where beer is made commercially. This company

has a water-treatment facility for drinking water, where chlorine dioxide (ClO_2) was used for water purification. Thus, it was a practical investigation using a patented non-invasive monitoring system. The system with crayfish has been used since April 2016, and exact data tracking has been started since February 2017. Chlorine dioxide was produced by hydrochloric-acid-sodium-chlorite reaction. In this reaction chlorine dioxide yield, and conversion had different values, where maximum yield is 100 % and maximum conversion is 80 % (Aieta and Berg, 1986).

Chlorine dioxide concentrations in water, in which crayfish were kept, were varied every day. Animals were in individual aquariums with a flow-through system which means pumping water disinfected by chlorine dioxide into aquariums and the flushing out of effluent (Appendix 2). Five crayfish were monitored simultaneously and the data about cardiac activity and motion were continuously logged onto a personal computer and then processed using a program MS Excel for further analyzing from created diagrams. Water characteristics such as pH, concentrations of ClO_2, Fe, Mn were measured daily.

3.4. Statistical analysis

The data recorded from treated individuals has been divided for observation on three experimental sets in accordance to time when crayfish were exposed to maximum concentration (MC) of disinfectant (MC; $ClO_2 > 0.2$ mg·l^{-1}): Group 1 got MC on days 1 to 6; Group 2 on days 11 to 15; and Group 3 was exposed by MC on days 30 to 43 after stocking to experimental aquarium system (Table 1). Grouping of the obtained data allowed their further statistical analysis.

All data presented as means ± standard deviation. Data were tested for normality using Shapiro-Wilk's test. One-way analysis of variance (ANOVA, Statistica 13, StatSoft, Inc.) with subsequent Tukey's post-hoc test was used to estimate differences between tested groups. Kruskal-Wallis non-parametric ANOVA was used for evaluation of the mortality latency between groups exposed by different level of contamination. The level of significance was set at $p < 0.05$.

4 Results

4.1. Pre-ecdysis period

One crayfish molted during the present study. The increasing of heart rate has been observed four hours before the molting up to 60 beats per minute (bpm) with the peak of 72 bpm (Fig. 3). The decline of heart rate was observed 35 min before molting with a few leaps. Increased locomotor activity was found four hours before molting, as well. It was a series of periodic movements with short duration.

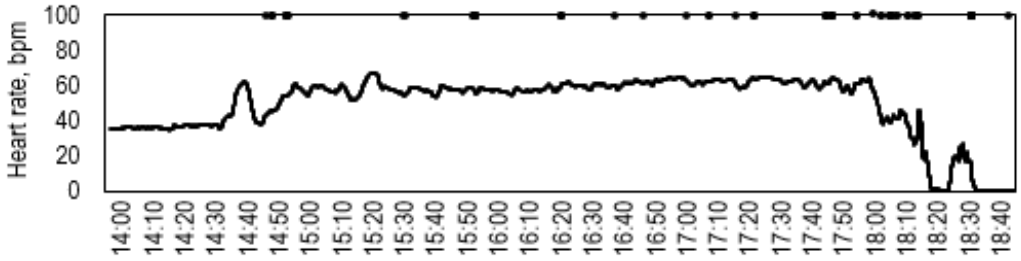


Figure 3. Heart rate and movement activity of crayfish *Pacifastacus leniusculus* four and half hours before the molting (— - heart rate, beats/min, ▪ - movement activity (on the upper side of the graph).

4.2. Diurnal rhythm

Crayfish were exposed to levels of chlorine dioxide concentrations which ranged from 0.01 to 0.29 $mg \cdot l^{-1}$. First 3 months (February-April) high concentration of ClO_2 (0.2-0.29) was occurred 4.6 times less than during the next 4 months (May-August), when this concentration was presented more often.

Day and night activity was different for each exposed crayfish. The daily cycle of animal heart rate was disturbed already in a lower level of chlorine dioxide concentration (less than 0.2 $mg \cdot l^{-1}$). There was a prevalence of disrupted heart rate with chaotic increase and decrease regardless of the time of day. Diurnal rhythm was disrupted, and rhythmicity was lost. It expressed in different heart rate fluctuations of animals exposed to the same concentration of ClO_2 (Fig. 4).

The locomotor activity was different for each crayfish individual. Some of them did not move around the aquarium for most of the twenty-for-hours period, while others were active at night and almost did not change a position during the day time. In general, cardiac and movement activity were disordered for all animals.

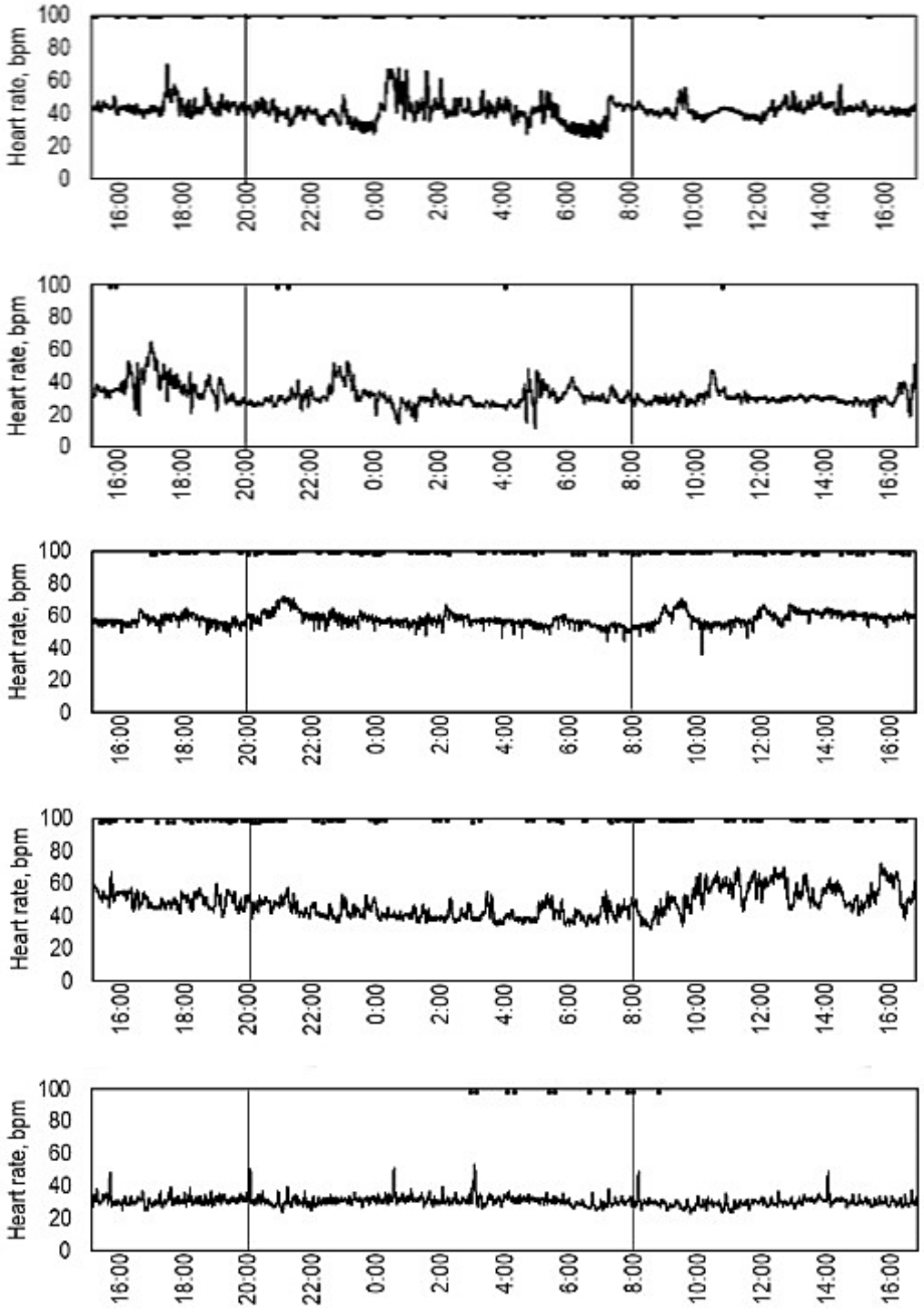


Figure 4. Examples of heart rate and movement activity of 5 crayfish *Pacifastacus leniusculus* during day and night period, 5th and 6th of June with ClO_2 concentrations 0.25 mg·l^{-1} and 0.19 mg·l^{-1}, respectively (— - heart rate, beats/min, ▪ - movement activity (on the upper side of the graph). Two vertical lines separate night time from day time.

4.3. Mortality

Presence of high concentration of chlorine dioxide (0.2-0.29 $mg \cdot l^{-1}$) resulted in disturbed diurnal rhythm for all individuals with rhythmicity loss and consequently led to death (Fig. 5). Mortality rose with an increasing number of ClO_2 high concentrations. In the period (89 days), when high ClO_2 concentration (up to 0.2 $mg \cdot l^{-1}$) was found 5 times, 4 crayfish died. In the second period (113 days), when high ClO_2 concentration occurred 23 times, 21 crayfish died. Thus, in first period mortality was 5.3 times less than in second. No individual has survived the experiment.

4.3.1. Mortality latency

Mortality latency (duration of life after exposure to MC) and ClO_2 cumulative concentration for each crayfish were determined (Table 1). Cumulative concentration was individual for each animal during its life period because of different living duration.

There was a significant difference in mortality latency between groups: individuals from Group 2 in general lived after exposure to MC more than twice longer (16±8 days after MC) compared to crayfish from Group 1 and Group 3, who died in 9±7 and 5±2 days respectively (Fig. 6). The cumulative concentration was: Group 1 - 1.58±0.76 mg; Group 2 - 3.91±0.62 mg; Group 3 - 6.07±1.16 mg. There were significant differences in cumulative concentration of ClO_2 between all tested groups (Fig. 7).

Table 1. Division of crayfish into 3 groups based on the ordinal day of exposure to maximum concentration (MC); mortality latency after MC treat and animal ClO_2 cumulative concentration over the entire exposure period; n – number of crayfish in each group. Mean ± SD.

Crayfish group (n)	MC of ClO_2, $mg \cdot l^{-1}$	Ordinal day, when MC was occurred	Exposure period before death, days	Mortality latency, days	Cumulative concentration, mg
1 (13)	0.21±0.04	4±2	13±8	9±7	1.58±0.76
2 (6)	0.26±0.02	13±1	29±8	16±8	3.91±0.62
3 (6)	0.29±0.01	38±6	43±7	5±2	6.07±1.16

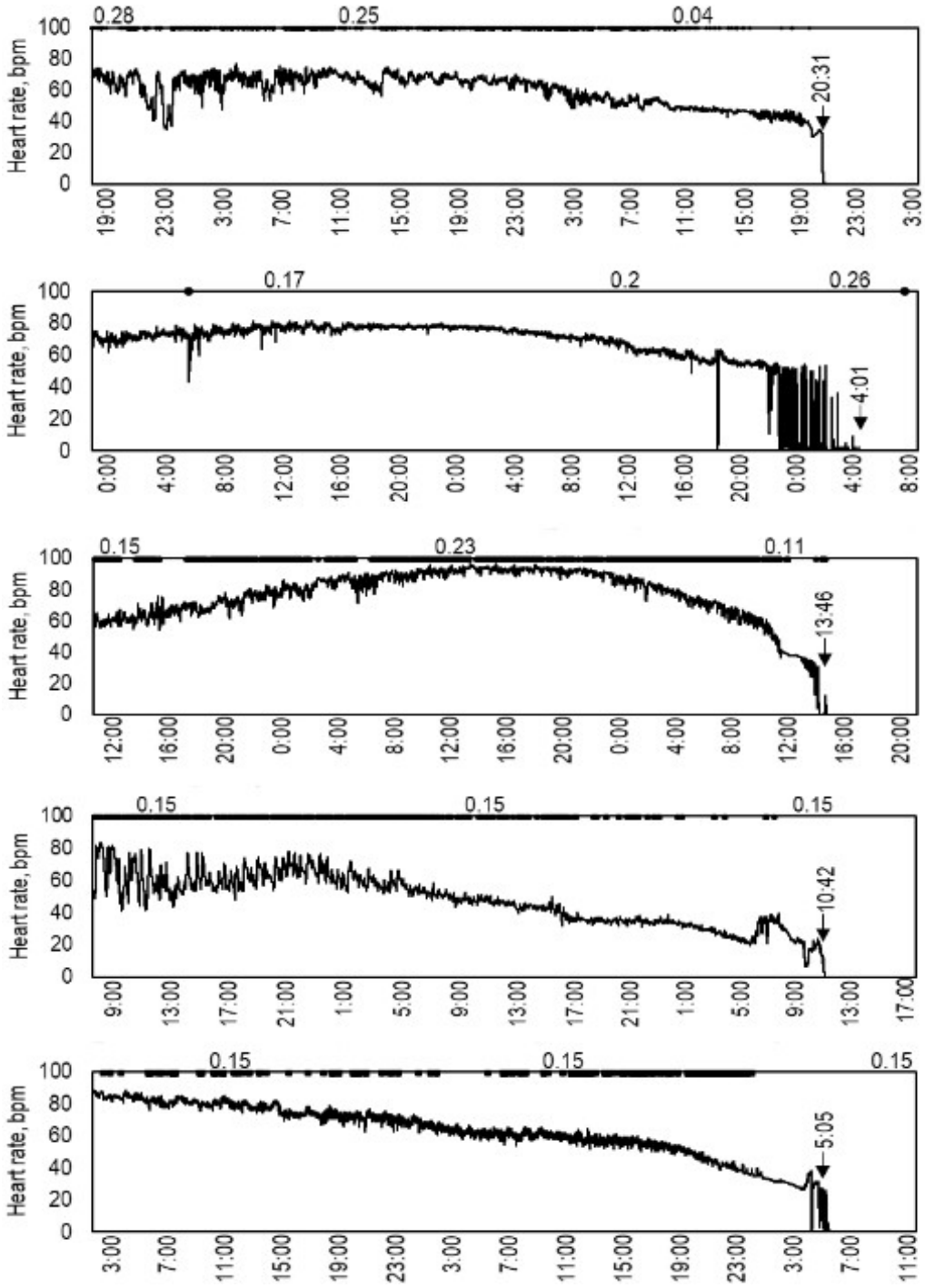


Figure 5. Examples of heart rate and movement activity of crayfish *Pacifastacus leniusculus* two days before death (—- heart rate, beats per min (bpm), ▪ - movement activity (on the upper side of the graph). Concentrations of ClO_2 ($mg \cdot l^{-1}$) per day are above the graphs, time of death is marked on each graph with vertical arrow.

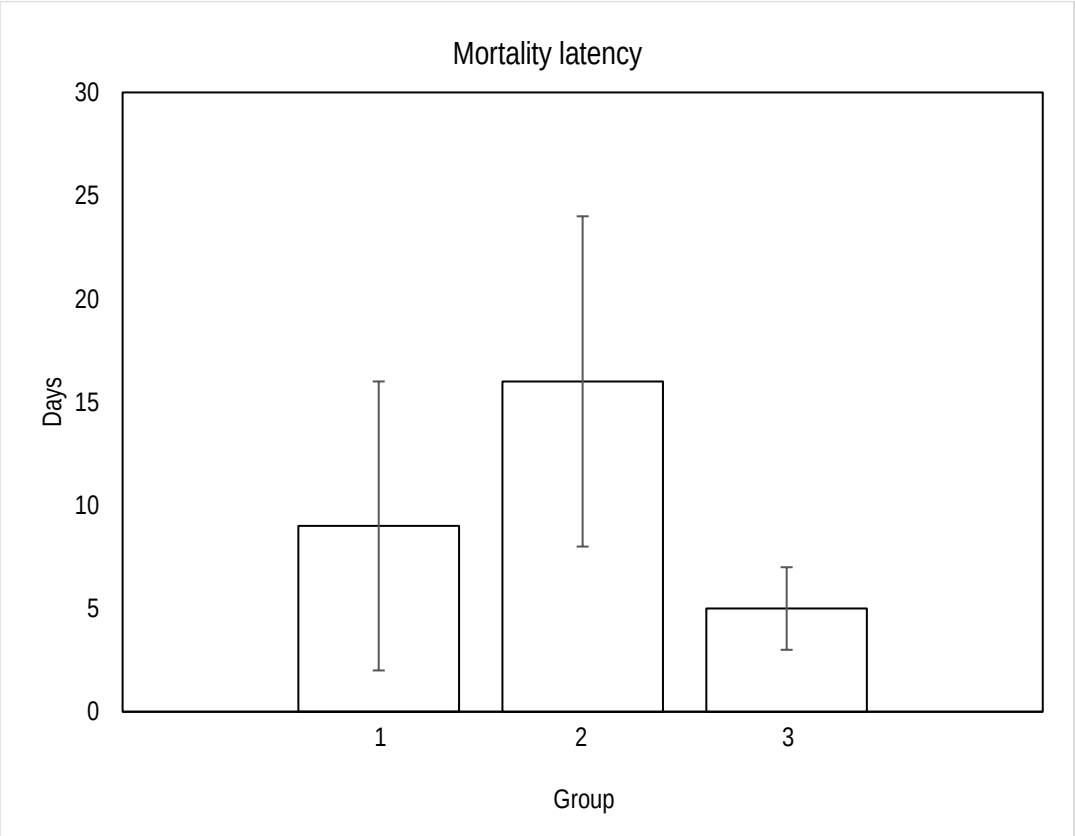


Figure 6. Mortality latency (duration of life after exposure to maximum concentration (MC)) of crayfish three experimental groups: Group 1 got MC on day 4±2; Group 2 on day 13±1; and Group 3 was exposed by MC on day 38±6 after stocking to experimental aquarium system. Mortality latency was: 9±7 days, 16±8 days and 5±2 days respectively. There was a significant difference in mortality latency between groups: individuals from Group 2 in general lived more than twice longer after exposure to MC compare to crayfish from Group 1 and Group 3.

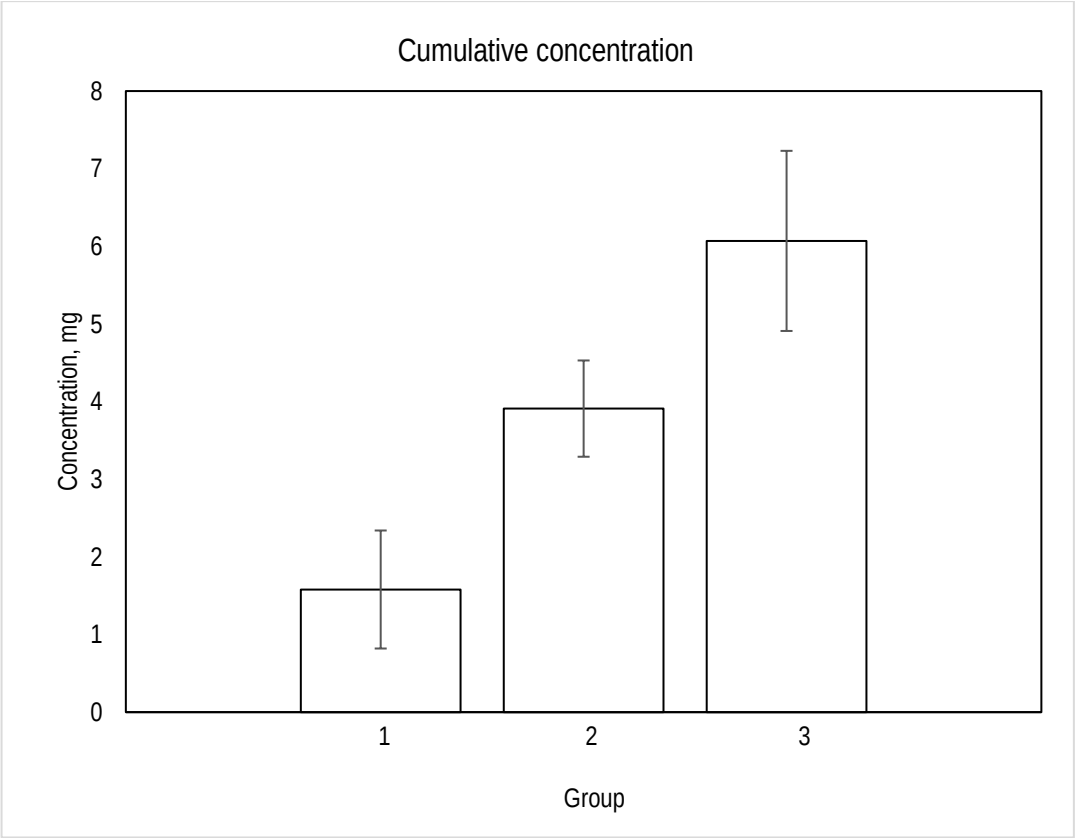


Figure 7. Cumulative concentration of crayfish three experimental groups: Group 1 - 1.58±0.76 mg; Group 2 - 3.91±0.62 mg; Group 3 - 6.07±1.16 mg. There were significant differences in cumulative concentration of ClO_2 between all tested groups.

5 Discussion

In the present study the effect of long-term exposure of chlorine dioxide concentration on signal crayfish has been investigated and evaluated through the observation of heart rate, diurnal rhythm and locomotor activity, which were the main point of monitoring.

Insignificant differences were found between heart rate of none-treated spiny lobster described in Kuramoto (1993) and chlorine dioxide exposed signal crayfish in premolting period. In a study by Kuramoto (1993) it was noted that the heart rate rises and falls during molting of lobster in the same way as in crayfish. The heart rate of an unaffected animal increased 1-2 hours before ecdysis to peak of 80-120 bpm and declined about 15 minutes before the beginning of molting. In this study during ClO_2 treatment the heat rate increase observed four hours before the molting up to 60 beats per minute (bpm) with the peak of 72 bpm and the heart rate decline detected 35 min before molting. For successful molting crayfish flips on its side and moves its limbs. Increased motion activity could be one of the reasons of heart rate rise. Kuklina et al. (2018) noticed, that crayfish heart rate disturbance was often simultaneously with initiated locomotion under chloramine-T impact.

The heart rate of crayfish in the natural condition is higher at night time and lower at day time, which explained by animal typical nocturnal activity (Fig. 8). Several studies were devoted to influence of different factors on crayfish circadian cardiac rhythm (e. g. Styrishave et al., 1995; Bojsen et al., 1998; Udalova et al., 2009; Kuznetsova et al., 2010).

Figure 8. Dynamics of HR (beats/min) in the crayfish. Vertical bars in graphs indicate the beginning of the nocturnal and diurnal periods. Obtained from (Udalova et al., 2009).

A present study showed a possible impact of chlorine dioxide on crayfish heart rate and nocturnal behavior. A disturbance of the circadian cardiac rhythm was observed in all individuals, expressed in random decline and rise of heart rate. Typical increased nocturnal locomotor activity almost was not noticed in lowest ClO_2 concentration, while in high concentrations it was completely disrupted for all animals. Therefore, diurnal rhythm was disturbed, a circadian rhythmicity was lost, and animals died (Fig. 6). Similar observation was described in Kuznetsova et al. (2010) as a reaction on hydroquinone solution, where crayfish before death had circadian rhythm disruption. In Styrishave et al. (1995) increase of heart rate at the day time and decrease at night was noticed as a response on copper and mercury exposure. In that case mortality was also detected.

Not only is the loss of circadian rhythmicity, but also changes in animal organs suspected to be the reason for crayfish mortality. Chupani et al. (2015) described effect of sub-acute peracetic acid exposure on crayfish. Animals had epithelial cell disorganization in gill tissues after 7 days of 2 $mg \cdot l^{-1}$ treat. In higher concentration (10 $mg \cdot l^{-1}$) of disinfectant hemocyte aggregation in vessels, infiltration of granular hemocytes, malformations of lamella tips were additionally detected. Also, the pathology of hepatopancreas and antennal gland was observed. In experiments, conducted with grass carp juveniles, peracetic acid induced pathological alterations in gill tissue of exposed fish. Mortality (71.5%) was observed only in the fish group with highly peracetic acid concentration, which was 3 $mg \cdot l^{-1}$ (Chupani et al., 2014). Straus et al. (2012) also found degeneration of the gill epithelium as result of fish fry exposure to 2.2 $mg \cdot l^{-1}$ peracetic acid. Furthermore, gill pathology was observed in 80% of the fish treated by 0.13 $mg \cdot l^{-1}$ of ClO_2 concentration. Epithelial damage expressed in epithelial lifting, hypertrophy, hyperplasia, and necrosis (Yonkos et al., 2000). The next confirmation of the destructive effect of the disinfectant is detected necrosis in gills, hepatopancreas and the antennal gland of the shrimp exposed to formalin. Reduction in the total number of haemocytes in haemolymph was noticed as well (Lamela et al., 2008).

Chlorine dioxide could cause changes in cardio-respiratory responses and affect growth of fish. This disinfectant induced decline of respiration and heart rate frequency of rainbow trout larvae even at lower concentrations (0.3-.06 $mg \cdot l^{-1}$) and reduced growth of their body weight. 96-hours exposure of adult fish led to their consequently mortality

(Svecevicius et al., 2005). Oxidative damage and antioxidant defenses changes were observed in the heart tissue of ClO_2 treated rainbow trout (Tkachenko et al., 2015).

Chlorine compounds affect the physiological condition of organisms more than peracetic acid. Elia et al. (2006) studied the influence of three disinfectants (chlorine dioxide, sodium hypochlorite, peracetic acid) on carp, which was expressed in obvious biochemical changes in the liver of treated animals. But it was noticed that antioxidant responses of fish to peracetic acid were slight in comparison with reaction to chlorine compounds. Moreover, chlorine dioxide is more toxic to aquatic organisms than chlorite. It was found, that it is 18 times more harmful to adult fish, which resulted in their higher mortality, and larvae are 3.8 times more sensitive to the chlorine dioxide acute impact than adult fish (Svecevicius et al., 2005).

In our study changes in heart rate and typical diurnal rhythm of treated animals and their locomotor activity were various for each organism which could be caused by their different functional state and individual physiological response to chlorine dioxide concentrations.

In Kuklina et al. (2018) male and female crayfish have approximately equal reaction, expressed in heart rate changes, on various stressors such as chloramine-T, food odor, predatory fish and conspecific crayfish. Obtained average heart rate of both sexes in the posttreatment state does not have significant difference between each other. Therefore, during the experiment, the data were recorded regardless of the animal sex.

In the present study crayfish were continuously exposed to ClO_2, which led to disturbance of circadian rhythmicity and consequently to mortality. Level of disruption of physiological parameters is depending on treatment duration. According to Kuklina et al. (2014) mean heart rate of crayfish, exposed to 50 $mg \cdot l^{-1}$ of chloramine-T during 1-24 h was 89 bpm in comparison with mean heart rate of animals, treated during 1 h, which was 68 bpm. Also, repeatedly exposed crayfish to physical and chemical stress had exhaustion of energy, which expressed in the lack of ability to increase heart rate as response on influencing stimuli. Treatment with progressive increase in the concentration of chloramine-T could lead to a detrimental influence on animal chemoreceptors, mainly respond to external stimuli.

Peak concentrations of ClO_2 (0.2-0.29 $mg \cdot l^{-1}$) observed during our experiment significantly influenced the life duration of animals. When crayfish got the maximum concentration, death was noticed after a certain number of days in each group. Crayfish

mortality in Group 1 already occurred during exposure to MC 0.21 $mg \cdot l^{-1}$. Crayfish from Group 3 had short mortality latency, which could be caused by longest exposure period before that, already disturbed cardiac and physiological processes in lowest ClO_2 concentrations and the largest MC. Crayfish from Group 2 had the longest mortality latency, which could be related to individual reaction on substance and function state.

Cumulative concentrations were significantly differenced in all groups. It was noted that the cumulative concentration was higher for animals that were exposed longer.

The evidence of crayfish tolerance to the exposure by various substances was found in several studies. Kouba et al. (2012) assumed that the crayfish tolerance to highly toxic to aquatic organisms Persteril 36 could be induced by calcium carbonate and calcium phosphate, partially formed a carapace, which help to withstand the effects of acidic and oxidizing compounds. Also, crayfish demonstrate high tolerance to chloride concentration, which express in heart rate normalization after a few minutes or hours of NaCl exposure (Kozák et al., 2009). Svecevicius et al. (2005) found, that cardio-respiratory responses of rainbow trout return to a normal state after increase during treatment period of chlorite. Thus, crayfish could tolerate certain lower concentration of used substances. In the present study high ClO_2 concentrations (0.2-0.29 $mg \cdot l^{-1}$) led to crayfish mortality. The more this acute concentration was present in the water, the higher was the mortality of crayfish.

Changes in heart rate and circadian rhythmicity could provide information about the functional state of crayfish and its reaction on changes in environment quality (Udalova et al. 2009). The presence of chlorine dioxide concentration in water, obviously, affects the expression of circadian rhythm in the heart rate. However, further work is needed to investigate whether chlorine dioxide exposure could influence internal organs of crayfish.

6 Conclusion

Changes of crayfish typical circadian cardiac rhythm may indicate a deterioration of the animal functional state. In the present study we have analyzed the data of continuous exposure by chlorine dioxide on heart rate and locomotor activity of signal crayfish (*Pacifastacus leniusculus*). This practical investigation was conducted under brewery conditions which has a water-treatment facility for drinking water, where chlorine dioxide was used for water purification. A patented non-invasive monitoring system was used for observation of crayfish cardiac and locomotor activity. Typical diurnal rhythm with increased night activity was almost not noticed in the lowest ClO_2 concentration, while in high concentrations it completely disturbed all animals. A possible lethal concentration (> 0.2 mg·l^{-1}) of this disinfectant, which caused animal mortality, was determined. Crayfish mortality occurred on average up to three-four weeks. An increase in mortality was observed after more frequent presence of high levels of chlorine dioxide in water. Observed crayfish response to the disinfectant varied among individuals, which could be explained by a different functional state and individual reaction on stimuli.

This study provides useful information for further investigations, which should be done in future to examine the effect of chlorine dioxide on internal organs of crayfish. In addition, present work can serve as a basis for the effective use of crayfish as bioindicators in disinfected water and, in particular, for rationalizing the parameters of chlorine dioxide.

7 Bibliography

Aagaard, A., 1996. In situ variation in heart rate of the shore crab *Carcinus maenasin* relation to environmental factors and physiological condition. Marine Biology 125, 765-772.

Ache, B.W., Sandeman, D.C., 1980. Olfactory-induced central neural activity in the Murray crayfish, *Euastacus armatus*. J. Comp. Physiol. 140, 295-301.

Ahern, M.D., Morris, S., 1999. Respiratory, acid – base and metabolic responses of the freshwater crayfish *Cherax destructor* to lead contamination. Comparative Biochemistry and Physiology Part A 124, 105–111.

Aieta, E.M., Berg, J.D., 1986. A review of chlorine dioxide in drinking water treatment. Am. Water Works Assoc. 78 (6), 62-72.

Alcorlo, P., Otero, M., Crehuet, M., Baltanas, A., Montes, C., 2006. The use of the red swamp crayfish (*Procambarus clarkii*, Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the River Guadiamar (SW, Spain). Science of the Total Environment 366, 380-390.

Anderson, M.B., Preslan, J.E., Jolibois, L., Bollinger, J.E., George, W.J., 1997. Bioaccumulation of lead nitrate in Red Swamp Crayfish (*Procambarus clarkii*). Journal of Hazardous Materials 54, 15-29.

Armienta, M.A., Rodriguez, R., Ceniceros, N., Juarez, F., Cruz, O., 1996. Distribution, origin and fate of chromium in soils in Guanajuato, Mexico. Environmental Pollution 91 (3), 391-397.

Aguilar-Betancourt, C.M., Gonzales-Sanson, G., Kidd, K.A., Munkittrick, K.R., Curry, R.A., Kosonoy-Aceves, D., Lucano-Ramirez, G., Ruiz-Ramirez, S., Flores-Ortega, J.R., 2016. Fishes as indicators of untreated sewage contamination in a Mexican coastal lagoon. Marine Pollution Bulletin 113, 100-109.

Barbee, G.C., McClain, W.R., Lanka, S.K., Stout, M.J., 2010. Acute toxicity of chlorantraniliprole to non-target crayfish (*Procambarus clarkii*) associated with rice–crayfish cropping systems. Pest Management Science 66, 996–1001.

Basil, J., Sandeman, D., 2000. Crayfish (*Cherax destructor*) use tactile cues to detect and learn topographical changes in their environment. Ethology 106, 247–259.

Belanger, R.M., Peters, T.J., Sabhapathy, G.S., Khan, S., Katta, J., Abraham, N.K., 2015. Atrazine exposure affects the ability of crayfish (*Orconectes rusticus*) to localize a food odor source. Arch Environ Contam Toxicol 68, 636–645.

Berglund, O., 2003. Periphyton density influences organochlorine accumulation in rivers. Limnol. Oceanogr. 48(6), 2106–2116.

Bergman, D.A., Moore, P.A., 2003. Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. Biol. Bull. 205, 26–35.

Bini., G., Chelazzi, G., 2006. Acclimatable cardiac and ventilatory responses to copper in the freshwater crayfish *Procambarus clarkii*. Comparative Biochemistry and Physiology, Part C 144, 235-241.

Birungi, Z., Masola, B., Zaranyika, M.F., Naigaga, I., Marshall, B., 2007. Active biomonitoring of trace heavy metals using fish (*Oreochromis niloticus*) as bioindicators species: the case of Nakivubo wetland along Lake Victoria. Physics and chemistry of the earth, Parts A/B/C 32 (15-18), 1350-1358.

Bloxham, M.J., Worsfold, P.J., Depledge, M.H., 1999. Integrated biological and chemical monitoring: In situ physiological responses of freshwater crayfish to fluctuations in environmental ammonia concentrations. Ecotoxicology 8, 225–231.

Bojsen, B.H., Witthofft, H., Styrishave, B., Andersen, O., 1998. In situ studies on heart rate and locomotor activity in the freshwater crayfish, *Astacus astacus* (L.) in relation to natural fluctuations in temperature and light intensity. Freshwater Biology 39, 455–465.

Bollinger, J.E., Bundy, K., Anderson, M.B., Millet, L., Preslan, J.E., Jolibois, L., Chen, H.-L., Kamath, B., George, W.J., 1997. Bioaccumulation of Chromium in Red SwampCrayfish (*Procambarus clarkii*). Journal of Hazardous Materials 54, 1-13.

Bowen, R.E., Depledge, M.H., 2006. Rapid Assessment of Marine Pollution (RAMP). Marine Pollution Bulletin 53, 631-639.

Brinkman, S.F., Johnston, W.D., 2012. Acute toxicity of zinc to several aquatic species native to the Rocky Mountains. Arch Environ Contam Toxicol 62, 272–281.

Browne, A.M., Moore, P.A., 2014. The Effects of Sublethal Levels of 2,4-Dichlorophenoxyacetic Acid Herbicide (2,4-D) on Feeding Behaviors of the Crayfish *O. rusticus*. Arch Environ Contam Toxicol 67, 234–244.

Bucas, G., Saliot, A., 2002. Sea transport of animal and vegetable oils and its environmental consequences. Marine Pollution Bulletin 44, 1388–1396.

Buscaino, G., Filiciotto, F., Gristina, M., Bellante, A., Buffa, G., Di Stefano, V., Maccarrone, V., Tranchida, G., Buscaino, C., Mazzola, S., 2011. Acoustic behavior of the European spiny lobster *Palinurus elephas*. Marine Ecology Progress Series 441, 177–184.

Buscaino, G., Filiciotto, F., Buffa, G., Di Stefano, V., Maccarrone, V., Buscaino, C., Mazzola, S., 2012. The underwater acoustic activities of the red swamp crayfish *Procambarus clarkii*. J. Acoust. Soc. Am. 132 (3), 1792-1798.

Chikamoto, K., Kagaya, K., Takahata, M., 2008. Electromyographic characterization of walking behavior initiated spontaneously in crayfish. Zoological Science 25, 783–792.

Chupani, L., Stara, A., Velisek, J., Zuskova, E., 2014. Evaluation of the toxic effect of peracetic acid on grass carp (*Ctenopharyngodon idella*) juveniles. Neuroendocrinology Letters 35 (2), 86-92.

Chupani, L., Zuskova, E., Stara, A., Velisek, J., Kouba, A., 2015. Histological changes and antioxidant enzyme activity in signal crayfish (*Pacifastacus leniusculus*) associated with sub-acute peracetic acid exposure. Fish & Shellfish Immunology 48, 190-195.

Cisar, P., Saberioon, M., Kozák, P., Pautsina, A., 2018. Fully contactless system for crayfish heartbeat monitoring: Undisturbed crayfish as bio-indicator. Sensors and Actuators B 255, 29-34.

Coimbra, C.N., Graca, M.A.S., Cortes, R.M., 1996. The effects of a basic effluent on macroinvertebrate community structure in a temporary Mediterranean river. Environmental Pollution 94 (3), 301-307.

Comber, S.D.W., 1999. Abiotic persistence of atrazine and simazine in water. Pesticide Science 55, 696-702.

Connon, R.E., Geist, J., Werner, I., 2012. Effect-based tools for monitoring and predicting the ecotoxicological effects of chemicals in the aquatic environment. Sensors 12: 12741-12771.

Cook, M.E., Moore, P. A., 2008. The Effects of the Herbicide Metolachlor on Agonistic Behavior in the Crayfish, *Orconectes rusticus*. Arch Environ Contam Toxicol 55, 94–102.

Cooke, I.M., 2002. Reliable, responsive pacemaking and pattern generation with minimal cell numbers: the crustacean cardiac ganglion. Biol. Bull. 202, 108-136.

Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change? Environmental Health Perspectives 107 (6), 907-938.

De la Sienra, E., Armienta, M.A., Gonsebatt, M.E., 2003. Potassium dichromate increases the micronucleus frequency in the crayfish *Procambarus clarkii*. Environmental Pollution 126, 367–370.

Denissenko, P., Lukaschuk, S., Breithaupt, T., 2007. The flow generated by an active olfactory system of the red swamp crayfish. The Journal of Experimental Biology 210, 4083-4091.

Depledge, M.H., Fossi, M.C., 1994. The role of biomarkers in environmental assessment (2). Invertebrates. Ecotoxicology 3, 161-172.

Depledge, M.H., Lundebye, A.K., Curtis, T., Aagaard, A., Andersen, B.B., 1996. Automated interpulseduration assessment (AIDA): a new technique for detecting disturbances in cardiac activity in selected macroinvertebrates. Mar. Biol. 126, 313–319.

Depledge, M.H. 1984. Photoplethysmography—a non-invasive technique for monitoring heart beat and ventilation rate in decapod crustaceans. Comp. Biochem. Phys. 77, 369–371.

Depledge, M.H., Andersen, B.B., 1990. A computer-aided physiological monitoring system for continuous, long-term recording of cardiac activity in selected invertebrates. Comp. Biochem. Phys. 96, 473–477.

Elia, A.C., Anastasi, V., Dorr, A.J.M., 2006. Hepatic antioxidant enzymes and total glutathione of *Cyprinus carpio* exposed to three disinfectants, chloride dioxide, sodium hypochlorite and peracetic acid, for superficial water potabilization. Chemosphere 64, 1633-1641.

Faria, M., Huertas, D., Soto, D.X., Grimalt, J.O., Catalan, J., Riva, M.C., Barata, C., 2010. Contaminant accumulation and multi-biomarker responses in field collected zebra mussels (*Dreissena polymorpha*) and crayfish (*Procambarus clarkii*), to evaluate toxicological effects of industrial hazardous dumps in the Ebro river (NE Spain). Chemosphere 78, 232-240.

Fedotov, V.P., Zhuravlev, V.L., Khalatov, A.N., Kholodkevich, S.V., 2009. Comparative Analysis of Heart Activity of the Crayfish *Pontastacus leptodactylus* by Methods of Plethysmography and Electrocardiography. J. Evol. Biochem. Phys. 45, 527—529.

Fedotov, V.P., Kholodkevich, S.V., Strochilo, A.G., 2000. Study of contractile activity of the crayfish heart with the aid of a new non-invasive technique. J. Evol. Biochem. Phys. 36, 288–293.

Fernandes, D., Potrykus, J., Morsiani, C., Raldua, D., Lavado, R., Porte, C., 2002. The Combined Use of Chemical and Biochemical Markers to Assess Water Quality in Two Low-Stream Rivers (NE Spain). Environmental Research 90, 169-178.

Füreder, L., Reynolds, J.D. 2003. Is *Austropotamobius pallipes* a good bioindicators? Bull. Fr. Peche Piscic. 370-371, 157-163.

Gilmour, C.C., Henry, E.A., 1991. Mercury methylation in aquatic systems affected by acid deposition. Environmental Pollution 71, 131-169.

Gore, A.C. 2010. Neuroendocrine targets of endocrine disruptors. Hormones 9, 16–27.

Goudkamp, J.E., Seebacher, F., Ahern, M., Franklin, C.E., 2004. Physiological thermoregulation in a crustacean? Heart rate hysteresis in the freshwater crayfish *Cherax destructor*. Comp. Biochem. Phys. 138, 399–403.

Graedel, T.E., Bertram, M., Kapur, A., Reck, B., Spatari, S., 2004. Exploratory data analysis of the multilevel anthropogenic copper cycle. Environ. Sci. Technol. 38, 1253–1261.

Hagner-Holler, S., Kusche, K., Hembach, A., Burmester, T., 2005. Biochemical and molecular characterisation of hemocyanin from the amphipod *Gammarus roeseli*: complex pattern of hemocyanin subunit evolution in Crustacea. J Comp Physiol. 175, 445–452.

Harper, S.L., Reiber, C.L., 2006. Cardiac development in crayfish: ontogeny of cardiac physiology and aerobic metabolism in the red swamp crayfish *Procambarus clarkii*. J Comp Physiol. 176, 405–414.

Helfrich, L.A., Weigmann, D.L., Hipkins, P., Stinson, E.R., 1996. Pesticides and aquatic animals: a guide to reducing impacts on aquatic systems. Virginia Polytechnic Institute and State University, 3–24.

Hirsch, R.M., Alexander, R.B., Smith, R.A., 1991. Selection of methods for the detection and estimation of trends in water quality. Water resources research 27 (5), 803-813.

Hodkinson, I.D., Jackson, J.K., 2005. Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. Environmental Management 35 (5), 649–666.

Holmqvist, N., Stenroth, P., Berglund, O., Nystorm, P., Graneli, W., Larsson, P., 2007. Persistent organic pollutants (POP) in a benthic omnivore – A comparison between lake and stream crayfish populations. Chemosphere 66, 1070-1078.

Imeh-Nathaniel, A., Okon, M., Huber, R., Nathaniel, T.I. 2014. Exploratory behavior and withdrawal signs in Crayfish: Chronic central morphine injections and termination effects. Behavioural Brain Research 264, 181–187.

Jamon, M., Clarac, F., 1995. Locomotor patterns in freely moving crayfish (Procambarus clarkii). The Journal of Experimental Biology 198, 683-700.

Jurcak, A.M., Gauthier, S.J., Moore P.A., 2015. The Effects of biodiesel and crude oil on the foraging behavior of rusty crayfish, *Orconectes rusticus*. Arch Environ Contam Toxicol 69, 557–565.

Kaddissi, S.A., Legeay, A., Elia, A.C., Gonzalez P., Camilleri, V., Gilbin, R., Simon, O., 2012. Effects of uranium on crayfish *Procambarus clarkii* mitochondria and antioxidants responses after chronic exposure: What have we learned? Ecotoxicology and Environmental Safety 78, 218–224.

Kane, A.S., Salierno, J.D., Gipson, G.T., Molteno, T.C.A., Hunter, C., 2004. A video-based movement analysis system to quantify behavioral stress responses of fish. Water research 38, 3993-4001.

Khan, N, Warith, M.A., Luk, G., 2007. A comparison of acute toxicity of biodiesel, biodiesel blends, and diesel on aquatic organisms. Journal of the Air & Waste Management Association 57, 286–296.

Kholodkevich, S.V., Ivanov, A.V., Kurakin, A.S., Kornienko, E.L., Fedotov, V.P., 2008. Real time biomonitoring of surface water toxicity level at water supply stations. Environmental Bioindicators 3, 23–34.

Knocke, W.R., Van Benschoten, J.E., Kearney, M.J., Soborski, A.W., Reckhow, D.A., 1991. Kinetics of manganese and iron oxidation by potassium permanganate and chlorine dioxide. J Am Water Works Assoc 83(7), 80–87.

Kouba, A., Kuklina, I., Niksirat, H., Machova, J., Kozák, P., 2012. Tolerance of signal crayfish (*Pacifastacus leniusculus*) to Persteril 36 supports use of peracetic acid in astaciculture. Aquaculture 350-353, 71-74.

Kozák, P., Kuklina, I., 2016. Crayfish as tools of water quality monitoring. In: Kawai, T., Faulkes, Z., Scholtz, G. (Eds.), Freshwater Crayfish: A Global Overview. Taylor & Francis Group, Boca Raton, pp. 275-297.

Kozák, P., Policar, T., Fedotov, V. P., Kuznetsova, T. V., Buřič, M., Kholodkevich, S. V., 2009. Effect of chloride content in water on heart rate in narrow-clawed crayfish (*Astacus leptodactylus*). Knowledge and Management of Aquatic Ecosystems 394(395), 1–10.

Kraak, M.H.S., Scholten, M.C.T., Peeters, W.H.M., Kock, W.C.H., 1991. Biomonitoring of heavy metals in the Western European rivers Rhine and Meuse using the freshwatermussel Dreissena polymorpha. Environ. Pollut. 74, 101-114.

Kuklina, I., Lozek, F., Cisar, P., Kouba, A., Kozák, P., 2018. Crayfish can distinguish between natural and chemical stimuli as assessed by cardiac and locomotor reactions. Environ. Sci. Pollut. Res. Published online.

Kuklina, I., Sladkova, S., Kouba, A., Kholodkevich, S., Kozák, P., 2014. Investigation ofchloramine-T impact on crayfish *Astacus leptodactylus* (Esch., 1823) cardiacactivity, Environ. Sci. Pollut. Res. 21, 10262–10269.

Kuramoto, T., 1993. Cardiac activation and inhibition involved in molting behavior of a spiny lobster. Experientia 49, 682-685.

Kuramoto, T., Yamagishi, H., 1990. Physiological Anatomy, Burst Formation, and Burst Frequency of the cardiac ganglion of crustaceans. Physiological Zoology 63, 102-116.

Kuznetsova, T.V., Sladkova, S.V., Kholodkevich, S.V., 2010. Evaluation of functional state of crayfish *Pontastacus leptodactylus* in normal and toxic environment by characteristics of their cardiac activity and hemolymph biochemical parameters. J. Evol. Biochem. Phys. 46(3), 241–250.

Lahman, S.E., Trent, K.R., Moore, P.A., 2015. Sublethal copper toxicity impairs chemical orientation in the crayfish, *Orconectes rusticus*. Ecotoxicology and Environmental Safety 113, 369-377.

Lalezary, S., Pirbazari, M., McGuire, M.J., 1986. Oxidation of five-musty taste and odor compounds. J Am Water Works Assoc 78 (3), 62-69.

Lamela, R.E.L., Quintana, Y.C., Coffigny, F.S., Martinez, M., Herrate, N.G., 2008. Effects of formalin on total haemocytes count and histopathological changes in the shrimp *Litopenaeus schmitti* (Perez-Farfante & Kensley 1997). Aquaculture research 39, 1316-1321.

Lee, S.Y., Lee, B.L., Soderchall, K., 2004. Processing of crayfish hemocyanin subunits into phenoloxidase. Biochemical and Biophysical Research Communications 322, 490-496.

Linder, M.C., 1991. Biochemistry of copper. Plenum Press, New York.

Listerman, L.R., Deskins, J., Bradacs, H., Cooper, R.L., 2000. Heart rate within male crayfish: Social interactions and effects of 5-HT. Comp. Biochem. Phys. 125, 251–263.

Loeb, S.L., 1994. An ecological context for biological monitoring. In: Loeb, S.L., Spacie, A. (Eds.), Biological monitoring of aquatic systems. CRC Press LLC, Boca Raton, Florida, pp. 3-7.

Lowe, K., Fitzgibbon, S., Seebacher, F., Wilson, R. S., 2010. Physiological and behavioral responses to seasonal changes in environmental temperature in the Australian spiny crayfish *Euastacus sulcatus*. J. Comp. Physiol. 180, 653–660.

Luebke, R.W., Hodson, P.V., Faisal, M., Ross, P.S., Grasman, K.A., Zelikoff, J., 1997. Aquatic pollution-induced immunotoxicity in wildlife species. Fundamental and applied toxicology 37, 1-15.

Maeda, S., Inoue, R., Kozono, T., Tokudam, T., Ohki, A., Takeshita, T., 1990. Arsenic metabolism in a freshwater food chain. Chemosphere 20, 101-108.

Martin-Diaz, M.L., Tuberty, S.R., McKenney, C.L., Sales, D., Del Valls, T.A., 2005. Effects of cadmium and zinc on *Procambarus clarkii*: simulation of the Aznalcollar mining spill. Scienc Mar. 31, 197–202.

Maynard, D.M., 1960. Circulation and heart function. In: Waterman, T.H. (Ed.), The Physiology of Crustacea, Volume: Metabolism and Growth. Academic Press, New York, pp. 161–226.

McIntyre, J. K., Beauchamp, D. A., 2007. Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. Science of the Total Environment 372, 571-584.

McLaughlin, P.A., 1983. Internal anatomy. In: Mantel, L.H. (Ed.), Internal Anatomy and Physiological Regulation, Volume 5. In: Bliss, D.E. (Ed.), Biology of Crustacea. Academic Press, New York, pp. 1–53.

McMahon, B.R., Burnett, L.E., 1990. The crustacean open circulatory system: a reexamination. Physiological Zoology 63 (1), 35–71.

McMahon, B.R., 2012. Comparative evolution and design in non-vertebrate cardiovascular systems. In: Sedmera, D., Wang, T. (Eds.), Ontogeny and Phylogeny of the vertebrate heart. Springer, New York, pp. 1-34.

Mirenda, R.J., 1986. Acute toxicity and accumulation of zinc in the crayfish, *Orconectes virilis* (Hagen). Bull. Environ. Contam. Toxicol. 37, 387-394.

Morris, S., van Aardt, W.J., Ahern, M.D., 2005. The effect of lead on the metabolic and energetic status of the Yabby, *Cherax destructor*, during environmental hypoxia. Aquatic Toxicology 75, 16–31.

Naqvi, S.M., Howell, R.D., 1993. Toxicity of Cadmium and Lead to Juvenile Red Swamp Crayfish, *Procambarus clarkii*, and Effects on Fecundity of Adults. Bull. Environ. Contam. Toxicol. 51, 303-308.

Nathaniel, T.I., Huber, R., Panksepp, J., 2012. Repeated cocaine treatments induce distinct locomotor effects in Crayfish. Brain Research Bulletin 87, 328– 333.

Nriagu, J.O., Pacyna, J.M., 1988., Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 333, 134-139.

Panksepp, J.B., Huber, R., 2004. Ethological analyses of crayfish behavior: a new invertebrate system for measuring the rewarding properties of psychostimulants. Behav. Brain Res. 153, 171–180.

Parks, J.W., Curry, C., Romani, D., Russell, D.D., 1991. Young northern pike, yellow perch and crayfish as bioindicators in a mercury contaminated watercourse. Environmental Monitoring and Assessment 16, 39-73.

Paul, E.A., Simonin, H.A., 2006. Toxicity of Three Mosquito Insecticides to Crayfish. Bull. Environ. Contam. Toxicol. 76, 614–621.

Pautsina, A. 2015. Noninvasive crayfish cardiac and behavioral activities monitoring system. Dissertation, University of South Bohemia, 168 pp.

Pautsina, A., Kuklina, I., Stys, D., Cisar, P., Kozák, P. 2014. Noninvasive crayfish cardiac activity monitoring system. Limnol. Oceanogr.: Methods 12, 670–679.

Pennuto, C.M., Lane, O.P., Evers, D.C., Taylor, R.J., Loukmas, J., 2005. Mercury in the Northern Crayfish, *Orconectes virilis* (Hagen), in New England, USA. Ecotoxicology 14, 149–162.

Rahmanpour, S., Ashtiyani, S.M.L., Ghorghani, N.F., 2016. Biomonitoring of heavy metals using bottom fish and crab as bioindicators species, the Arvand River. Toxicology and Industrial Health 32 (7), 1208-1214.

Randall, D.J., 1970. The circulatory system. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology. New York: Academic, pp. 133–172.

Reddy, P.S., Devi, M., Sarojini, R., Nagabhushanam, R., Fingerman, M., 1994. Cadmium chloride induced hyperglycemia in the red swamp crayfish, *Procambarus clarkii*: possible role of crustacean hyperglycemic hormone. Comp. Biochem. Physiol. 107C (1), 57-61.

Reddy, P.S., Tuberty, S.R., Fingerman, M., 1997. Effects of cadmium and mercury on ovarian maturation in the red swamp crayfish, *Procambarus clarkii*. Ecotoxicology and environmental safety 37, 62-65.

Reiber, C.L. 1997. Ontogeny of cardiac and ventilatory function in the crayfish *Procambarus clarkia*. American Zoologist 37, 82-91.

Rice, C.D., Kergosien, D.H., Adams S. M., 1996. Innate immune function as a bioindicator of pollution stress in fish. Ecotoxicology and environmental safety 33, 186-192.

Robinson, C.A., Thom, T.J., Lucas, M.C., 2000. Ranging behavior of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. Freshwater Biol. 44, 509–521.

Sandeman, D.C., Sandeman, R.E., 1998. Orthodromically and antidromically evoked local field potentials in the crayfish olfactory lobe. The Journal of Experimental Biology 201, 1331-1344.

Schapker, H., Breithaupt, T., Shuranova, Z., Burmistrov, Y., Cooper, R.L., 2002. Heart and ventilatory measures in crayfish during environmental disturbances and social interactions. Comp. Biochem. Phys. 131, 397–407.

Schilderman, P.A.E.L., Moonen, E.J.C., Maas, L.M., Welle, I., Kleinjans, J.C.S., 1999. Use of Crayfish in Biomonitoring Studies of Environmental Pollution of the River Meuse. Ecotoxicology and Environmental Safety 44, 241-252.

Sherba, M., Dunhman, D.W., Harvey, H.H., 2000. Sublethal copper toxicity and food response in the freshwater crayfish *Cambarus bartonii* (Cambaridae, Decapoda, Crustacea). Ecotoxicol. Environ. Saf. 46, 329–333.

Simon, O., Boudou, A., 2001. Simultaneous experimental study of direct and direct plus trophic contamination of the crayfish *Astacus astacus* by inorganic mercury and methylmercury. Environmental Toxicology and Chemistry 20 (6), 1206–1215.

Simon, O., Floriani, M., Camilleri, V., Gilbin, R., Frelon, S., 2013. Relative importance of direct and trophic uranium exposures in the crayfish *Orconectes limosus*: implication for predicting uranium bioaccumulation and its associated toxicity. Environmental Toxicology and Chemistry 32 (2), 410–416.

Soedarini, B., Klaver, L., Roessink, I., Widianarko, B., van Straalen, N.M., van Gestel, C.A.M., 2012. Copper kinetics and internal distribution in the marbled crayfish (*Procambarus* sp.). Chemosphere 87, 333-338.

Spicer, J.I., 2001. Development of Cardiac Function in Crustaceans. American Zoologist 41, 1068–1077.

Straus, D.L., Meinelt, T., Farmer, B.D., Beck, B.H., 2012. Acute toxicity and histopathology of channel catfish fry exposed to peracetic acid. Aquaculture 342-343, 134-138.

Strickman, R.J., Mitchell, C.P.J., 2017. Methylmercury production and accumulation in urban stormwater ponds and habitat wetlands. Environmental Pollution 221, 326-334.

Styrishave, B., Rasmussen, A.D., Depledge, M.H., 1995. The influence of bulk and trace metals on the circadian rhythm of heart rates in freshwater crayfish, *Astacus astacus*. Mar. Pollut. Bull. 31, 87–92.

Suzuki, K., Takagi, T., Hiraishi, T., 2003. Video analysis of fish schooling behavior in finite space using a mathematical model. Fisheries Research 60, 3–10.

Svecevicius, G., Syvokiene, J., Stasiunaite, P., Mickeniene, L., 2005. Acute and Chronic Toxicity of Chlorine Dioxide (ClO_2) and Chlorite (ClO_2^-) to Rainbow Trout (*Oncorhynchus mykiss*). Environ. Sci. Pollut. Res. 12 (5), 302-305.

Taylor, R.M., Watson, G.D., Alikhan, M.A., 1995. Comparative sub-lethal and lethal acute toxicity of copper to the freshwater crayfish, *Cambarus robustus* (Cambaridae, Decapoda,

Crustacea) from an acidic metal-contaminated lake and a circumneutral uncontaminated stream. Water Research 29, 401-408.

Terwilliger, N.B., Ryan, M., 2001. Ontogeny of Crustacean Respiratory Proteins. American Zoologist 41, 1057-1067.

Tkachenko, H., Kurhaluk, N., Grudniewska, J., 2015. Biomarkers of oxidative stress and antioxidant defences as indicators of different disinfectants exposure in the heart of rainbow trout (*Oncorhynchus Mykiss* Walbaum). Aquaculture Research 46, 679-689.

Torreblanca, A., Ramo J. D., Diaz-Mayans, J., 1991. Effects of cadmium on the biochemical composition of the freshwater crayfish *Procambarus clarkii* (Girard, 1852). Bull. Environ. Contam. Toxicol. 47, 933-938.

Triebskorn, R., Adam, S., Casper, H., Honnen, W., Pawert, M., Schramm, M., Schwaiger, J., Kohler, H.-R., 2002. Biomarkers as diagnostic tools for evaluating effects of unknown past water quality conditions on stream organisms. Ecotoxicology 11, 451-465.

Tsuchida, Y., Hama, N., Takahata, M., 2004. An optical telemetry system for underwater recording of electromyogram and neuronal activity from non-tethered crayfish. J. Neurosci. Meth. 137, 103–109.

Udalova, G.P., Khodasevich, S.V., Sladkova, S.V., Ivanov, A.V., Rymsha, V.A., 2009. Study of circadian activity in the crayfish *Pontastacus Leptodactylus* during their multimonth maintenance in the river water flow. J. Evol. Biochem. Phys. 45, 372-381.

Van der Schalie, W.H., Shedd, T.R., Knechtges, P.L., Widder, M.W., 2001. Using higher organisms in biological early warning systems for real-time toxicity detection. Biosensors & Bioelectronics 16, 457–465.

Velisek, J., Kouba, A., Stara, A., 2013. Acute toxicity of triazine pesticides to juvenile signal crayfish (*Pacifastacus leniusculus*). Neuroendocrinology Letters 34 (2), 31-36.

Vogt, G., 2002. Functional anatomy. In: Holdich, D.M. (Ed.), Biology of Freshwater Crayfish. Backwell Science Ltd., Oxford, pp. 53-151.

Waye, A., Trudeau, V.L., 2011. Neuroendocrine disruption: more than hormones are upset. Journal of Toxicology and Environmental Health, Part B 14, 270-291.

Webb, M.H., Richardson, A.M.M., 2004. A radio telemetry study of movement in the giant Tasmanian freshwater crayfish *Astacopsis gouldi*. Freshwater Crayfish. 14, 197–204.

WHO (World Health Organization), 2001. Depleted Uranium: Sources, exposure and health effects. Geneva, Department of protection of the human environment. WHO 12, 964-969.

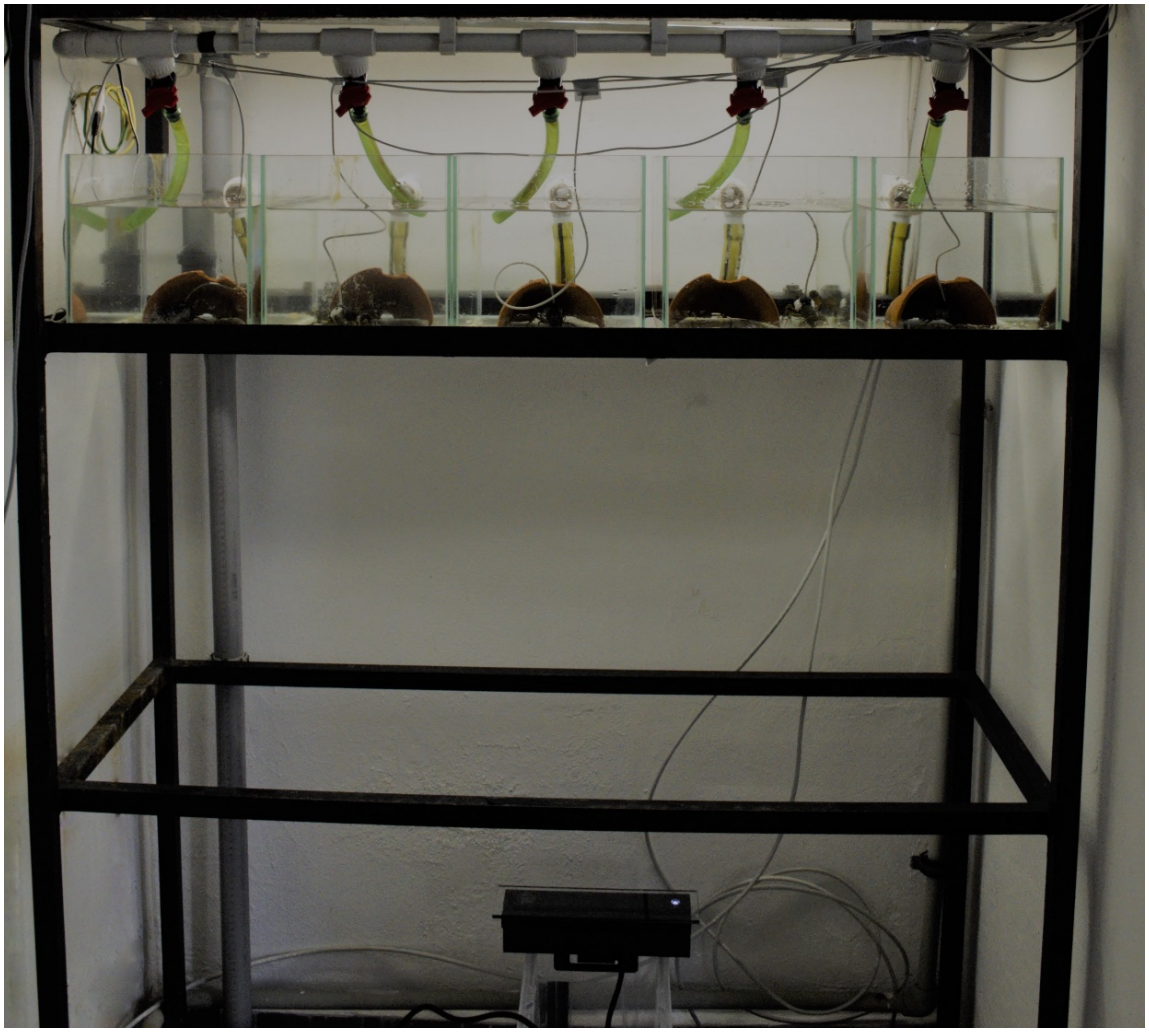
Wolf, M.C., Moore, P.A., 2002. Effects of the herbicide metolachlor on the perception of chemical stimuli by *Orconectes rusticus*. Journal of the North American Benthological Society 21(3), 457–467.

Yonkos, L.T., Fisher, D.J., Wright, D.A., Kane, A.S., 2000. Pathology of fathead minnows (*Pimephales promelas*) exposed to chlorine dioxide and chlorite. Marine Environmental Research 50, 267-271.

8 Appendixes

Appendix 1: Crayfish *Pacifastacus leniusculus* with attached sensor on the carapace.

Appendix 2: Five crayfish *Pacifastacus leniusculus* with attached sensors in water-flow aquariums with ClO_2 concentrations during monitoring process. The motion detection module is located at the lower part of the picture.



9 Abstract

Effect of hygienically treated water on crayfish heart rate and their subsequent mortality

The study is focused on the evaluation of crayfish physiological reaction on hygienically treated water with chlorine dioxide (ClO_2). A patented non-invasive monitoring system was used for observation of crayfish cardiac and locomotor activity. Monitoring was conducted from February to August 2017 under conditions of private commercial enterprise "Pivovar Protivín" in Czech Republic. Adult individuals of signal crayfish, *Pacifastacus leniusculus*, were kept separately in water-flow aquariums directly after the water treatment device producing ClO_2 in concentration from 0.01 to 0.29 $mg \cdot l^{-1}$. Observed crayfish response to the disinfectant varied among individuals which could be explained by a different functional state and individual reaction on stimuli. Diurnal rhythm of some crayfish was disturbed even at a lower concentrations of chlorine dioxide (0.01-0.2 $mg \cdot l^{-1}$), while higher concentrations affected all animals. In addition to that, higher levels of chlorine dioxide (> 0.2 $mg \cdot l^{-1}$) significantly increased mortality. Maximum concentrations (0.2-0.29 $mg \cdot l^{-1}$) were observed 28 times in total during 202 days of monitoring, which resulted in 25 mortality cases occurred several days after exposure. In average, mortality of crayfish occurred three-four weeks after stocking to the experimental system. Possible lethal concentration of ClO_2, which caused animal mortality, is > 0.2 $mg \cdot l^{-1}$. Results suggested that crayfish exposure to ClO_2, obviously, negatively affect their physiological processes; however, further studies are needed to examine specific effects of chlorine dioxide on internal organs of crayfish.

Key words: cardiac activity, signal crayfish, chlorine dioxide, *Pacifastacus leniusculus*, disinfection, circadian rhythm, water treatment

10 Abstract in Czech

Vliv hygienicky ošetřené vody na srdeční frekvenci raka a jejich následnou mortalitu

Cílem této práce bylo vyhodnocení fyziologické odezvy u raků nacházejících se ve vodě bezprostředně po hygienickém ošetření pomocí oxidu chloričitého (ClO_2). K zaznamenání pohybové a srdeční aktivity byl použit patentovaný neinvazivní monitorovací systém. Monitorování probíhalo od února do srpna 2017 v provozních podmínkách soukromého podniku "Pivovar Protivín, a.s." v České republice. Dospělí jedinci raka signálního *Pacifastacus leniusculus*, byli individuálně drženi v průtočných akváriích nacházejících se za vyústěním ze zařízení k hygienickému ošetření vody produkujícím ClO_2 v koncentraci od 0,01 do 0,29 $mg \cdot l^{-1}$. Pozorovaná reakce raků na dezinfekční prostředek se u jednotlivců lišila, což lze vysvětlit odlišným funkčním stavem a individuální reakcí na podněty. Denní rytmus některých raků byl narušen i při nejnižších koncentracích ClO_2 (0,01-0,2 $mg \cdot l^{-1}$), zatímco vyšší koncentrace (> 0,2 $mg \cdot l^{-1}$) ovlivnila všechny jedince a výrazně zvýšila mortalitu. V průběhu celého sledování byli raci vystaveni maximálním koncentracím (0,2-0,29 $mg \cdot l^{-1}$) dvacet osmkrát, kdy došlo po několika dnech k úmrtí celkově u 25 jedinců. V průměru raci uhynuli 3-4 týdny po umístění do experimentálního systému. Výsledky naznačují, že vystavení raků oxidu chloričitému ovlivňuje jejich fyziologii, nicméně je zapotřebí dalšího studia ke zjištění vlivu oxidu chloričitého na vnitřní orgány raků.

Klíčová slova: srdeční aktivita, signální rak, oxid chloričitý, *Pacifastacus leniusculus*, dezinfekce, cirkadiánní rytmus, úprava vody