

Unconstrained and Noninvasive Measurement of Bioelectric Signals from Small Fish

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Abstract

Recently, the technique of fish bioassay has attracted attention as a method for constant monitoring of aquatic contamination. The respiratory rhythms of fish are considered an efficient indicator for the monitoring of water quality, since they are sensitive to chemicals and can be indirectly measured from bioelectric signals generated by breathing. However, no method has yet been established to measure signals in small free-swimming fish.

In this paper, we propose a system to measure bioelectric signals in small fish and monitor the frequency component in real time. To cover the large measurement range required in a free-swimming environment, the signals are measured using multiple electrodes. Further, the system focuses on the frequency component of the signal to assess the condition of fish using frequency analysis and a band-pass filter. Experiments were conducted with the purpose of enabling remote sensing and environment estimation. First, it was verified that the measured signals were synchronized with breathing. Then, a remote sensing experiment was performed using medaka (*Oryzias latipes*) that were allowed to swim freely in a measurement aquarium. The results confirmed that bioelectric signals synchronized with breathing could be measured in unconstrained and noninvasive conditions.

1 Introduction

Currently, incidents involving the contamination of water sources by industrial effluent are reported every year in Japan. Accordingly, the quality of tap water is monitored in water treatment plants to prevent contaminated water from being supplied to homes. In this monitoring, chemical concentration levels in water are analyzed and checked to ensure that safety standards for tap water are met. Not all tests, however, can be performed frequently because of limitations in terms of time and cost. As a result, only three items are inspected each day, and other checks are carried out just once a month [1]. Consequently, aquatic contamination may not be discovered until it causes a health

hazard after a contamination incident occurs. This situation has led the Ministry of Health, Labour and Welfare to recommend introducing the bioassay system together with chemical analysis [2].

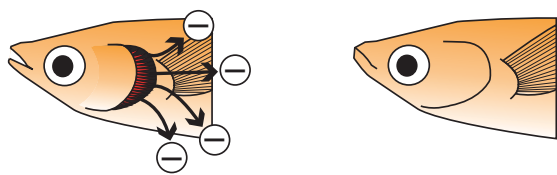
Bioassay is a method of estimating environmental changes from biological responses. In general, fish are used in the examination of water. Since it has been reported that bioelectric signals from fish are sensitive to changes [3], these signals are expected to make early detection possible, and several research projects on a bioassay system using bioelectric signals have therefore been conducted. Shedd et al. [4], for example, proposed a system that calculates ventilatory frequency from signals and evaluates aquatic contamination from changes in breathing. However, this system limits the movement range of fish to improve the quality of signal measurement, which can cause stress and influence breathing conditions. Additionally, Taue et al. [5] proposed a system using small fish in free-swimming conditions. However, the system can only assess whether fish are dead or alive, as it relies solely on the amplitude information of the signal for assessment.

The aim of this study was to develop a bioassay system using bioelectric signals from small fish in free-swimming conditions. As the first step, this paper proposes a method to measure the bioelectric signals of medaka (*Oryzias latipes*) in unconstrained and noninvasive conditions to minimize their levels of stress. Instead of amplitude information, the system utilizes frequency information that has been proven to be quite stable in free-swimming conditions.

2 Ventilatory signals

The medaka is suitable as a test fish for the bioassay system because it is relatively sensitive to chemicals, and is recommended in the OECD Guidelines for the Testing of Chemicals [6]. In our research, ventilatory signals were selected as the measurement target.

It is already known that it is possible to observe the electrical field around a fish's body by which peri-



(a) Opened gill cover condition (b) Closed gill cover condition

Figure 1: Relationship between gill cover movement and ion movement

odic potential is generated [7]. The main source of the potential difference is considered to be the ionic concentration difference between the inside and outside of the body caused by the osmotic mechanism [8].

As shown in Fig. 1(a), when the gill cover is opened, ions move to the outside of the body, generating electric potential. On the other hand, when the gill cover is closed, ionic movement is shut off (Fig. 1(b)). The potential around the fish is thus synchronized with the open-close movement of gill covers [9].

3 The ventilatory signal measurement system

The system established to measure the ventilatory signals of the medaka is shown in Fig. 2, and consists of a signal measuring part and a signal processing part. This section describes the system configuration.

3.1 Signal measuring part

The signal measuring part plays the role of inputting measured signals into a PC, and is composed of a measurement aquarium, electrodes, amplifiers and A/D converters.

Signals are measured using disposable medical electrodes (Ag-AgCl). n pairs of active electrodes (+, -) and a reference electrode (GND) are placed in the aquarium to enable differential amplification for signal denoising. Since the measured signals are faint (i.e., in μV order), they are amplified using a bioelectric amplifier (time constant: 3 [ms], high cutoff frequency: 30 [Hz]; Nihon Kohden Corporation). AD processing (sampling frequency: 1,000 [Hz]) is then conducted to input the signals into a PC using an interface module (PCI-3521, Interface Inc.).

3.2 Signal processing part

In the signal processing part, the input signals are filtered and converted into the frequency domain, and both are monitored on the PC screen.

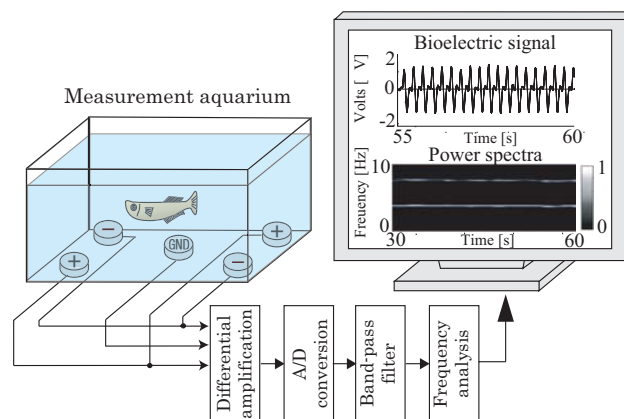


Figure 2: Structure of the measurement and signal analysis system

First, input signals are filtered by band-pass filters (low cutoff frequency: 0.053 [Hz]; high cutoff frequency: 10 [Hz]). Then, frequency analysis is conducted using an AR model, which is less influenced by unexpected noise. The AR model is given by the following equation:

$$x(n) = - \sum_{k=1}^K a(k)x(n-k) + \varepsilon(n), \quad (1)$$

where $x(n-k)$ is the measured signal and $\varepsilon(n)$ is the prediction error (white noise). This model predicts future data $x(n)$ from measured signals by appropriately adjusting AR parameter $a(k)$.

Power spectrum density (PSD) $P(f)$ is calculated for every second using an AR model of order $K = 200$ using equation (2).

$$P(f) = \frac{\sigma_{\varepsilon}^2}{\left| 1 + \sum_{k=1}^K a(k)e^{j2\pi kf} \right|^2}, \quad (2)$$

where σ_{ε}^2 is the prediction error variance. $P(f)$ is normalized in the range of 0 to 10 [Hz] using equation (3) to monitor peak frequency per unit of time.

$$P_n(f) = \frac{P(f)}{\max(P(f))} \quad : (f = [0, 10]), \quad (3)$$

Normalized PSD $P(f)$ is displayed in grayscale on the PC screen, as shown in Fig. 2. This system can measure the ventilatory signals of medaka in real time and monitor the frequency component.

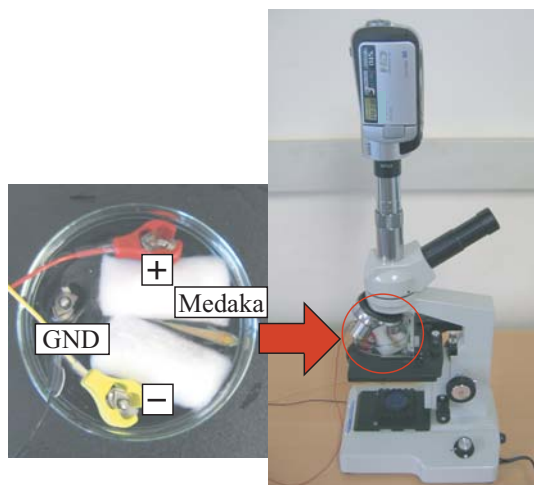


Figure 3: Experimental apparatus for the verification experiment

4 Measurement under constrained conditions

Ventilatory signal measurements of medaka were conducted under constrained conditions as a basis for later experiments in free-swimming conditions. First, synchronization between measured signals and breathing was confirmed. Then, the influence of electrode distance on the signal quality was examined to enable remote sensing. The water used for the experiment was dechlorinated ahead of time. The temperature and electrical conductivity were also measured before the experiment, and the temperature was kept constant during the testing period.

4.1 Experiment to verify ventilatory signals

The correlation between the measured signals and gill cover movement was examined to verify that the measured signals are synchronized with breathing. First, a medaka was placed on a petri dish, and its range of movement was limited using absorbent cotton as shown in Fig. 3. Then, a pair of active electrodes (+, -) and a reference electrode (GND) were placed in the petri dish for signal measurement. At the same time, the gill cover movements were recorded using a video camera (frame rate 29.97 [fps]) mounted on a microscope to quantify the movements by image analysis.

The video images were analyzed using Cosmos32 image analysis software (Library Inc.). The picture was converted into a binary image, and the area of gill cover corresponding to the number of black pixels

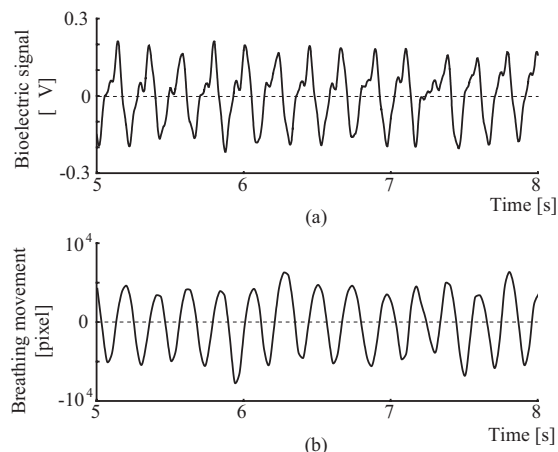


Figure 4: Examples of the results experimental verification

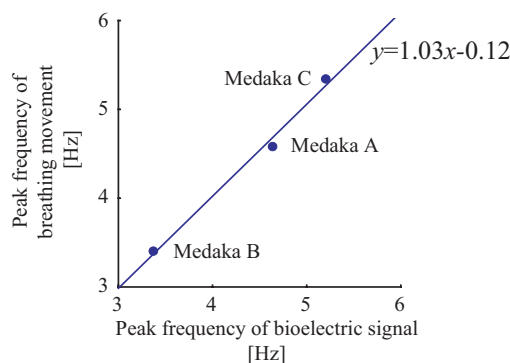


Figure 5: Relation between signal frequency and ventilatory frequency

was calculated. The same process was performed on all frames of the video, and the number of black pixels in each frame was obtained. Then, the number data were processed using a band-pass filter (low cutoff frequency 1 [Hz], high cutoff frequency 10 [Hz]), and the data thus obtained were used to define the breathing movement.

Fig. 4 shows an example of the experimental results obtained from three subjects. (a) shows a measured signal. The horizontal axis represents time, and the vertical axis is the electric potential. (b) shows the breathing movement. The horizontal axis represents time, and the vertical axis is the number of black pixels. Both the measured signal and the gill cover movements showed a periodic wave pattern, as seen in Fig. 4.

Fig. 5 shows the correlation between the peak frequencies of the signals and gill cover movements as calculated using data from a 60-second period. The result for the correlation coefficient of each point shows a value of 0.995. This high correlation verified that the

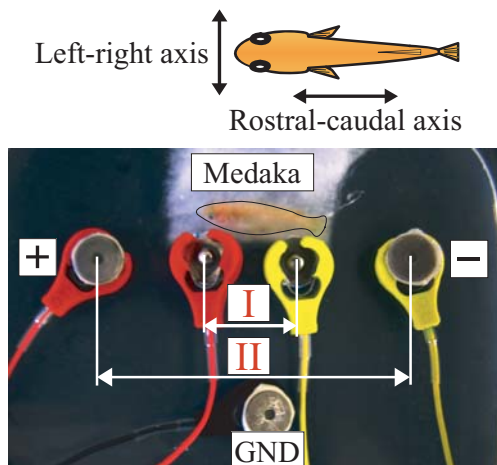


Figure 6: Electrode arrangement for the measuring experiment with different interelectrode distances

measured signals were synchronized with breathing.

4.2 The measuring experiment in different interelectrode distance

Next, we conducted a measuring experiment with different interelectrode distances to confirm the influence of this distance with medaka. The fish was constrained using absorbent cotton to confine the motion of its fins. The size of the aquarium used for measurement was 500(W)×350(D)×200(H) [mm], and two pairs of active electrodes were used, as shown in Fig. 6. Electrode pair I was attached to the fish to measure the low-noise standard signal with an interelectrode distance of 20 [mm]. The interelectrode distance of electrode pair II was changed from 40 to 300 [mm] in increments of 20 [mm]. The experiments were conducted under two sets of conditions: one with the direction of the electrode pair along the rostral-caudal axis, and the other along the left-right axis against the axis of the fish's body.

Experiments were conducted with four subjects. Figs. 7, 8 and 9 show the results when the electrode pairs were placed along the rostral-caudal axis. Fig. 7 shows an example of the ventilatory signals measured when the interelectrode distance of electrode pair II was 100 [mm]. The relationship between the interelectrode distance and the signals is shown in Fig. 8. The vertical axis denotes the ratio of effective value (V_2/V_1). V_1 and V_2 are the effective values of electrode pairs I and II. Fig. 9 shows the relationship between the interelectrode distance and the peak frequency of the signals. The differences in the signal amplitude were confirmed by the differences in distance, as shown

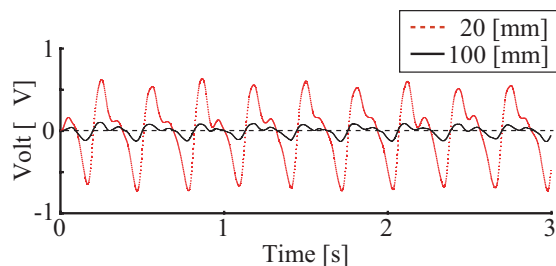


Figure 7: An example of the experimental results under constrained conditions

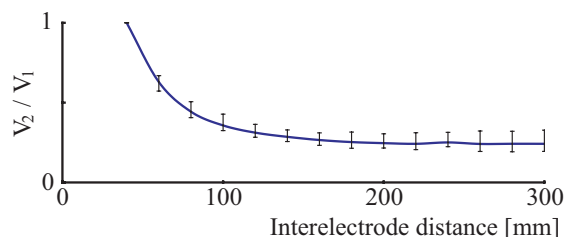


Figure 8: Effective value of measured bioelectric signals with different interelectrode distances

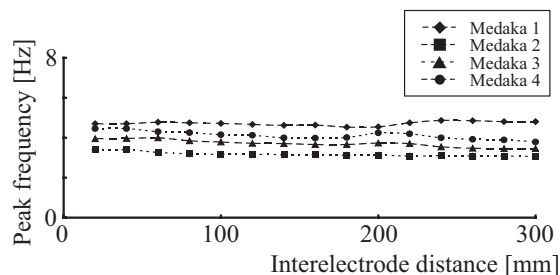


Figure 9: Peak frequency with different interelectrode distances

in Fig. 7, and the amplitude decreased as the electrode distance increased, as shown in Fig. 8. This is due to increased electrical resistance between the subject and the electrodes. In contrast, the peak frequency bore no relation to the distance, and remained almost constant as shown in Fig. 9. Similar results were obtained under the condition in which the electrode pairs were placed on the left-right axis. From the results described above, it can be considered that the information on signal frequency is more suitable for evaluating the changes in the conditions of fish than amplitude in unconstrained and noninvasive conditions, because medaka would move freely around the aquarium causing constant changes in amplitude information.

5 Measuring experiment under free-swimming conditions

It is necessary to measure ventilatory signals under no-stress conditions from the viewpoint of developing a

practical bioassay system. Accordingly, we conducted an experiment under free-swimming conditions using a medaka in a polyethylene resin measurement aquarium with dimensions 150(W) \times 100(D) \times 50(H) [mm] (Fig. 10). The size was determined based on the measurement results described in the previous section and further preliminary experimentation. Electrodes were placed on the four lower corners of the aquarium to keep the medaka between the active electrodes (+, -). The other experimental conditions were the same as those described in Section 4.2. The water temperature and electrical conductivity were 20.2 [°C] and 12.08 [μ S/mm], respectively.

5.1 Ventilatory signals under free-swimming conditions

We performed an experiment to verify the feasibility of measuring ventilatory signals from medaka allowed to swim freely using the proposed system. Fig. 11 shows an example of the experimental results. (a) shows the signals measured in the period between 300 and 310 [s]. (b) is the spectrum variation of the signals from 290 - 320 [s]. The magnitude of PSD is expressed in grayscale. (c) shows the trajectory of the medaka from 290 - 320 [s]. It was confirmed that ventilatory signals could be measured even from medaka under free-swimming conditions, as shown in Fig. 11. It was also confirmed that the amplitude of signals changed according to the position of the medaka as shown in (a), but the signal frequency was almost constant during swimming, as shown in (b). An unexpected change in frequency was recorded when the medaka rushed to the aquarium wall (310 - 320[s]). These results imply that signal frequency can be affected by mechanical stimulus and possibly utilized to monitor abnormal behavior in the subject.

5.2 Response of signals to breach exposure

A water contamination experiment was performed to confirm the response of ventilatory signals to chemical irritation. The experiment started with the same conditions as those described in the previous subsection. Then, 4 [ml] of household bleach mainly consisting of NaClO was poured into the aquarium at 180 [s].

Fig. 12 shows an example of the experimental results after the exposure. (a) shows the signals measured from 330 - 340 [s], and (b) shows the spectrum variation of the signals from 320 - 350 [s]. (c) shows the trajectory of the medaka from 320 - 350 [s]. In comparison with Fig. 11, it can be seen that both the

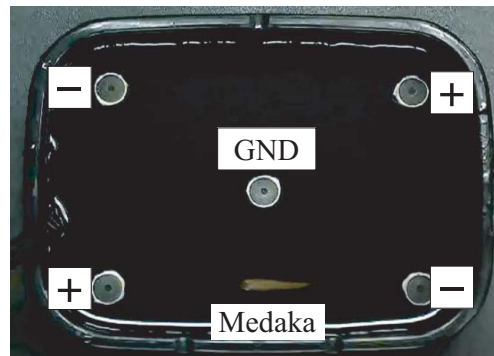


Figure 10: Measuring aquarium for the experiment under free-swimming conditions

spectrum and the amplitude of the signals showed a marked change after exposure. Note that the signal changes were not evoked by movement; the medaka stayed in the same position, as shown in Fig. 12 (c). It is therefore suggested that aquatic contamination can be detected using ventilatory signals.

6 Conclusion

In this paper, we propose a system to measure the ventilatory signals of medaka and report on the experiment conducted to measure these signals. The results confirm that the system can successfully measure signals from medaka in unconstrained and noninvasive conditions. Further, it was suggested that signal frequency could be an important factor in estimating levels of water contamination. In the future, we plan to analyze changes in the patterns of bioelectric signals in line with exposure to different toxic substances, and to develop a system that can discriminate aquatic contamination from bioelectric signals.

Acknowledgements

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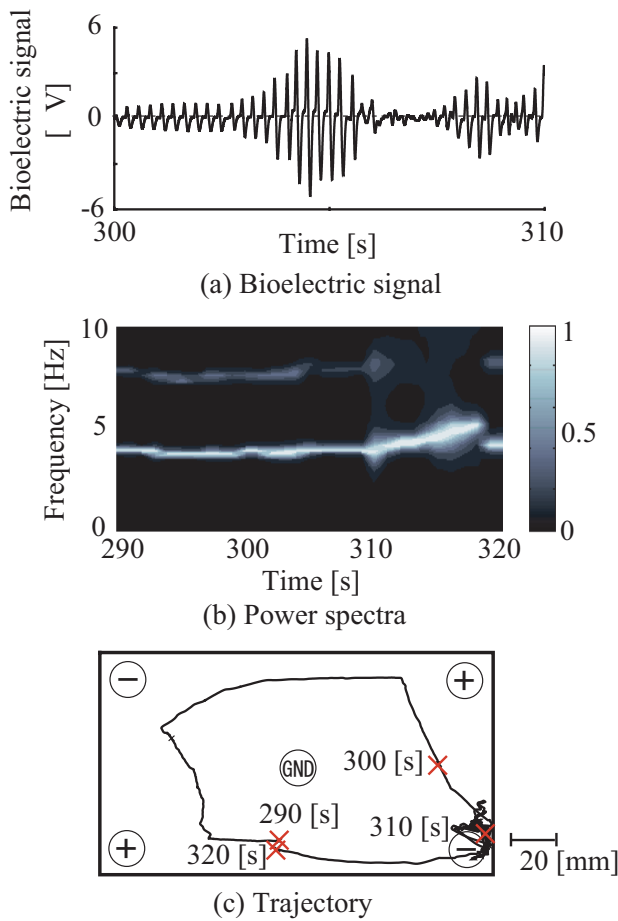


Figure 11: An example of the experimental results under free-swimming conditions

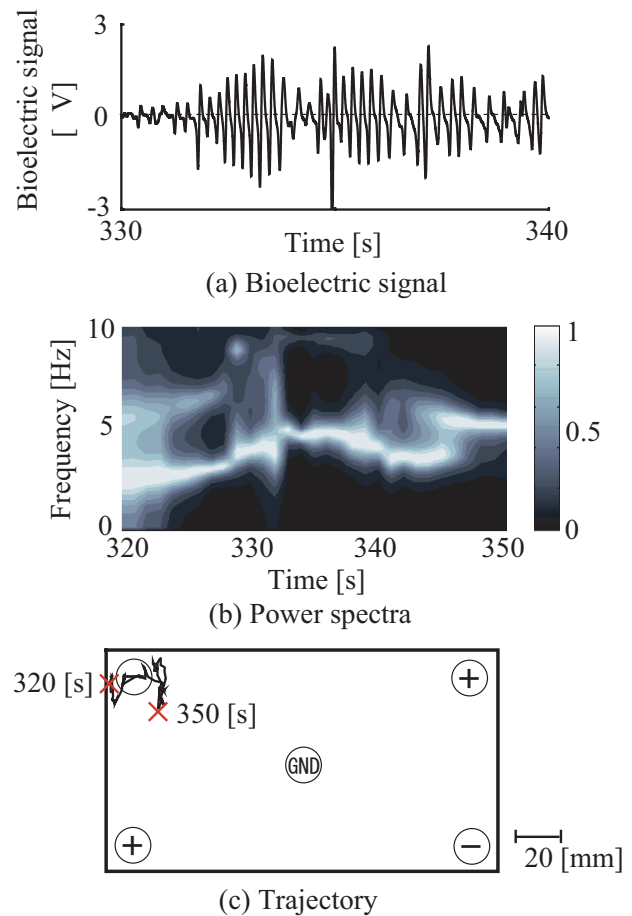


Figure 12: An example of the results from the bleach exposure experiment

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