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Research Article

Branchial Motion Assessment in Abalone Using Photoplethysmography

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The abalone, a gastropod mollusk belonging to the Haliotidae family, has been a subject of research with a focus on its behavior in response to various environmental factors, including temperature fluctuations and hypoxia. While existing studies have primarily explored abalone behavior in relation to environmental changes, there remains a significant amount to be discovered regarding their behavioral patterns. There are studies in which recordings of readings have been made (indirect methods) of the heart rate of the abalone since it is believed that it is one of the parameters affected due to the changes in temperature and dissolved oxygen in its environment. The main disadvantage of some procedures to measure heart rate in abalone is that an incision is made near the heart, through this incision the measurement is made using the photoplethysmography technique. Another parameter that is affected by these changes (temperature and hypoxia) is respiratory rate. Temperature is an important factor that can reduce oxygen solubility and thus alter abalone respiration; so in this work, we propose to use the technique of photoplethysmography: a method involving the measurement of changes in volume within a biological organ, which offers a unique opportunity to monitor these changes. We adapt this technique to measure gill movements in abalone, a critical indicator of respiratory activity in these marine mollusks. By utilizing a pulse oximetry setup and synchronous detection, we establish an innovative approach to gill movement analysis. This approach employs a pulse train and synchronous demodulation technique, the train of pulses is guided through an optical fiber to one of the respiratory orifices of the abalone to effectively detect and analyze the gill movements of abalone. Additionally, the role of frequency of its gill movements is highlighted as another critical variable of interest within the broader study of *Haliotis fulgens*, shedding light on the multifaceted nature of abalone behavior and responses to their surroundings. This research contributes to a deeper understanding of abalone physiology and behavior, offering insights into their adaptation to changing environmental conditions.

1. Introduction

Abalone holds significant commercial and economic importance in Mexico, with a particular focus on the west coast of the Baja California Peninsula [1]. This region represents the sole area within the Mexican Republic where abalone is both harvested from the wild and cultivated [2]. In recent years, there has been a noticeable decline in abalone catches. This decline can be attributed to a combination of factors, including the overexploitation of abalone fishing and the influence of temperature fluctuations resulting from changing environmental conditions [3]. These issues have raised concerns about the sustainability and long-term viability of the abalone industry in the region. Efforts to address these challenges and manage abalone resources effectively are crucial to maintain the economic and ecological balance in this area. Studies have shown that temperature is a significant factor due to the vulnerability of abalone to thermal changes and the ability of temperature to penetrate physical barriers, having potentially serious effects on the structure of all macromolecules [4]; likewise and to a lesser extent, temperature causes effects on abalone behavior, such as the selection of an ideal thermal habitat [5]. Indeed, abalone behaviors and their distribution can be significantly impacted by a range of
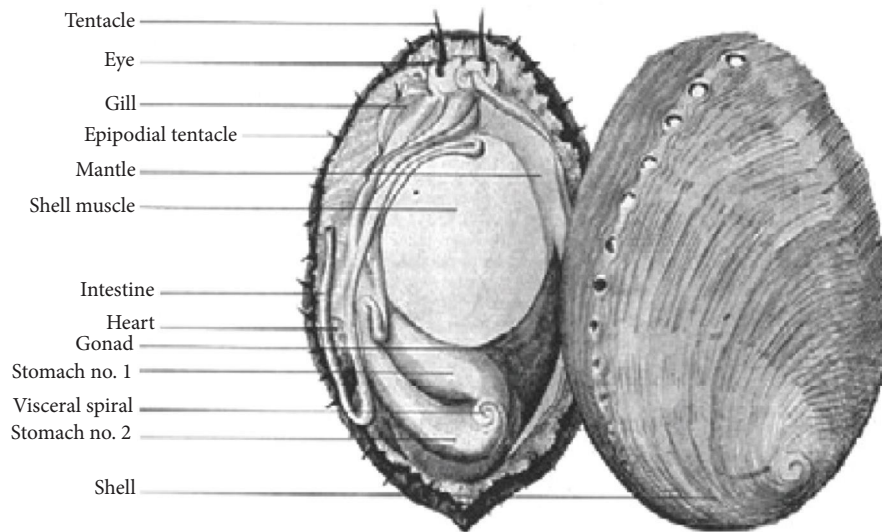
FIGURE 1: Physiological diagram of the abalone. Figure obtained from [24].

environmental factors. Deoxygenation, for instance, can have a profound effect on abalone distribution and may limit population growth, as noted by Calderón-Liévanos et al. [6]. Furthermore, various studies have explored how abalones, including other species, respond to different environmental variables. Some of these studies have investigated responses to factors such as light quality (as shown in Gao et al.'s [7] study) and food availability [8, 9]. Additionally, there have been efforts to document the causes associated with different stressors, such as high temperatures, elevated ammonium content, low levels of dissolved oxygen, and high levels of other environmental factors, [10–12]. These research efforts collectively contribute to a more comprehensive understanding of how abalone populations respond to their ever-changing environments, shedding light on the complex interplay between abalone behavior and ecological factors. However, there is a poor understanding of abalone behavior due to the lack of continuous observation of environmental variables and a lack of biological information of the species [13]. Therefore, it is important to determine the biological characteristics of abalone to describe its behavior. The blue abalone *Haliotis fulgens* is the object of this study because there are reports of environmental change effects such as detachment of the organism from its substrates and even mortality. Depledge et al. [14] proposed a noninvasive method of measuring cardiac activity using an infrared emitter (LED) and a phototransistor in selected invertebrates through an automatic computer-controlled system. Employing the same principle, but making some modifications, Chelazzi et al. [15] and Dong and Williams [16] performed measurements in tropical limpets. Through this noninvasive method, Chen et al. [17] investigated heat tolerance in abalone. Morash et al. [18] analyzed abalone exposure to stressful events. These five studies have the following characteristics: (a) they use a noninvasive method, (b) the device requires a computer to perform the measurements, and (c) an incision in the carapace (near the heart) is necessary for the interaction between the specimen's body and the sensors.

For the present study, data collection was carried out at the Centro de Investigaciones Biológicas del Noroeste (CIB-NOR), Baja California, Mexico, in collaboration with CIATEC A.C.

One of the main characteristics of the techniques used for the measurement of physiological parameters in living beings is not to discomfort or damage them or otherwise cause them the least possible discomfort, which is why the technique used par excellence is a noninvasive technique, specifically for this case is photoplethysmography.

Photoplethysmography is an optical measurement technique that is used to measure volume changes in microvascular tissue [19] and has wide clinical application, for example, pulse oximetry and cardiac pulse signaling. The principle of operation is very simple: it requires a light source placed on the skin, and once the light has interacted with the skin, either by transmission or by reflection, it is collected and processed. The interaction of light with living tissue is somewhat complex (reflection, transmission, absorption, and scattering) [20], and there are many factors that affect the amount of light received by the sensor, such as the effect of pressure exerted on the sensor [21]. Plethysmography techniques are well-known; e.g., for obtaining cardiac pulse, a single light source is used, and for pulse oximetry, two light sources at different wavelengths are needed. We propose a noninvasive device that uses the method of photoplethysmography as its operating principle, without the need to use a computer for measurement and without the need to make an incision in the abalone shell. This device works as follows: a train of pulses is generated by emitters, this signal passes through a pair of optical fibers to interact with the surface of the abalone skin, and the collected data are analyzed using the synchronous demodulation technique, due to which it is possible to recover the gill movement of the abalone [22, 23].
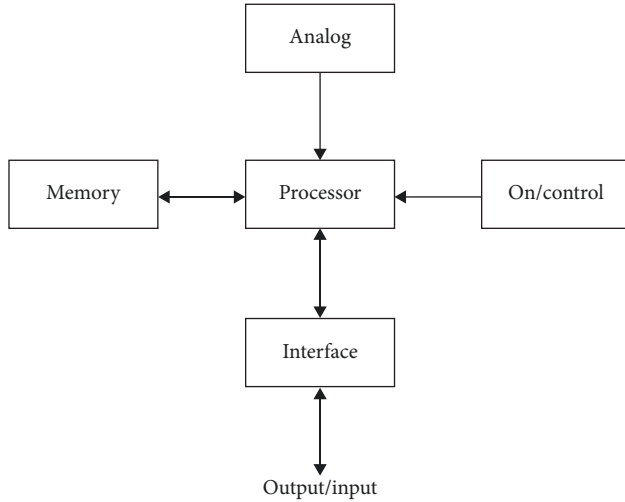
FIGURE 2: Basic diagram of the circuit used for data collection.

FIGURE 3: Basic connection diagram for the analog data collection system, where IR is an infrared LED, r is a red LED, R is the resistance, Op is the operational amplifier, and GND is a ground line.

The purpose of this research is to evaluate gill movements in abalone. The paper is organized as follows. Section 1 presents some previous studies related to the biological characteristics of abalone. Section 2 details the design of the system used to measure abalone, the processing to analyze the data generated by the system, and how it interacts with abalone. Finally, Section 3 displays the information obtained.

1.1. Abalone Structure. It is important to know the internal structure of the abalone to be able to locate the point where the measurement will be taken. Figure 1 shows its physiological structure.

The external structure (exoskeleton) has holes that allow part of the skin of the abalone to be in contact with the external environment. These holes were used to introduce optical fibers into the abalone and measure signals. In particular, data collection was performed close to the gills (Gill) of the abalone.

2. Materials and Methods

2.1. Principle of Operation. Figure 2 provides a basic flow diagram of the circuit designed for data collection in the abalone. The microprocessor (PIC16F1615), through the pulse width modulation (PWM) function, generated a pulse train at a frequency of 122 Hz. The same microprocessor controlled an on and off system of red and infrared LEDs (VSMD66694). This control allowed one LED to remain on for a period of eight pulses (data sampling period and its storage in memory) while the other remained off, and vice versa (Figure 3). The emission of the beams affected the surface of the abalone through an optical fiber. Once it interacted with the skin of the abalone, the reflected light was collected by another optical fiber and processed by a sensor (TEMD7000) connected to a transimpedance circuit (ADA4505-2ARMZ) and amplified to be converted into a digital signal. The processor sent the data to the memory (CY15V104QN).
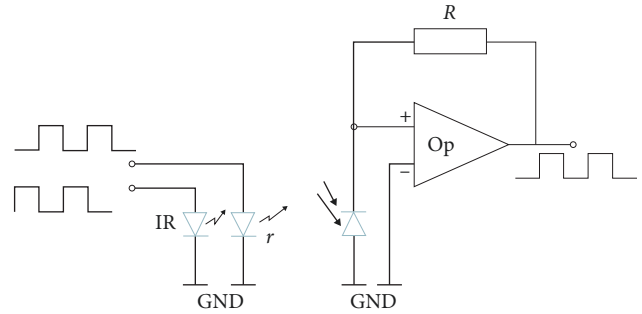
The process of LED control, data collection, and storage were performed for a period of 128 s (2.13 min). The sampling frequency of the signal was 1,950 Hz. Once the data were stored, they were retrieved or emptied into a computer and processed by PYTHON.

The configuration of the analog system used is shown in Figure 3.

Figure 4 illustrates the designed circuit mounted on a system that allows retaining the circuit and moving it downwards until the optical fibers are in one of the holes of the abalone.

2.2. Signal Processing Method. A pulse train was generated by a microcontroller through the PWM technique. Each LED (red and infrared) was turned on alternately by a synchronization circuit; while one LED was active, there were eight pulses of reading and storage in memory, and the other LED was inactive, and vice versa, as shown in Figure 5.

Equation (1) describes a pulse train [25]:

$$t_p(t) = \text{sgn}(\cos(2\pi f t)) = \text{sgnsgn}(\cos(\omega t)). \tag{1}$$

For this case, a pulse train is applied that is defined by the following form:

$$t_p(t) = \text{sgn}(\cos(2\pi f t)) = A + \text{sgnsgn}(\cos(\omega t)), \tag{2}$$

where t_p is the pulse train function, t is time, ω is the principal or fundamental frequency, and A is a constant.

Plethysmography [26–28] is a technique that allows visualizing the variation in blood volume changes as a result of flow variations, thus allowing the detection and measurement of cardiac pulse, oxygen levels, and other biomedical variables [29]. Herein, plethysmography is used to measure gill movement.

The light beams were applied in the form of a pulse train, as indicated in Equation (2). The light beam (red and infrared) had a frequency of 122 Hz and was sampled at a rate of 1,950 Hz ($t = 5.12 \times 10^{-4}$ s). The signal was recovered by reflection.

If a convolution low-pass filter is applied to the sampled signal [30], the following equation is obtained:
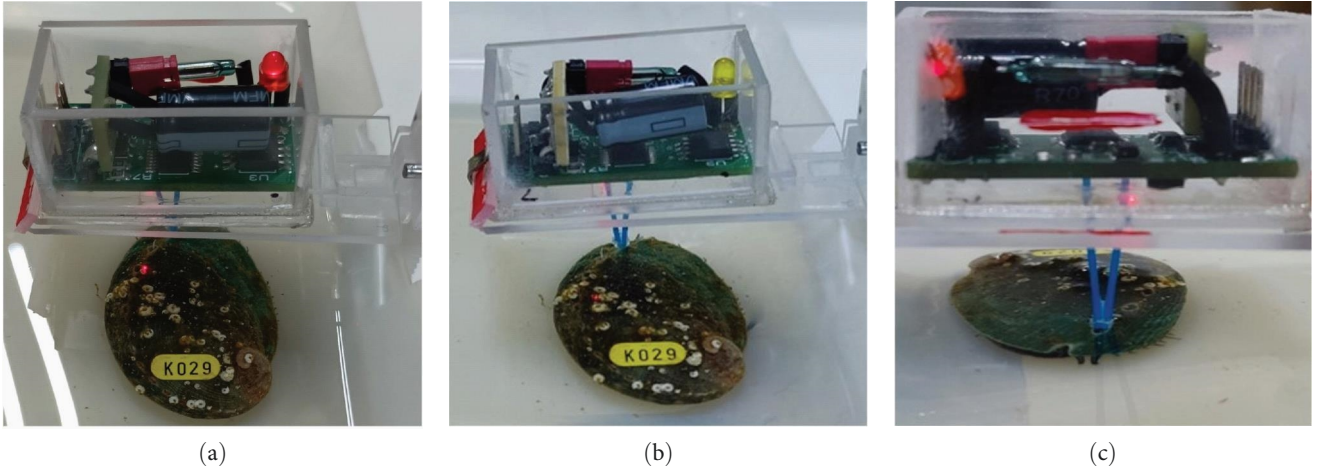
(a)

(b)

(c)

Figure 4: Circuit designed for reading data in abalone (K029). The classification is a record that is assigned by CIBNOR. (a) Side view of test sensor 1. (b) Side view of test sensor 2. (c) View of the fibers inserted in one of the pores of the abalone.
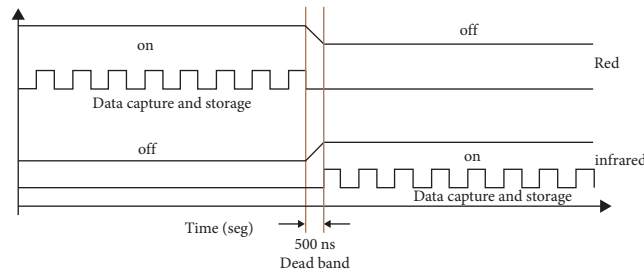

Figure 5: Pulse train: on and off sequence for the LEDs. The pulse train frequency is 122 Hz.
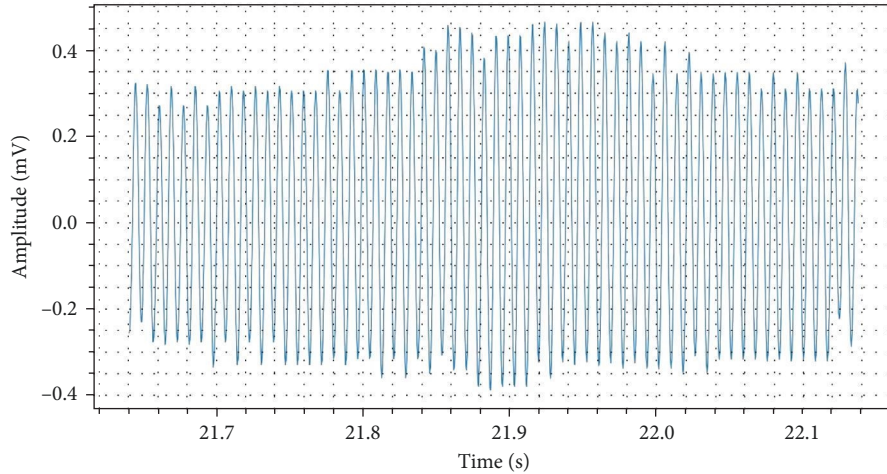

Figure 6: Signal recovered by the sensor due to the light (red and infrared LEDs) reflected on the incident surface.

$$x(t) = \left[t_p(t)\right] \times F_{pb} = A + B\cos\left(\omega_p t\right) = A + B\cos\left(2\pi f_p t\right), \quad (3)$$

where ω_p and f_p are the angular frequency and the spatial frequency of the carrier signal ($t_p(t)$), respectively, and A and B are constants. Figure 6 shows the signal recovered by applying the low-pass filter, and Figure 7 shows the Fourier spectrum of the recovered signal, displaying the oscillation frequency of the light beams.

2.3. Synchronous Detection [23]. The synchronous detection method is based on the theory of radio communication, where the phase of an unknown signal is recovered by the correlation of this signal with a sinusoidal signal of the same frequency [22].
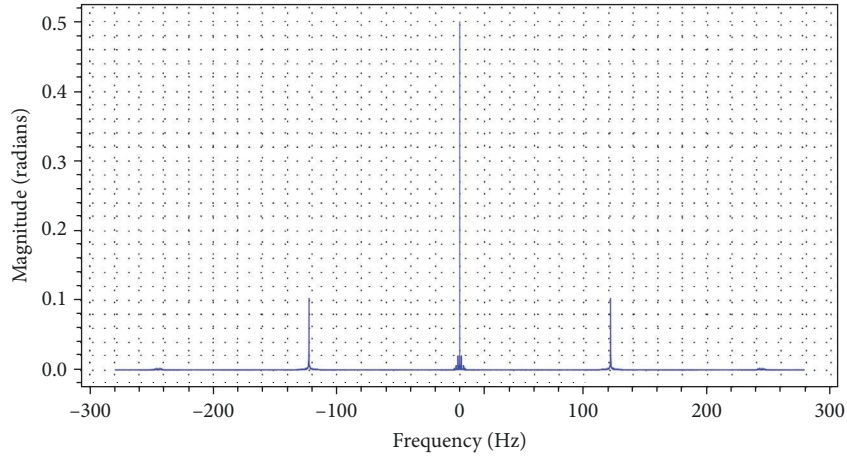
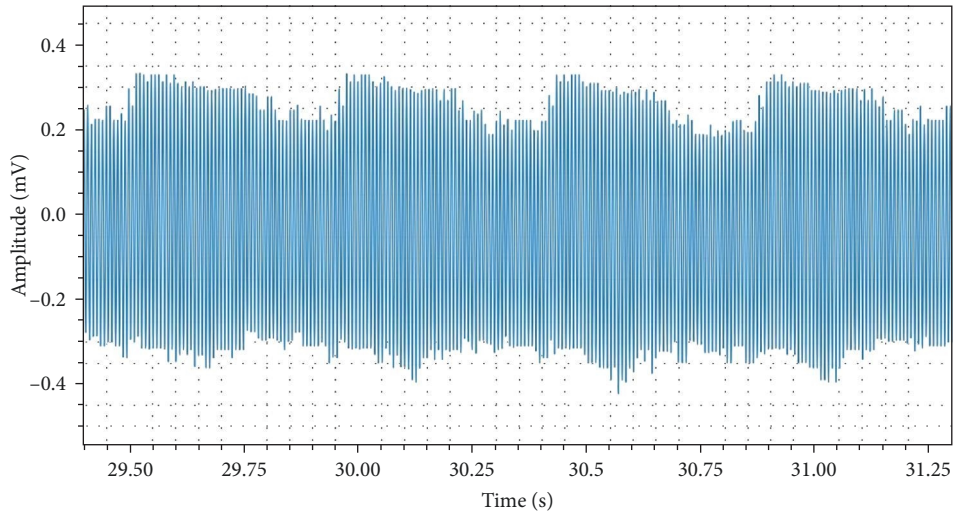FIGURE 7: Frequency of oscillation of red and infrared LEDs.

FIGURE 8: Recovered signal to which the low-pass filter has been applied (Equation (3)).

If we consider that the signal derived from the movement of the gills, $P(t)$ can modulate a train of pulses, as indicated in Equation (2), whose frequency (f_p, p: carrier) is much higher than that of the frequency of the branchial movement (f_r, r: branchial rhythm) and that this signal can be considered as a carrier signal, such that:

$$f_r \ll f_p. \tag{4}$$

A relationship can be considered as follows:

$$\begin{aligned} y(t) &= [P(t)][x(t)] \\ y(t) &= P(t)\left[A + B\cos\left(2\pi f_p t\right)\right]. \end{aligned} \tag{5}$$

If this signal ratio is multiplied by two signals (sine and cosine) of frequency almost equal to the carrier frequency, the following equation is obtained:

$$[y(t)]\left[\cos\left(2\pi f_{p'} t\right)\right] = P(t)\left[A + B\cos\left(2\pi f_p t\right)\right]\cos\left(2\pi f_{p'} t\right). \tag{6}$$

If the previous expression is developed, the following equation is obtained:

$$\begin{aligned} [y(t)]\left[\cos\left(2\pi f_{p'} t\right)\right] &= AP(t)\cos\left(2\pi f_{p'} t\right) \\ &\quad + BP(t)\cos\left(2\pi f_p t\right)\cos\left(2\pi f_{p'} t\right), \end{aligned} \tag{7}$$

where:

$$\begin{aligned} &BP(t)\cos\left(2\pi f_p t\right)\cos\left(2\pi f_{p'} t\right) \\ &= BP(t)\left[\frac{1}{2}\cos\left(2\pi\left(f_p - f_{p'}\right)t\right) + \frac{1}{2}\cos\left(2\pi\left(f_p + f_{p'}\right)t\right)\right] \\ &= B'P(t)\cos\left(2\pi\left(f_p - f_{p'}\right)t\right) + B'P(t)\cos\left(2\pi\left(f_p + f_{p'}\right)t\right), \end{aligned} \tag{8}$$

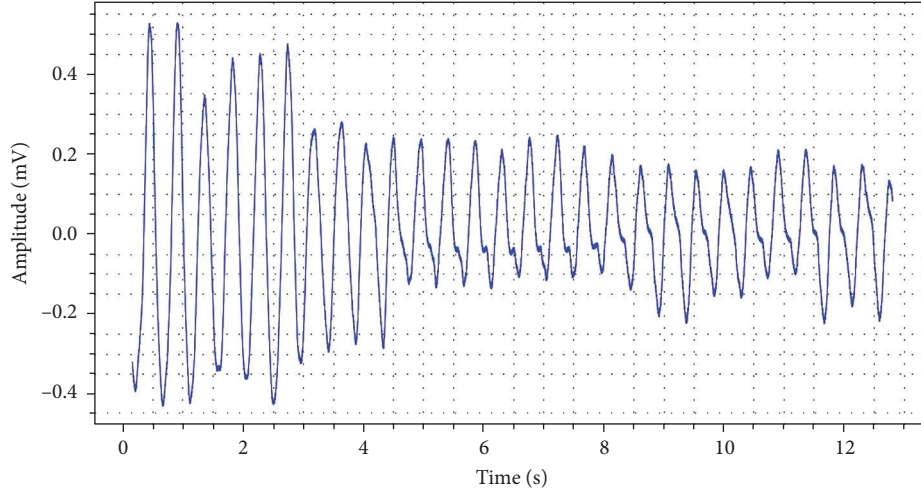where $B' = \frac{B}{2}$ is a constant term.

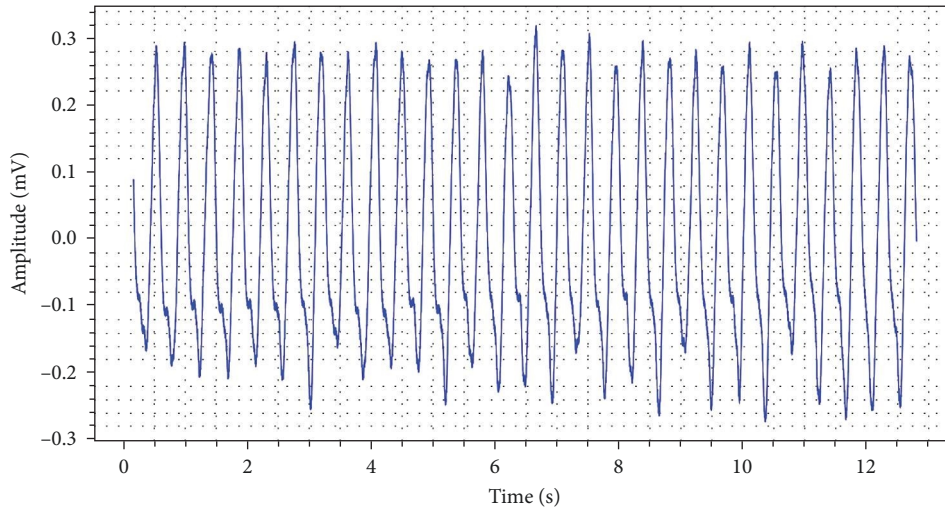FIGURE 9: Gill movement measured using the synchronous detection method.

FIGURE 10: Branchial movement in abalone.

The term $f_p - f_{p'}$ corresponds to low frequency, and $f_p + f_{p'}$ and $2\pi f_{p'} t$ correspond to high frequencies, if it is considered that $f_r \ll f_p$.

By applying a low-pass filter (Butterworth type [28]), the following equation is obtained:

$$\left[y(t)\cos\left(2\pi f_{p'} t\right)\right] \times \left[F_{bp}\right] = B'P(t)\cos\left(2\pi\left(f_p - f_{p'}\right)t\right). \tag{9}$$

A similar analysis is applied for the following relationship:

$$\left[y(t)\right]\left[\sin\left(2\pi f_{p'} t\right)\right] = P(t)\left[A + B\cos\left(2\pi f_p t\right)\right]\sin\left(2\pi f_{p'} t\right). \tag{10}$$

In addition, if $f_{p'} \approx f_p$ and after applying a low-pass filter, then, the following equation is obtained:

$$\left[y(t)\sin\left(2\pi f_{p'} t\right)\right]\left[F_{bp}\right] = B'P(t)\sin\left(2\pi\left(f_p - f_{p'}\right)t\right). \tag{11}$$

There is no interest in recovering the phase but rather the magnitude of $P(t)$. From the previous equation, the following equation is obtained:

$$|P(t)| = \sqrt{\left(B'P(t)\cos\left(2\pi\left(f_p - f_{p'}\right)t\right)\right)^2 + \left(B'P(t)\sin\left(2\pi\left(f_p - f_{p'}\right)t\right)\right)^2}. \tag{12}$$

Therefore, we have the following equation:

$$|P(t)| = \sqrt{\left(\left[y(t)\cos\left(2\pi f_{p'} t\right)\right] \times \left[F_{bp}\right]\right)^2 + \left(\left[y(t)\sin\left(2\pi f_{p'} t\right)\right]\left[F_{bp}\right]\right)^2}. \tag{13}$$

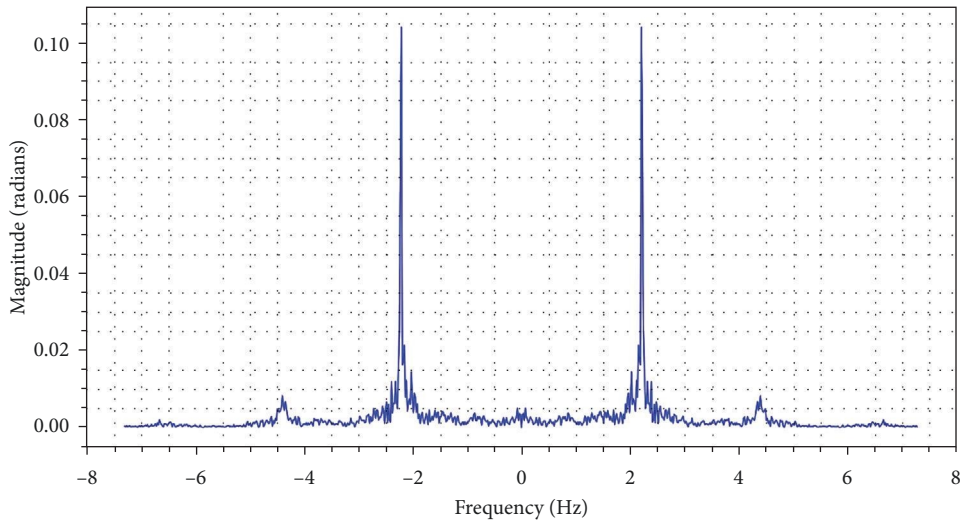From Equation (13), gill movement is calculated.

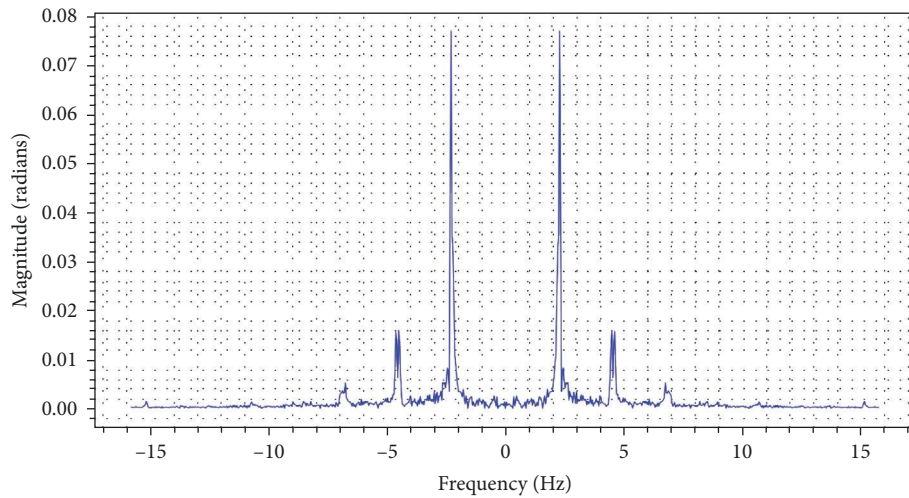FIGURE 11: Fourier spectrum of the data obtained by applying the synchronous detection method.

FIGURE 12: Frequency spectrum of gill movement.

3. Results and Discussion

Applying the described data analysis procedure, the following results were obtained. Figure 8 shows the processed signal obtained from the sensor, i.e., after applying Equation (3). It can be seen that the signal is modulated by the second signal which is the gill movement (Equation (5)).

After applying the procedure and Equations (9), (11), and (13), the following result was obtained.

Figures 9 and 10 show the recovered signal and gill movement. Applying Fourier transform to the data obtained, the following frequency spectrum was obtained (Figures 9 and 10, frequencies of 2.19 and 2.27 Hz, respectively).

The Fourier specter was obtained at frequencies of 2.19 and 2.27 Hz (Figure 11). A total of 17 data points were collected, and the analysis indicated a frequency of gill (Figure 12) movement between 2 and 2.3 Hz. Table 1 shows all the frequencies obtained from the 17 measurements made, the arithmetic mean of the data is 2.20176471 and its standard deviation is 0.08216411, the standard deviation is very small

TABLE 1: Recorded gill frequencies of the 17 measurements.

Number of readings recorded	Frequency obtained (Hz)
1	2.3
2	2.25
3	2.224
4	2.293
5	2.262
6	2.087
7	2.201
8	2.041
9	2.194
10	2.163
11	2.102
12	2.278
13	2.214
14	2.171
15	2.087
16	2.27
17	2.293

which indicates that there is not much variability in the data and that on the contrary, there is stability in the respiration of the specimen.

While temperature is one of the very important physical factors affecting marine animals, its increase reduces oxygen solubility thereby altering respiration, or metabolic rate in marine organisms [17]. In addition, it has been demonstrated that gills are responsible for oxygen consumption in anormoxic and hypoxic conditions and that they extract oxygen efficiently [31]. Therefore, the proposed device presented here may allow a broader understanding of the behavior of the gills in the studies that have been carried out on the gill plates.

4. Conclusions

Abalone is a species that is being studied given its challenges with regard to environmental changes. A method of detecting the movement of gills in abalone was proposed. By means of plethysmography and through a pulse train using the synchronous demodulation technique, gill movement was measured (frequency of 2.19 Hz). The measurement of this variable can provide better knowledge of the behavior of abalone, and it is possible that other variables can be measured using photoplethysmography.

Data Availability

All data analyzed in this paper were processed with MATLAB R2015a and PyCharm 2022.1. Data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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