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Fakulta rybnářství
a ochrany vod
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of Waters

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Crayfish heart rate monitoring with an accelerometer

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Abstract

Crayfish are widely used as a bioindicator of water pollution levels and as a model organism to study environmental changes. Existing non-invasive systems for water pollution detection using the crayfish use the optical principle of heart rate monitoring. The aim of this study is to develop an alternative method to crayfish heart rate monitoring which solves the problems associated with the current methods. The introduced solution is based on the use of crayfish vibration measurement using an accelerometer. The method enables us to determine the heart rate and provide a quantitatively similar signal to the existing systems. The advantage of this method is the direct detection of crayfish locomotor activity from a signal measured by one sensor only; optical sensors require an additional sensor for crayfish motion detection. The method is an alternative to the existing methods for water pollution detection and ethological experiments.

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1. Introduction

Bioindication is one of the methods of determining the state of the environment and revealing positive or negative changes therein (Bartram et al. 1996, Gunatilaka and Diehl 2001, Parmar and Rawtani 2016). The main advantage of bioindicators is their rapid (seconds), non-specific detection of low concentrations of pollutants. This is due to the high sensitivity and rapid reaction time of the organisms used. Thus, compared to standard chemical analysis methods, which are specific but time-consuming, bioindicators are used as early detection systems. Water quality monitoring is one of the main areas where bioindicators are applied. Crayfish, as a result of their high sensitivity to changes in the aquatic environment, especially pollution, and thanks to the simplicity of monitoring them in experimental conditions, are widely used for biomonitoring of water quality (Kholodkevich et al. 2008, Kozák et al. 2015, Kuklina et al. 2013, Kuklina 2014 et al. 2014). Additionally, the simplicity of their nervous system compared to vertebrates makes the crayfish model useful for studying basic behavior (Li et al. 2000). The basic principle of crayfish use for bioindication is the measurement of crayfish heart activity. There are several systems for monitoring the cardiac activity of crayfish for the detection of various chemicals (Bini and Chelazzi 2006, Kouba et al. 2010, Kozák et al. 2011) and various physiological states (Fedotov et al. 2006, Cooper et al. 2011).

A crayfish's heartbeat activity provides a general indication of the status of the crayfish's metabolism (Depledge and Galloway 2005). A change in this activity can signal the integrated impact of natural and/or anthropogenic stressors and may reflect the availability of energy needed for normal life, growth, and reproduction of crayfish (Depledge and Galloway 2005). The detection of crayfish water pollutants is based on a correlation between crayfish heart activity and environmental pollutants (Depledge and Andersen 1990). A common approach is heart rate frequency analysis. In other words, the beats per minute (bpm) is a critical parameter of the cardiac activity needed to successfully determine the presence of pollutants or physiological states. Previous studies have shown the reaction of the crayfish heart system to selected chemical agents in water, such as potassium nitrate and ammonium phosphate (Kholodkevich et al. 2008), sodium chloride (Styrishave et al. 1995; Kozak et al. 2009), ammonia (Bloxham et al. 1999), and chlorides and nitrites together (Kozák et al. 2011). Several authors have described the results of their studies on crayfish cardiac activity in various physiological states (Fedotov et al. 2006; Cooper et al. 2011).

Monitoring of the cardiac activity of crayfish is carried out by the indirect measurement of the contractions of the crayfish's heart. There are two basic principles used in various chemical detection studies: The principle of invasive monitoring of heart area resistance changes by implanted electrodes (Pollard and Larimer 1977, Li et al. 2000, Schapker et al. 2002); and the non-invasive monitoring of changes in the volume of blood in the heart by reflected infrared radiation from the blood in the heart (Depledge and Andersen 1990, Fedotov et al. 2000, Burnett 2013) using a sensor or light guides placed on the crayfish's carapace.

The disadvantage of the invasive approach is the need to implant electrodes into the body of the crayfish, which is a source of stress and can cause injury and unnatural behavior. The non-invasive method of measuring the amount of light reflected from the blood in the crayfish heart after passing through the carapace does not require direct intervention in the crayfish's body. However, it uses a sensor that has to be firmly attached to the crayfish's body by means of a saddle or additional brackets. The CAPMON system proposed by Depledge and Andersen (1990) and the NICCAM system proposed by Pautsina (Pautsina et al. 2014) utilize a transducer consisting of an infrared (IR) light-emitting diode (LED) and a phototransistor

fixed on the animal's dorsal carapace. The signal measured by the sensor provides indirect information about the heartbeat frequency. Another photoplethysmograph system proposed by Fedotov et al. (2000) is based on the same operating principle as the CAPMON system but utilizes optical fibers as light guides between the crayfish and an externally placed IR semiconductor laser and photo receiver. The disadvantage of this system is the need to properly position the sensor on the crayfish carapace to measure the reflected light from the heart as well as the accurate fixing of the sensor.

The crayfish heartbeat frequency is not only correlated with the water pollution levels but is also highly correlated with the crayfish movement activity (Pautsina et al. 2014) which can mask the response of the crayfish to changes in the water quality. Therefore, the existing systems use additional methods to detect crayfish locomotor activity through an additional sensor or camera. These sensors must be synchronized with the heartbeat-monitoring sensor to allow for them to be disabled during signal analysis for water quality monitoring.

In this study, we introduce an alternative method of crayfish heartbeat monitoring based on the measurement of the crayfish body vibrations using an accelerometer. The method enables us to qualitatively measure similar signals to those measured by the existing systems; however, our system has a lower sensitivity to sensor placement and the possibility of crayfish movement detection from the measured signal.

2 Material and methods

2.1 Crayfish and conditions

The experimental data for the assessment of the system and the software algorithms were obtained from signal crayfish (*Pacifastacus leniusculus*), hand-collected/captured in the summer of 2017 from Křesanovský brook (385 01 - 49.060945, 13.756824), a non-polluted brook near (Šumava National Park) Vimperk, the South Bohemian region, the Czech Republic. After capture, they were transferred for pre-experimental acclimatization to the recirculation system in the Faculty of Fisheries and Protection of Waters. One adult male crayfish was used for the experiments (total length: 103 mm; carapace length: 72 mm).

The experiments were conducted in July 2018. For acclimatization, the crayfish was maintained for 2 weeks in an individual 20 L glass aquarium. The experiments were performed in the experimental 5 L aquarium.

2.2 Data acquisition

The crayfish was able to move freely in the experimental aquarium filled with water. The aquarium was placed on a piece of foam with a width of 10 cm to avoid any transmission mechanical vibrations. A miniature, lightweight (1.22 gm) ceramic shear Integrated Circuit-Piezoelectric (ICP) accelerometer PCB, model 352A74, with a sensitivity of 10.71 mV/(m/s²) was attached to the crayfish's back, positioned near its heart, with an elastic band. The rubber band was placed in front of the last pair of legs. The

accelerometer signal was amplified using a conditioning amplifier, ICP Signal Conditioner PCB 482C16, whose gain was set to 100. The signal was acquired using an RME Fireface 400 sound card and a personal laptop. The sampling rate of the sound card was set to 32 kHz. A schematic representation of the measurement setup is depicted in **Error! Reference source not found.**. A picture of the crayfish with the attached accelerometer is shown in **Error! Reference source not found.**.

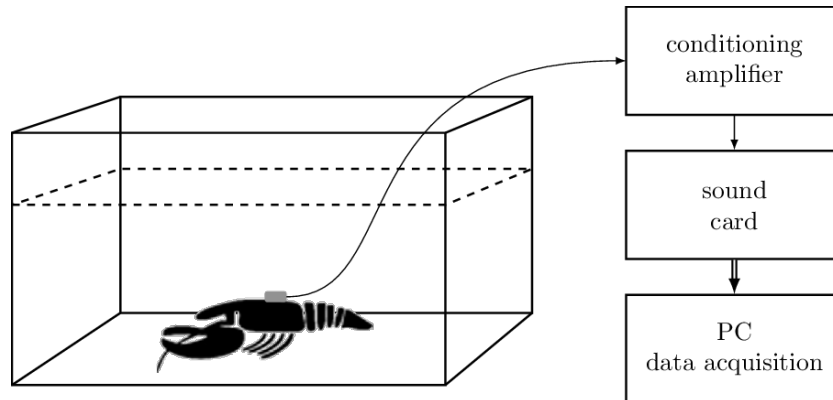


Figure 1: Schematic representation of the measurement setup. The accelerometer is placed on the crayfish's back, attached using a rubber band.



Figure 2: Crayfish with the accelerometer attached to it.

Experiment conditions

The accelerometer-based heart rate measurement method was tested using the following experiment, which aims to take advantage of the relationship between crayfish stress levels and cardiac activity (Depledge & Galloway, 2005). The crayfish was first removed from the cultivation aquarium and the accelerometer was attached to its back. Although this procedure only took about a dozen seconds, it was long enough to produce high levels of stress in the crayfish and increase its cardiac activity. The crayfish was then released into the water-filled experimental aquarium and left alone in a quiet room (without stress stimuli) for three hours. During this long period of time without stress, the cardiac activity of the crayfish was expected to decrease. A 60-second interval was recorded every 10 minutes for further processing. After three hours, its stress levels were increased by a small amount by tapping on the aquarium wall. Finally, stress was increased again by removing the crayfish from the aquarium and putting it back in place. After each stress event, a 60-second interval of accelerometer signal was recorded.

2.3 Data processing

Each recorded 60-second signal was manually searched to select a 10-second segment in which the crayfish had not moved and in which the heartbeat signal was visible (see **Error! Reference source not found.**). The mathematical–statistical autocorrelation function was used to estimate the heart rate in each selected 10-second segment of the acceleration signals (Favret & Caputo, 1966; Semmlow, 2008).

An example of an accelerometer signal is shown in **Error! Reference source not found.**. In this signal, two distinct time-segments can be recognized. The first segment, from 0 to 15 seconds, shows the crayfish's heartbeat, which indicates about 18 repetitive beats in the first 10 seconds corresponding to a heart rate of 108 beats per minute. In the second segment, the signal is much stronger and less periodic. This part of the signal corresponds to the movement of the crayfish and is distinguishable from the time-segment where the crayfish does not move and only the heartbeat is visible. In such an example, the first ten seconds of the signal can be selected to estimate the heart rate.

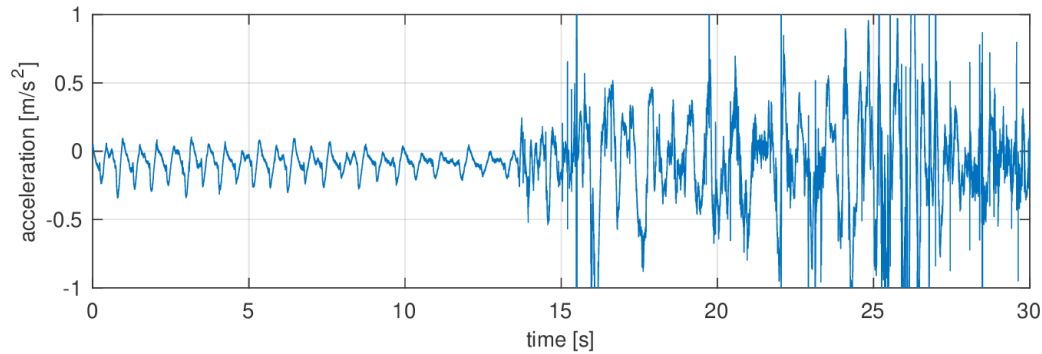


Figure 3: An example of the accelerometer signal measuring the heartbeat of the crayfish. The heart rate is visible in the first half (0–15 seconds) of the signal. The movement activity of the crayfish during the second half of the signal (15–30 seconds) is also easily detectable.

3 Results and discussion

The heart rate of the crayfish was evaluated from the accelerometer signals every 10 minutes for three hours using the autocorrelation method. The estimated heart rate is shown in **Error! Reference source not found.** as a function of time. First, the accelerometer was attached to the crayfish, then the crayfish was released into the tank filled with water, and the measurement was started. The estimated heart rate of the crayfish at the beginning of the measurement was very high (155 bps) due to the stress caused by the accelerometer fixation procedure. During the next three hours, the crayfish's heart rate was monitored every 10 minutes. As the crayfish was left to calm, without any stress stimuli, the heart rate decreased from its initial 155 bpm to values below 100 bpm (blue circles). Note that some measurement points were skipped, this is due to crayfish heart rate monitoring being impossible while the crayfish is moving. As shown in **Error! Reference source not found.**, when the crayfish is moving, the heartbeat signal is masked by the signal corresponding to the motion activity, and the heart rate is almost impossible to extract. After the three hours, the walls of the aquarium were again tapped to increase the crayfish's stress levels, resulting in an immediate increase in the heart rate (to 121 bpm) (pink X-mark). Finally, the crayfish was removed from the aquarium and put back in place in order to cause additional stress, leading to an increase in its heart rate (to 150 bpm) (red triangle).

Error! Reference source not found. shows two accelerometer signals to demonstrate the differences in the acceleration signals measured at two different heart rates. The first, associated with a heart rate of 138 bpm (bottom left figure) contains between four and five cycles in the two-second window, while the one associated with a heart rate of 99 bpm (bottom right figure) contains a little over three cycles.

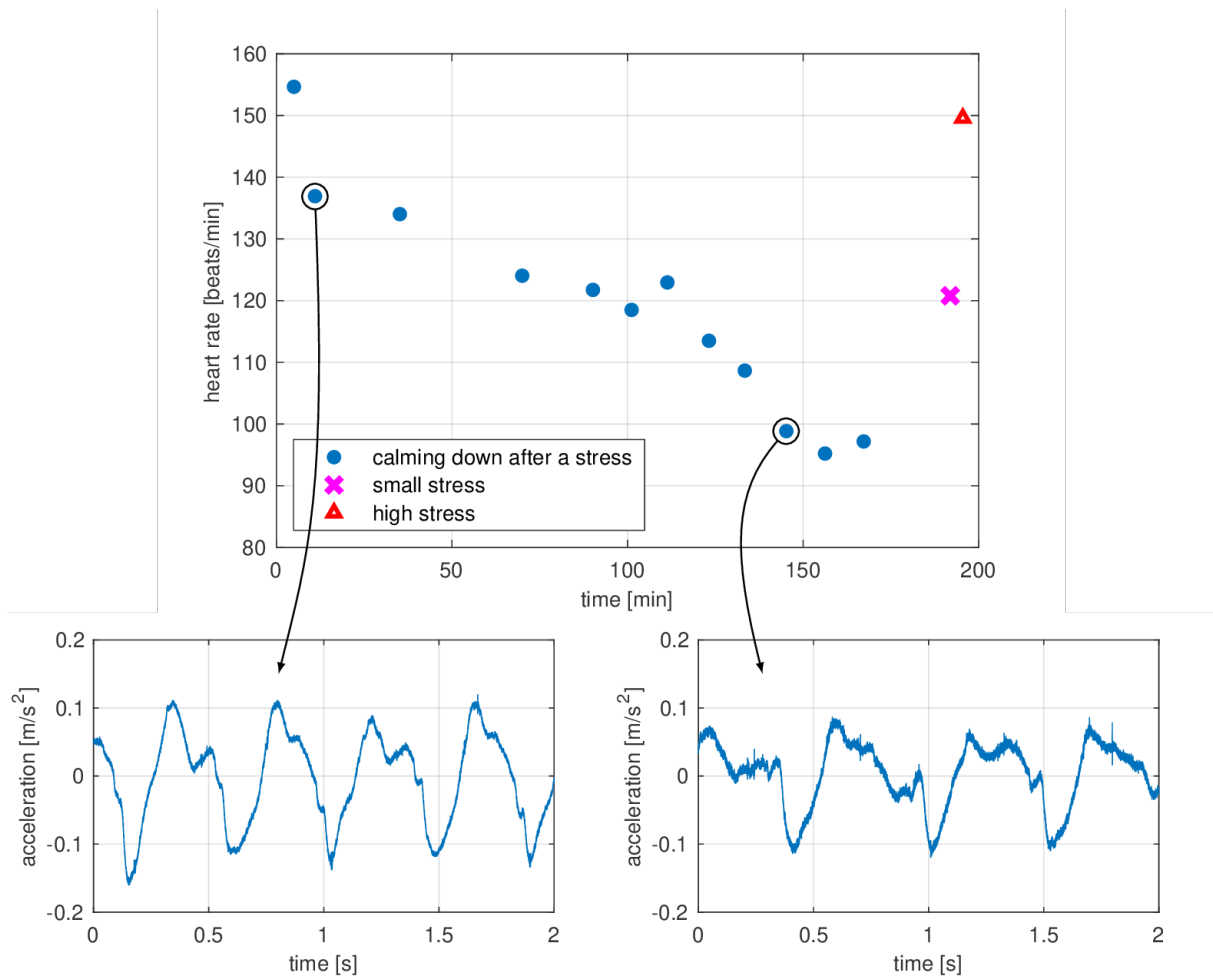


Figure 4: Crayfish heartbeat estimated from the accelerometer signals. After a stress event at time zero (155 bpm) the crayfish calmed down, and its heart rate decreased to under 100 bpm. Small stress and high stress events after 180 minutes increased the heart rate. Two 2-second accelerometer signal segments are depicted for two different heart rates.

The heart rate measured by the accelerometer corresponds to the heart rate of the stressed crayfish in terms of the frequency as well as the shape of the signal. Pautsina [4] described the systole and diastole phase of the heartbeat signal, which is also detectable in the signal measured by the accelerometer. The measured heartbeat frequency is correlated with the stress introduced to the crayfish, and therefore, no evaluation experiments with the optical-based systems were performed.

The experience presented above shows that measurement with an accelerometer attached to the back of a crayfish can be easily used for heart rate monitoring. Compared to other non-invasive methods based on measuring the amount of light scattered by the crayfish heart (Pautsina, Kuklina, Štys, Císař, & Kozák, 2014), this method has both several advantages and disadvantages. Firstly, since the method is based on vibration measurements, any other disturbance from outside can disturb the measurement. As shown in **Error! Reference source not found.**, the movement of the crayfish causes vibrations that are detected by

the accelerometer. On the one hand, this disrupts the heartbeat signal being measured and makes it almost impossible to detect the heart rate. On the other hand, monitoring and analysis of water pollution using crayfish cannot be carried out during their movement because the movement of crayfish influences their cardiac activity (Michael H. Depledge & Galloway, 2005). Another motion sensor (e.g., a camera) is often used to detect the movement of the crayfish and to ensure heart rate monitoring is stopped during movement. When using an accelerometer to measure crayfish activity, no other sensors are required for motion monitoring.

Heart rate monitoring using the existing non-invasive methods is sensitive to the positioning of the sensor on the crayfish back. Different placements of the accelerometer on the crayfish carapace were tested to determine the sensitivity of the method. The best results of accelerometer heart rate monitoring were achieved when it was placed at the end of the cephalothorax, before the beginning of the abdomen. The quality of the signal was not sensitive to the positioning of the sensor in this area. The rubber band used for the sensor attachment did not influence crayfish movement, and the crayfish quickly adapted to it.

The weight of the accelerometer used in this study was 1.22 g, which is equivalent to the weight of the standard optical sensors used in other studies (Pautsina et al., 2014). The accelerometer and conditioning amplifier used in this study were high-performance laboratory devices that were used for proof of concept. A much cheaper solution would be a MEMS accelerometer with a similar sensitivity and noise floor, and with a signal-conditioned voltage output, which can be bought for a price of about \$15. Considering the fact that there is no need to use any additional sensors to detect the crayfish motion activity, the final cost of the solution with the accelerometer comes out lower than that of optical sensors.

Conclusions

The main goal of this study was to develop an alternative method of crayfish heart rate monitoring to the existing optical- and electrode-based methods. A system for non-invasive heart rate monitoring and crayfish movement detection, using only a single accelerometer sensor, was developed. The method was tested using a freely moving, stressed signal crayfish. The system provides information about the crayfish's heart rate, which is qualitatively similar to the information gathered by existing systems, as well as information about the crayfish's movement activity. The lightweight sensor does not cause any additional stress during monitoring and is not affected by varying positions on the crayfish carapace. The introduced method for heart rate measurement based on the accelerometer is an alternative approach to existing non-invasive methods and can be used for studies where both the heart rate and the crayfish locomotor activity are analyzed. Future studies will be focused on the testing of low-cost accelerometers, in a hope to make the system cost-effective and available for routine usage.

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