A portable infrared photoplethysmograph: heartbeat of *Mytilus galloprovincialis* analyzed by MRI and application to *Bathymodiolus septemdierum*

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**ABSTRACT**

Infrared plethysmogram (IR-PPG) and magnetic resonance image (MRI) of the *Mytilus galloprovincialis* heart were obtained simultaneously. Heart rate was varied by changing temperature, aerial exposure and hypoxia. Higher heart rates (35-20 beat min⁻¹) were usually observed at 20°C under the aerobic condition, and typical IR-PPG represented a single peak (peak v). The upward and downward slopes of the peak v corresponded to the filling and contracting of the ventricle, respectively. A double-peak IR-PPG was observed in a wide range of heart rates (5 to 35 beats min⁻¹) under various conditions. The initial peak v corresponded to the filling of the ventricle, and the origin of the second peak (v') varied with the heart rate. A flat IR-PPG with a noise-level represented cardiac arrest. Although large movement of the shells and the foot caused slow waves or a baseline drift of the IR-PPG, the heart rate can be calculated from the v-v' interval. Based on these results, we assembled a portable IR-PPG recording system, and measured the heartbeats of *Bathymodiolus septemdierum* (*Mytilidae*) for 24 h on a research vessel just after sampling from the deep sea, showing that IR-PPG is a noninvasive, economical, robust method that can be used in field experiments.

**KEY WORDS:** Cardiac cycle, Cardiac arrest, Arrhythmia, Bivalve, Plethysmography, MRI

**INTRODUCTION**

The heartbeat is one of the four vital signs that reflect a body in a homeostatic balance (Carroll, 2007). Changes in cardiac activity are considered as integrative measures of the physiological fitness of organisms (Hagger et al., 2008). Indeed, the physiology and pharmacology of the hearts of bivalve molluscs have been studied for many years (Bayne, 1976), investigating a variety of important factors, such as the effects of temperature (Zittier et al., 2015; Bayne et al., 1976), salinity (Sara and De Pirro, 2011), aerial exposure (Coleman and Trueman, 1971), CO₂ tension (Zittier et al., 2015) and exposure to heavy metal ions (Curtis et al., 2000; Bakhmet et al., 2012). By measuring the heartbeat in field experiments and outside of the laboratory, we may be able to clarify the vivid activity of bivalves, and furthermore, minimize the delay of starting the heartbeat measurements after sampling of bivalves.

Two invasive techniques have been used for measuring the heartbeat in bivalves: (a) direct observation of the heartbeat through a small window cut in the shell, and (b) observation of changes in impedance using a pair of small electrodes implanted along the side of the pericardium (Bayne, 1976). Laser Doppler and ultrasound imaging are essentially noninvasive techniques, and have been applied in studies of the heart of zebrafish (Sun et al., 2008). However, in bivalves, due to a thick shell wall we have to make a small hole to insert the laser probe near the heart (Lannig et al., 2008). Infrared plethysmography (Bakhmet et al., 2015; Curtis et al., 2000; Depledge and Andersen, 1990) has advantages for application in field experiments, as it is possible to conduct (a) real-time monitoring and (b) day-long continuous measurements non-invasively and also (c) because it requires only low-cost hardware (Depledge and Andersen, 1990); however, until this study, the relationship between the results obtained by infrared plethysmogram (IR-PPG) and the actual heartbeat has not been confirmed in bivalves. This is a major reason why usage of the IR-PPG is limited. In this study, the cardiac cycle of the *Mytilus galloprovincialis* was observed by infrared plethysmograph (IR-PP) and T₁-weighted gradient-echo magnetic resonance image (T₁w-MRI) simultaneously. Then, the waveform of the IR-PPG was analyzed by comparing it with the cardiac cycle determined by T₁w-MRI (Seo et al., 2014a,b). In addition, we assembled a portable IR-PPG recording system, and tried to measure the heartbeat of a deep-sea bivalve, *Bathymodiolus septemdierum* (*Mytilidae*), on a research vessel just after sampling from the deep sea.

**RESULTS AND DISCUSSION**

**IR-PPG during regular heartbeat**

Previously, Depledge proposed measuring the heart rate of the crab by using IR-PP (Depledge, 1984). Compared with direct observation of the heartbeat via a small hole drilled in the carapace, it has been confirmed that reflection of IR light decreased during the systolic phase, while reflection of the IR light increased during the diastolic phase (Depledge and Andersen, 1990). However, the IR-PPG waveform of *Mytilus galloprovincialis* varied from a single peak to a double peak, and sometimes there was a split into more than three peaks (Figs 1 and 3).

The heartbeats of 11 mussels were measured under various conditions, such as immersed, emersed, and at rapid exposure of lower temperature. Among the 139 measurement sessions, a normal
regular rhythm was observed in 64.7% of the sessions. At temperatures around 20°C, higher heart rates (35-20 bpm) were observed, and the IR-PPG usually showed a single peak consisting of a slow increase (a) and a sharp peak (v) (Fig. 1I). Compared with sagittal T1w-MRI, the upward slope of the peak v coincided with the filling of the hemolymph into the ventricle, and the downward slope of the peak v coincided with the contraction of the ventricle (Fig. 1IA). The slow increase a coincided with the filling of the hemolymph into the auricles (Fig. 1IB).

Double peak IR-PPGs were observed in a wide range of heart rates, from 35 bpm to 5 bpm. Even at a high heart rate (around 35 bpm), the peak of the IR-PPG often split into two peaks (Fig. 1II). Compared with sagittal T1w-MRI (Fig. 1II; Movie 1), the upward slope of the peak v coincided with the filling of the hemolymph into the ventricle, and the peak v coincided with the end of the filling of the hemolymph into the ventricle and the Auricle. The small peak v coincided with the ventricular contraction (Fig. 1IIB). In this case, the filling of the hemolymph into the auricles (Fig. 1IIIC) coincided with the minimum of the IR-PPG waveform.

The intermediate heart rates (10-20 bpm) were usually observed during a decrease in temperature to 10°C, as reported by Zittier et al. (2015). The IR-PPG consisted of two peaks (v, v') and a slow increase (a) (Fig. 1III). Compared with transverse T1w-MRI (Fig. 1III; Movie 2), the upward slope of the peak v started from the opening of the AV valve (Fig. 1IIID), and the peak v coincided with the end of the filling of the hemolymph into the ventricle and auricles (Fig. 1IIIA). The second peak (v') was delayed, appearing at the start of ventricular contraction (Fig. 1IIIB). The systolic period continued until just before the opening of the AV valve (Fig. 1IIID). During the systolic period not only the flow in the posterior aorta, but also the flow in the anterior oblique veins, increased significantly and the later coincided with the slow increase a of the IR-PPG waveform.

At an even slower heart rate (5 bpm), usually observed at 10°C in the emersed hypoxic condition, the separation between the peaks v and v' became significant, so that the v-v' interval became similar to the v'-v interval (data not shown). Compared with the sagittal T1w-MRI, the upward slope of the peak v started from the opening of the AV valve, and the peak v coincided with the filling of the hemolymph into the ventricle. The second peak (v') coincided with the filling of the hemolymph into the auricles.

From these results, the initial peak v corresponded to the filling of the ventricle, and the origin of the second peak (v') varied with the heart rate. Therefore, the heart rates can be calculated from the v-v' interval (Fig. 1I). Indeed, compared with the heart rates calculated from the v-v interval and that from the interval of the ventricular contraction detected by MRI, the slope approaches unity, with a high correlation coefficient (Fig. 2).
Arrhythmia, cardiac arrest and restart of the heartbeat

Arrhythmia could be detected by change of the v-v interval. Among the 139 measurements sessions, arrhythmia appeared in 16.5% of the sessions. The results obtained by MRI and IR-PPG agreed well with each other (data are not shown). Mussels spontaneously arrested or restarted heartbeat during 18.7% of the sessions. A typical case of a restart of the heartbeat after cardiac arrest (2.5 min), arrested or restarted heartbeat during 18.7% of the sessions. The results obtained by MRI and IR-PPG agreed well with each other (data are not shown). Mussels spontaneously arrested or restarted heartbeat during 18.7% of the sessions.

Fig. 2. Heart rate observed by MRI and IR-PPG. Correlation of the heart rate calculated from the v-v interval of the IR-PPG (HRppg) and that from the interval of ventricular contraction detected by MRI (HRmri). Means and s.d. of seven sessions from four mussels are shown. Data labeled #1 and #3 were obtained in the sessions shown in Fig. 1 and Fig. 3, respectively. The number (n) represents the number of heartbeats used for the calculations. R² is a coefficient of determination of the regression line estimated from the seven sessions.

Artifacts in the IR-PPG

On occasion, peaks, slow waves or a drift in the baseline appeared in the IR-PPG waveform. However, there was little change in the T1w-MRI, except for an increase of flow in the gill vessels (Fig. 3IB). An increase in the flow in vessels and the lower mantle cavity had a minimum effect on the IR-PPG (Fig. 3IC). Therefore, broad peaks, slow waves or a drift in the baseline that appeared in the IR-PPG could be assigned as artifacts. However, the sharp peaks shown during cardiac arrest (indicated by an asterisk in Fig. 3) made it difficult to discriminate between the peaks and artifacts. This is a limitation of the IR-PPG method for measuring the heartbeat, and this limitation illustrated the need for T1w-MRI to judge whether a peak represented the heartbeat or an artifact.

Measurement of the heartbeat of Bathymodiolus septendecim, a research vessel

From the basis of results shown in the above, a combination of IR-PPG and T1w-MRI allowed us to analyze IR-PPG waveforms in a wide range of heart rates in Mytilus, and it was possible to calculate the heart rate from the v-v interval. The origin of artifacts shown on the IR-PPG could be determined by using T1w-MRI; therefore, we tried to assemble a portable IR-PPG recording system for measuring heartbeat of deep sea bivalves on a research vessel.

Recording of the heartbeats of two Bathymodiolus mussels were started 4 h after sampling, and measured for 24 h. Even with the sway and vibration of the vessel, we could detect fairly stable IR-PPG. A regular heartbeat at around 7 bpm (Fig. 4C), a slow heartbeats (bradycardia) (Fig. 4D), and arrhythmia including cardiac arrest (Fig. 4E) were observed, and these seemed to be appeared at random. It is also likely that variation of heartbeats was larger than that observed at 2 weeks after the sampling (data are not shown). Bradycardia and cardiac arrest were reported in bivalves, such as Mytilus, Isognomum and Anodonta (Curtis et al., 2000; Seo et al.,
The decrease of heart rate was caused by not only the metabolic suppression by hypoxia, but also was linked to various activities of bivalves such as closure of shells and extension of the pedal (de Zwaan and Wijsman, 1976; Curtis et al., 2000; Brand, 1976). Therefore, like patients of arrhythmia, continuous monitoring of the heart rate must be useful to find factors responsible to control the heartbeat.

From results obtained by this pilot experiment, the portable IR-PPG recording system is cheap, simple and robust and can be used in field experiments. In future, we will develop an IR-PP for in situ experiment in the deep sea.

**MATERIALS AND METHODS**

**Experimental animals**

*Mytilus galloprovincialis* Lamarck 1819 were supplied by Fishing Ito Limited (Chita, Aichi, Japan). These mussels were collected from a tidal area along the shore of Nagoya harbor, on July 2015. At the laboratory, in two separate 5 liter baths, 10 mussels were kept in each bath for a week in aerated synthetic seawater (salinity 3.6%) at room temperature (20-24°C) (Seo et al., 2014b). A total of 11 mussels were used in this MRI study. The length, height and width of the mussels were 27.4±0.43 mm, 14.6±0.34 mm, 8.7±0.27 mm (means and s.e.m.), respectively.

*Bathymodiolus septemdierum* Hashimoto and Okutani 1994, a family of Mytilidae, were collected at a depth of 1182 m at a seep site around Myojin Knoll in the Northern Ogasawara Islands (32° 7.48′N, 139° 50.534′E) during the KY15-07 cruise (27 April 2015), using the remotely operated vehicle (ROV) *Hyper-Dolphin* (Dive HPD#1810), operated by the research vessel *Kaiyo* of the Japan Agency of Marine-Earth Science and Technology (JAMSTEC). Two of these mussels were used in this MRI study. The length, height and width of the mussels were 46.5 and 46.2 mm, 28.6 and 28.4 mm, 18.0 and 18.4 mm, respectively.

All of the animal experiments conducted in this study were carried out under the rules and regulations of the ‘Guiding Principles for the Care and Use of Animals’ set by the Physiological Society of Japan, and approved by the Animal Research Councils at Dokkyo University School of Medicine (#840).

**Infrared photoplethysmography**

An infrared photoplethysmograph (IR-PP) was made using an infrared-sensor (CNY70; wavelength 950 nm, Vishay, Malvern, PA, USA) and the circuit reported by Burnett (Burnett et al., 2013). The output of IR-PP was digitized and stored in a digital data recorder (MR8870, Hioki, Nagano, Japan) every 20 ms. A typical heartbeat pattern (Fig. 4 in Depledge and Andersen, 1990) was presented with this polarity; however, in order to avoid counting error of the heart rate caused by double peaks, they put an inversion circuit in their plethysmograph. Burnett also employed this inversed polarity, and the output voltage (Vi) increased when reflected light decreased (Burnett et al., 2013). Therefore, we simply re-inversed the polarity by numerical calculation: 5-Vi, since the dynamic range of the circuit is around 5 volts.
The IR-PP function was tested by an infrared (IR) pulse (1 s) driven by a block-pulse generator (Fig. S1A). We observed a slow decay, which might have been caused by transient response of the circuit and the phototransistor of CNY70. It is also true that the waveform obtained have been caused by transient response of the circuit and the
non-magnetic fiber optic probe was used for photoplethysmography (POXS-D; fiber bundle diameter: 3 mm; wavelength 940 nm, SA Instruments Inc., New York, USA). The emission of IR light and the
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Competing interests

The authors declare no competing or financial interests.

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