Computers and Electronics in Agriculture xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Computers and Electronics in Agriculture

journal homepage: www.elsevier.com/locate/compag

Synthesis and measurement of valve activities by an improved online clam-based behavioral monitoring system

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ARTICLE INFO

Article history:
Received 26 March 2012
Received in revised form 11 August 2012
Accepted 25 September 2012
Available online xxxx

Keywords:
Valvometric technique
Clam *Corbicula fluminea*
Valve movement measurement
Bivalve behavioral rhythm
Siphon extension

ABSTRACT

The purpose of this study was to develop an improved clam-based online behavioral monitoring system. The improved system consists of a valvometry apparatus and three kinds of valve closure analytic programs that can offer a real-time and cost-effective valvometric technique to effectively detect valve activities of clams *Corbicula fluminea*. The improved valvometry apparatus mainly applied electromagnetic mutual-inducting actions and principles to measure the distance between two inductances as the sensing theory of determining the magnitude of the bivalve shell gape. It evolved into a noninvasive and unconstrained valvometric technique, allowing freshwater bivalves to move freely and burrow in the bottom sands of observed tanks with minimal experimental constraint. By employing a compiled LabVIEW graphic control program on a personal computer, the clam-based online behavioral monitoring system can monitor and analyze the daily valve activities of a group of clams in real time. A strict laboratory procedure associated with an improved valvometric technique was performed in the bioassay experiment to observe the magnitudes of shell gape as the determining thresholds of valve closing (VC) and siphon extension (SE) statuses. A statistics-based approach describing bivalve behavioral movements of *C. fluminea* exposed to unpolluted environment was performed. Our results demonstrated that the magnitudes of shell gape of 20% and 50% can be adopted as the determining thresholds of VC and SE statuses, respectively, to digitalize the valve movements in bivalves, allowing a well-established sequence framework for quantifying the characterization of the daily valve behavioral rhythm.

1. Introduction

The behavioral activity of valves acclimated in a laboratory has been used as a way to measure biological responses to exposure to various aquatic environmental conditions to quantify the environmental influence or toxicity effect to freshwater bivalves (Sluyts et al., 1996; Curtis et al., 2000; Kadar et al., 2001; Markich, 2003; Ortmann and Grieshaber, 2003; Tran et al., 2003; Fournier et al., 2004; Liao et al., 2009; Chen et al., 2010). Several bivalve-based biomonitoring systems using the valve-closure response or rhythmic gaping pattern as an early warning indicator of monitoring water quality have also been developed and implemented to diagnose the presence of pollution (Borcherding and Volpers, 1994; Sluyts et al., 1996).

Borcherding and Wolf (2001) used the Dreissena-Monitor valvometry apparatus to process toxicological behavior studies for observing valve behavioral response exposed to 2-chloro-4-nitroaniline, Cd and pentachlorophenol under laboratory conditions. The technology of Dreissena-Monitor is to measure valve movements of each individual accordingly registered by implementing a reed switch associated with a small magnet glued onto one of the free moving valves of bivalve, whereas another valve is fixed to a Plexiglas plate. An alarm can be triggered after a sudden increase in the number of valve movements or an abrupt decrease in the percentage of open mussels.

Both the Dreissena-Monitor and the Mosselmonitor were also used to monitor the valve-closure behavior of the Asian clam *Corbicula fluminea* (Ortmann and Grieshaber, 2003). The Mosselmonitor is based on a high-frequency (HF) electromagnetic induction system that can measure the varying distance between two electric coils that are glued onto the valves. The Mosselmonitor can determine the gape of the bivalves and record the trace at any revealed positions of bivalve shell movements. Curtis et al. (2000) used both the Mosselmonitor (valve activity) and the modified version of the computer-aided physiological monitoring system (CAPMON; cardiac activity) to simultaneously and non-invasively determine valve and cardiac activity of the blue mussel (*Mytilus edulis*) exposed to copper. Sluyts et al. (1996) also used the

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http://dx.doi.org/10.1016/j.compag.2012.09.008

Mosselmonitor to observe the valve position and the activities of eight *D. polymorpha* for monitoring the water quality.

Markich (2003) used valve movement response, measured in terms of the duration of valve opening (DVO) and frequency of valve adductions (FVAs), of the Australian tropical freshwater unionid bivalve *Velesunio angasi* exposed to sublethal uranium (U) concentrations in standard synthetic water. Markich et al. (2000) used a computer-based data acquisition system to measure the valve movement behavior (VMB) of the immobilized bivalve *V. angasi*. The difference between the Mosslmonitor and the VMB measuring system are that the latter uses a linear variable displacement transducer (LVDT) attached to a clasp upon the valve, which was connected to the spring-loaded lever arm, to determine the magnitudes (%) of shell gape of each bivalve. The same measuring means of VMB were also used to process toxicological behavior studies of other bivalve species by Markich et al. (2000) and Markich (2003).

Kadar et al. (2001) studied and examined the effect of aluminum on the filtering behavior (shell opening or gape) of the freshwater bivalve *Anodonta cygnea*. Doherty et al. (1987) indicated that a high correlation between the valve movement patterns was found in freshwater clam *C. fluminea* exposed to Cd and Zn. Tran et al. (2003) developed an original measuring system based on the impedance valvometry technique using light-weight platinum electrodes to study the behavioral reaction of unconstrained and glued bivalves in same low-stress conditions for obtaining the optimal testing period of valve exposed to contaminants. Further, a new analytical approach was presented to describe valve *C. fluminea* closure response (the closing percentage in a group of bivalves) as a function of integration time of response (ITR) and concentration of contaminant (Cd) (Tran et al., 2003). Similar laboratory procedures and analytical approaches describing valve-closure behavioral reaction in response to Cu and U were also employed by Tran et al. (2004) and Fournier et al. (2004), respectively.

The valvometry measuring apparatus with allowing the natural and unhindered movement of each bivalve in the sand substrate can measure and record the daily valve opening period and time-varying distance between the two electrodes glued to each valve by assembling a purpose-built computer-based data acquisition system and LabVIEW software. Specifically, the impedance electrodes of attaching bivalves were stimulated to proportionately induce various signal voltages according to the openness of bivalves' valve activities. With this equipment, the overall bivalves' valve movements were recorded by connecting the processed voltage signals to a PC.

The valvometry measuring apparatus should be required to better allow a low-stress determining condition to measure and record the daily valve opening period and the time-varying magnitude (%) of bivalve shell gape in near unhindered and spontaneous situations. Therefore, the objective of this paper was to develop an improved bivalve-based online behavioral monitoring system, including an inductance-based unconstrained valvometry apparatus and three kinds of valve-closure analytic programs compiled by the LabVIEW software, to measure valve activities of *C. fluminea*.

A strict laboratory procedure associated with an improved valvometric technique was performed in the bioassay experiment to observe the magnitudes of shell gape as the determining thresholds of valve closing (VC) and siphon extension (SE) status. We conducted and calibrated a statistics-based approach to precisely describe bivalve behavioral movements of *C. fluminea* exposed to an unpolluted environment. The determining thresholds of VC and SE statuses can be adopted to digitalize the valve movements in bivalves, allowing a well-established sequence framework for quantifying the characterization of the daily valve behavioral rhythm. The improved valvometric technique can reduce the stress from determining conditions (e.g., using light-weight valvometry sensors and with an unhindered/free-range movement) for observed bivalves to obtain recordings of valve movements in a near spontaneous status.

2. Materials and methods

2.1. Measurement

Our protocol for implementing synthetic measurement of valve activities with an improved online clam-based behavioral monitoring system is shown in Fig. 1. A measuring protocol and sensing principles for determining the magnitude of shell gape of a bivalve is shown in Fig. 2.

First, we selected 100 freshwater clams (*C. fluminea*) as test animals. The average values of body weight and shell length of tested adult specimens were 6.79 ± 0.78 g (mean ± sd) wet weight and 28.5 ± 0.26 mm, respectively. We considered that the weight of a valvometry sensor was confined to 10% of the clam weight. Thus, according to the specification of commercialized inductor coils, we selected a 50-mH inductance (diameter: 4 ± 0.001 mm, length: 7 ± 0.002 mm, naked weight: 0.46 ± 0.005 g) with the characteristics of lightweight and better induction as a sensing device to measure the magnitudes of bivalve shell gape.

We used a 10-mm length of heat-shrink tubing (diameter: 6 mm) to coat each electric coil to form a watertight and insulated cover. Enamel-insulated wires (diameter: 0.2 mm, length: 60 cm) were used to weld electric coils to transmit source/signal voltage. We employed heat-melt adhesive to process the conjunctional interface between an electric coil coated by heat-shrink tubing and enamel-insulated wires to insulate electric conduction from water. Fig. 2A shows that a pair of coated/insulated electric coils was glued onto the according shell of a clam.

When these tested specimens were in filtering plankton and unburrowing statuses, we used a picture processing technique to obtain an approximate observation of the maximum magnitudes (mm) of bivalve shell gape. Primary results revealed that the maximum openness (mm) of clams is not higher than 2 mm. Each pair of coated/insulated electric coils was required to be glued onto the according shell of each clam within a 5-mm range along the valve edge. On such a glued valve position, the average value of shell depth of tested adult clams was 6.56 ± 0.52 mm (mean ± sd) (Fig. 2A). According to the central distance between each pair of coated/insulated electric coils, maximum openness of clams and shell depth on a specific valve position (within a 5-mm range along the valve edge), we can estimate the varying distance (mm) between two electric coils glued onto a clam to obtain a measurable range between 5 and 13 mm.

Our sensing principle was based on a high-frequency (HF) electromagnetic inducting apparatus that can measure the varying distance between two coated/insulated electric coils glued onto the according bivalve shells (Fig. 2A and B).

Tsai (2002) depicted that a current-carrying solenoid or coil can be regarded as a uniform magnet. Fig. 2C shows that magnetic flux density of position P above the middle of a uniform magnet can be expressed as (Tsai, 2002)

$$\vec{B}_1 = \left(-\frac{\mu}{4\pi}\right)\frac{A_1 d_1 M}{(G^2 + d_1^2/4)^{3/2}}\hat{i}, M = n_1 I_1 A_1, \quad (1)$$

where $\vec{B}_1$ is magnetic flux density (Wb/m^2), μ is the permeability (H/m) of the medium, G is a vertical distance (m) from the magnet center along the x-axis to position P along the y-axis, d_1 is the length (m) of a magnet, A_1 is a cross-sectional area (m^2) of a magnet or coil, M is magnetic moment (A m^2) per unit volume magnet, $\hat{i}$ is a unit vector of x-direction, n_1 is the number of magnetic dipole (or

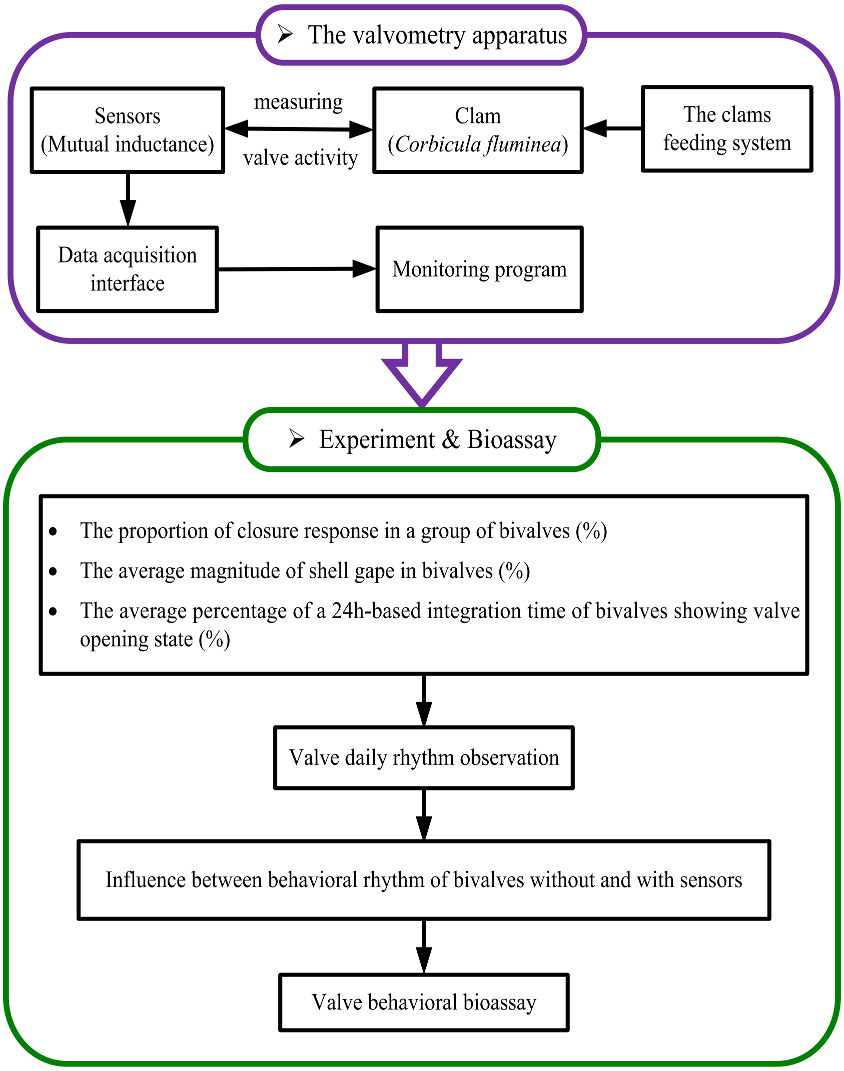

Fig. 1. An experimental protocol of preliminary studies showing the improved online clam-based behavioral monitoring system to assay valve activities of bivalves.

viewed as a current-carrying n_1-turn coil) per unit volume magnet and I_1 is current (A) flowing through a coil.

The current-carrying primary coil was viewed as a uniform magnet to generate a uniform magnetic field coupling the secondary coil. Fig. 2B illustrates that the secondary coil can link a time-varying magnetic field generated by the primary coil subject to alternating current (AC) sinusoidal excitation ($V_1 = V_{m1}\sin(2\pi ft)$) to induce a transformer voltage (V_2) across the secondary coil. Based on Faraday's law and AC circuit analysis, the expression of a transformer voltage (V_2) induced by the secondary coil can be derived as follows:

$$V_2 = n_2\frac{d\phi}{dt} = n_2 n_1 A_1^2 A_2 \cos\gamma\left(\frac{\mu}{4\pi}\frac{d_1}{(G^2+d_1^2/4)^{3/2}}\right) \times 2\pi f\frac{V_{m1}}{\sqrt{R_1^2+(2\pi fL_1)^2}}\cos(2\pi ft) = V_{m2}\cos(2\pi ft), \quad (2)$$

where ϕ is the magnetic flux generated by the primary coil, n_2 and A_2 are the turn number and cross-sectional area of the secondary coil, respectively, γ is the angle between the vectors B_1 and A_2, V_{m1} and f are the amplitude and frequency of voltage source V_1, respectively, R_1 and L_1 are the resistance (Ω) and inductance (H) circuit parameters of the primary coil, respectively, t is the time, and V_{m2} is the amplitude of a transformer voltage (V_2). From expression (2), the amplitude (V_{m2}) of the induced voltage V_2 can be written in the following form:

$$V_{m2} = n_2 n_1 A_1^2 A_2 \cos\gamma\left(\frac{\mu}{2}\right)\frac{d_1}{(G^2+d_1^2/4)^{3/2}}\cdot$$
$$V_{m1}\cdot\left(\frac{f}{Z}\right),$$
$$Z = \sqrt{R_1^2+(2\pi fL_1)^2} \quad (3)$$

where Z is the impedance value of the primary coil.

When n_1, n_2, A_1, A_2, γ, μ, d_1, G, V_{m1}, R_1 and L_1 are all constant, V_{m2} is only proportional to the frequency of excitation voltage (V_1). Through the measurement of the electromagnetic inducting apparatus (Fig. 2B), we fixed two experimental conditions (i.e., G = 4 mm and V_{m1} = 10 V) to obtain the variation between the induced peak voltage V_{m2} of the secondary coil and excitation frequency f of a primary coil (Fig. 2D). For R_1 = 343 Ω and L_1 = 50 mH in the primary coil, Fig. 2D shows that the ratio of frequency f to impedance Z and the induced peak voltage (V_{m2}) accordingly tended to 3.1 and 0.504 values when the excitation frequency f exceeded 4 kHz. If excitation frequency f is extremely high, the limit ratio of frequency f to impedance Z can be regarded as the expression: $1/(2\pi L_1)$ (i.e., nearly 3.18). Through the verification of Fig. 2D, we adopted 4-kHz excitation frequency and 10-V voltage amplitude as the circuit parameters of the primary coil.

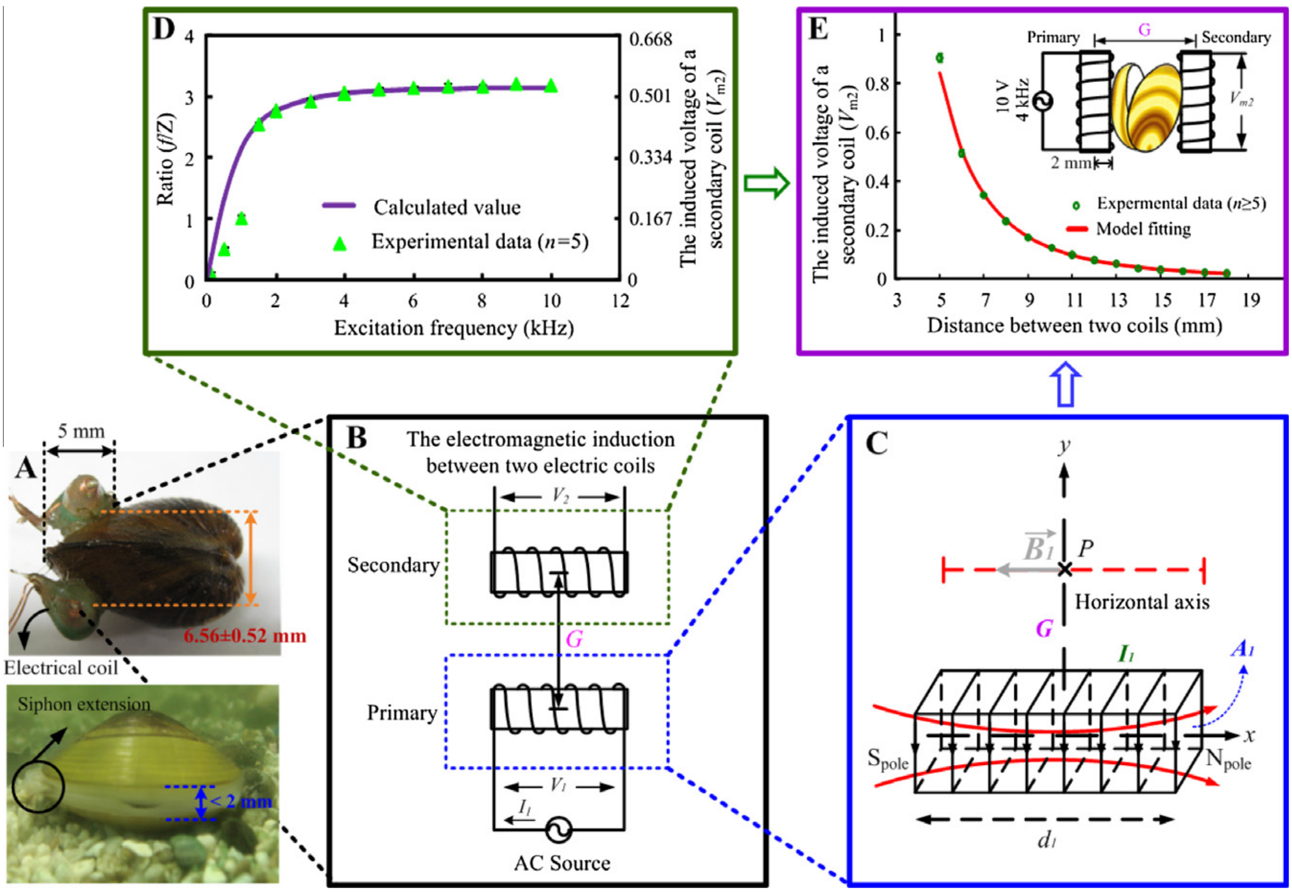

Fig. 2. A measuring protocol for determining the magnitude of shell gape of a clam diagramming: (A) how to glue a pair of coated/insulated inductances onto the according shell of a clam and sensing principles, (B) a primary coil was used as a transmitter of a magnetic field generated by an alternating current (AC) sinusoidal excitation (V_1) so that the secondary coil can couple part of this magnetic field to induce a transformer voltage (V_2) which was inversely proportional to the varying distance between two electric coils, (C) a current-carrying coil can be regarded as a uniform magnet, as a result, the magnetic flux density (B_1) of position P above the middle of a uniform magnet was obtained as the expression (1), (D) the experimental data and calculated values of the electromagnetic inducting apparatus showing the varying relation between the induced peak voltage V_{m2} across the secondary coil and excitation frequency f of a primary coil, and (E) using a two-parameter quadratic function (expression (5)) to fit the experimental data of the induced voltage V_{m2} across the secondary coil according as the varying distance between a pair of electric coils.

To obtain the relationship between the induced voltage V_{m2} of the secondary coil and the varying distance G between two inductances, expression (3) was written as the following:

$$(V_{m2})^{-\frac{2}{3}} = \left(n_2 n_1 A_1^2 A_2 \cos\gamma \frac{\mu}{2} d_i V_{m1} \frac{f}{\sqrt{R_1^2 + (2\pi f L_1)^2}}\right)^{-\frac{2}{3}} \cdot G^2 + \left(n_2 n_1 A_1^2 A_2 \cos\gamma \frac{\mu}{2} d_i V_{m1} \frac{f}{\sqrt{R_1^2 + (2\pi f L_1)^2}}\right)^{-\frac{2}{3}} \cdot \left(\frac{d_1^2}{4}\right). \quad (4)$$

All parameters n_1, n_2, A_1, A_2, γ, μ, d_1, V_{m1}, f, R_1 and L_1 in expression (4) are regarded as constants so that expression (4) can be simplified as

$$(V_{m2})^{-\frac{2}{3}} = a \cdot G^2 + b,$$

$$a = \left(n_2 n_1 A_1^2 A_2 \cos\gamma \left(\frac{\mu}{2}\right) d_i V_{m1} \frac{f}{\sqrt{R_1^2 + (2\pi f L_1)^2}}\right)^{-\frac{2}{3}},$$

$$b = \left(n_2 n_1 A_1^2 A_2 \cos\gamma \frac{\mu}{2} d_i V_{m1} \frac{f}{\sqrt{R_1^2 + (2\pi f L_1)^2}}\right)^{-\frac{2}{3}} \left(\frac{d_1^2}{4}\right). \quad (5)$$

Fig. 2E shows that the experimental data of the induced voltage V_{m2} across the secondary coil according to the varying distance between a pair of electric coils were fitted by a two-parameter quadratic function (expression (5)). The fitted regression was obtained as follows:

$$(V_{m2})^{-2/3} = 0.037579033 \times G^2 + 0.1810587, \quad R^2 = 0.9971, \; p < 0.001. \quad (6)$$

Thus the expression of the varying distance G between a pair of electric coils can be written as a function of the induced voltage V_{m2} of the secondary coil as the following formula:

$$G = \left(\frac{V_{m2}^{-\frac{2}{3}} - b}{a}\right)^{\frac{1}{2}}, a = 0.037579033, b = 0.1810587. \quad (7)$$

The sensing principle with respect to expression (7) was used as a determining formula of measuring the magnitude (%) of bivalve shell gape.

2.2. Signal processing and logging devices

Tran et al. (2003) demonstrated that by using 15 observed specimens in 135 d as a test group is enough to estimate precisely the valve rhythm of clams. We used a 16-channel differential input (sample rate: 1.25 MS/s, resolution: 16 bits) data acquisition

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interface card (NI DAQPad-6259) with the measurable range of ±10 V to simultaneously and in real time acquire the varying signal voltages of valve activities in 16 clams. Through the primary measurement, the general range of the induced voltage across the secondary coil glued onto the valve shell was between ±0.1 and ±0.5 V. For increasing the resolution of the sensing signal, we used 16 differential amplifiers (voltage gain: 20), amplifying the differential signal and synchronously rejecting the noise to process the induced voltage across the secondary coil glued onto the valve shell (see Fig. A1 in Supporting information).

2.3. Synthesis of a clam-based behavioral monitoring system

The synthetic schema of our developed clam-based behavioral monitoring system is shown in Fig. 3. The LabVIEW graphic control programming language (Version 8.0, NI Inc., North Mopac Expressway, Austin, TX, USA) was used to compile the calculating formulas and measurement of valve activities in 16 clams as the computational core program of the developed valvometry measuring system to achieve a real-time observation of valve activities in an unconfined status. The measuring technique of the electromagnetic inducting action in the AC circuit was applied to the inductance-based valvometry apparatus using an oscillating sinusoidal potential difference produced by a function generator at a frequency of 4 kHz with a voltage amplitude of 10 V connected to one of the two electric coils. Another electric coil attached to the clam valve was induced to accordingly output various signal voltages in accordance to the openness of valve opening activities.

The induced voltage V_{m2} across the secondary coil glued onto the valve shell was connected to the differential amplifier. Through a data acquisition interface card (NI DAQPad-6259), the voltage signal (V_i where i is the marked number of the according differential amplifier or clam for i = 1–16) processed by the differential amplifier was transmitted to a PC as a measurable physical parameter in the computational mechanism for determining the time-varying distance between two electric coils glued onto the according bivalve shells to subsequently quantify the magnitude of bivalve shell gape.

2.4. Compiler of bivalve behavioral monitoring programs

The programming for synthesis and measurement of valve activities in bivalves is shown in Fig. 4. The processed time-varying voltage signal ($V_i(t)$) was divided by the voltage gain of its corresponding differential amplifier to return the original accordingly induced voltage (V_{m2}) across the secondary coil, allowing a calculation for obtaining the time-varying distance ($G_i(t)$) between two electric coils glued onto the valves using the calibration protocol of expression (7) (Fig. 2A). Practically, the time-varying distance ($G_i(t)$) between two electric coils glued onto the valves is the sum of the distance between the two electrical coils at the valve closing state (G_{min}), the distance G_c between two electrical-coil sensing points inside the inductances (about 4 mm), and the distance of openness (G_o) between the valves. Thus, we obtain $G_i(t) = G_c + G_{min} + G_o$.

The difference in distance between G_{max} and G_{min} was used as the maximum magnitude (mm) of shell gape of a clam so that the valve opening magnitude (%) of a clam can be computed by the following expression:

$$m_i t = \frac{G_i(t) - (G_c + G_{min})}{G_{max} - (G_c + G_{min})}\%, G_{max} = G_c + G_{min} + G_{omax}, \tag{8}$$

where $m_i(t)$ is the time-varying valve opening magnitude (%) of a clam and G_{omax} is the maximum distance of shell gap between the valves. In expression (8), integrating a computational mechanism of the relative quantification can normalize the valve opening magnitude (%) of each clam without respect to the difference in body size among individuals.

In the compiler of the monitoring program, when the measured valve opening magnitude (%) of each clam ranges from 0 to 100 %, the program for determining the siphon extension/withdrawal and valve closing/opening statuses is performed and executes in real time the recording function of four time-varying values of each tested clam: (1) the induced voltage ($V_{i-m2}(t)$, V) across the secondary coil, (2) the distance ($G_i(t)$, mm) between two electric coils glued onto the valves, (3) the valve opening magnitude ($m_i(t)$, %) and (4) its according integration time. When $m_i(t) \geqslant X$, the status of valve activity is viewed as siphon extension. Contrary to this condition is regarded as siphon withdrawal. If $m_i(t) \leqslant Y$, the status of valve activity is determined to be in the valve closing state; the opposite condition signifies a valve opening state.

When $100 \leqslant m_i(t) \leqslant 105$ or $-5 \leqslant m_i(t) \leqslant 0$, the compiled program is set to carry out the revision of the maximum/minimum distance (G_{max} and G_{min}, mm) between each pair of inductances glued onto the valves. When $m_i(t) > 105$ or $m_i(t) < -5$, the compiled program commands that let the former value ($m_i(t-1)$) of valve opening magnitude replace the present abnormal value ($m_i(t)$), and the according induced voltage ($V_{i-m2}(t)$, V) across the secondary coil cannot be calculated and analyzed by the expressions (7) and (8), but only saved into the Excel file. Synchronously, the monitoring program is set to display such an abnormal status in the window so that observers can discover and maintain them every day.

2.5. Quantification of valve rhythms

The compiled analytic program including three subprograms is interpreted in the following description (Fig. 4B).

(1) The percentage of clams showing various valve activities in a group of clams: According to the recording of past observations in daily valve rhythm of clams (Tran et al., 2003; Ortmann and Grieshaber, 2003), Jou and Liao (2006) indicated that a periodical step function can be used as an analogous mathematical pattern to represent the variation of the daily valve rhythm of a clam. Thus, we define and analyze the valve rhythm of a clam using the following mathematical expression:

$$f_i(t) = [u(t - a_i) - u(t - b_1)], \tag{9}$$

where $f_i(t)$, $u(t - a_i)$ and $u(t - b_i)$ are step functions, a_i is the start time of valve opening and b_i is the end time of valve opening. For calculating the total number of clams showing the same valve activity state at the same time, we used the following expressions:

$$N^{SE}(t) = \sum_{i=1}^{N} f_i(t), N^{SW}(t) = N - N^{SE}(t), \tag{10a}$$

$$N^{VC}(t) = \sum_{i=1}^{N} f_i(t), N^{VO}(t) = N - N^{VC}(t), \tag{10b}$$

where N is the total number of observed specimens and $N^{SE}(t)$, $N^{SW}(t)$, $N^{VC}(t)$ and $N^{VO}(t)$ are the according total number of clams showing the siphon extension/withdrawal and valve closing/opening state at the same time, respectively.

The time-varying percentages of various valve activities in a group of clams can be calculated as based on Eq. (9) as

$$\psi_j^{SE}(t) = \frac{N^{SE}(t)}{N}, \varphi_j^{SW}(t) = 1 - \psi_j^{SE}(t), \tag{11a}$$

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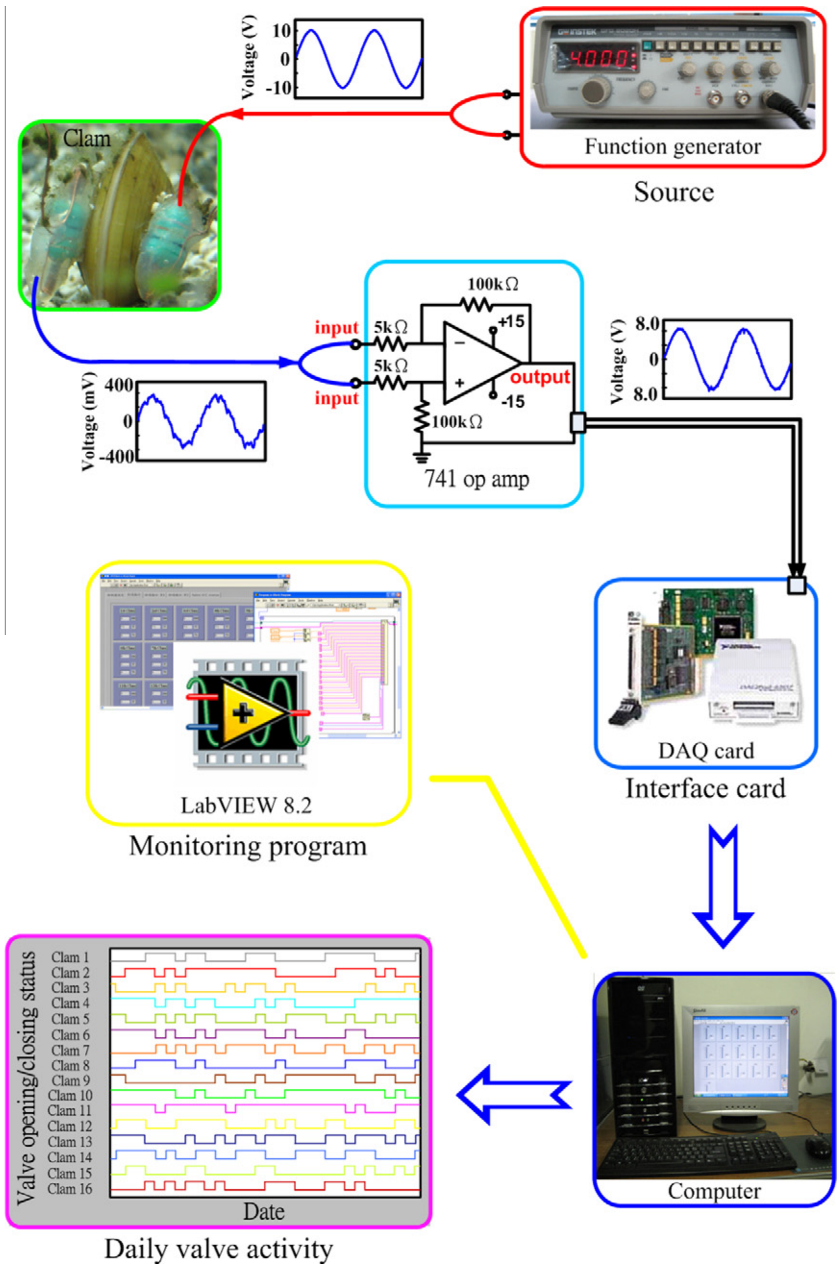

Fig. 3. Schematic showing the configuration of the improved valvometry measuring system to achieve a real-time observation for behavioral activities of clams in a free-range status.

$$\varphi_j^{VC}(t) = \frac{N^{VC}(t)}{N}, \psi_j^{VO}(t) = 1 - \varphi_j^{VC}(t), \tag{11b}$$

where $\psi_j^{SE}(t)$, $\psi_j^{SW}(t)$, $\varphi_j^{SW}(t)$, $\varphi_j^{VC}(t)$ are the time-varying proportion of a given number of clams showing the siphon extension/withdrawal and valve closing/opening state at the same time to the total number of clams, respectively, and suffix j is the order number of the according day in an observational period. During the observational period, the time-varying mean percentages of various valve activities in a group of clams can be calculated as

$$\Psi^{SE}(t) = \frac{\sum_{j=1}^{N_{day}} \psi_j^{SE}(t)}{N_{days}}, \quad \Phi^{SW}(t) = 1 - \Psi^{SE}(t), \tag{12a}$$

$$\Phi^{VC}(t) = \frac{\sum_{j=1}^{N_{day}} \varphi_j^{VC}(t)}{N_{days}}, \quad \Psi^{VO}(t) = 1 - \Phi^{VC}(t), \tag{12b}$$

where N_{days} is the total number of observational days and $\Psi^{SE}(t)$, $\Phi^{SW}(t)$, $\Phi^{VC}(t)$ and $\Psi^{VO}(t)$ are the mean time-varying proportion of a given number of clams showing the siphon extension/withdrawal and valve closing/opening state at the same time to the total number of clams, respectively.

(2) The average magnitude (%) of shell gape in a group of clams: The time-varying average magnitude ($\overline{m_j}(t)$) of shell gape in a group of clams can be expressed as

$$\overline{m_j}(t) = \frac{\sum_{i=1}^{N} m_j(t)}{N}. \tag{13}$$

During the observational period, the daily time-varying mean valve opening magnitude ($\overline{M}(t)$) can be computed as follows:

$$\overline{M}(t) = \frac{\sum_{j=1}^{N_{day}} \overline{m_j}(t)}{N_{days}}. \tag{14}$$

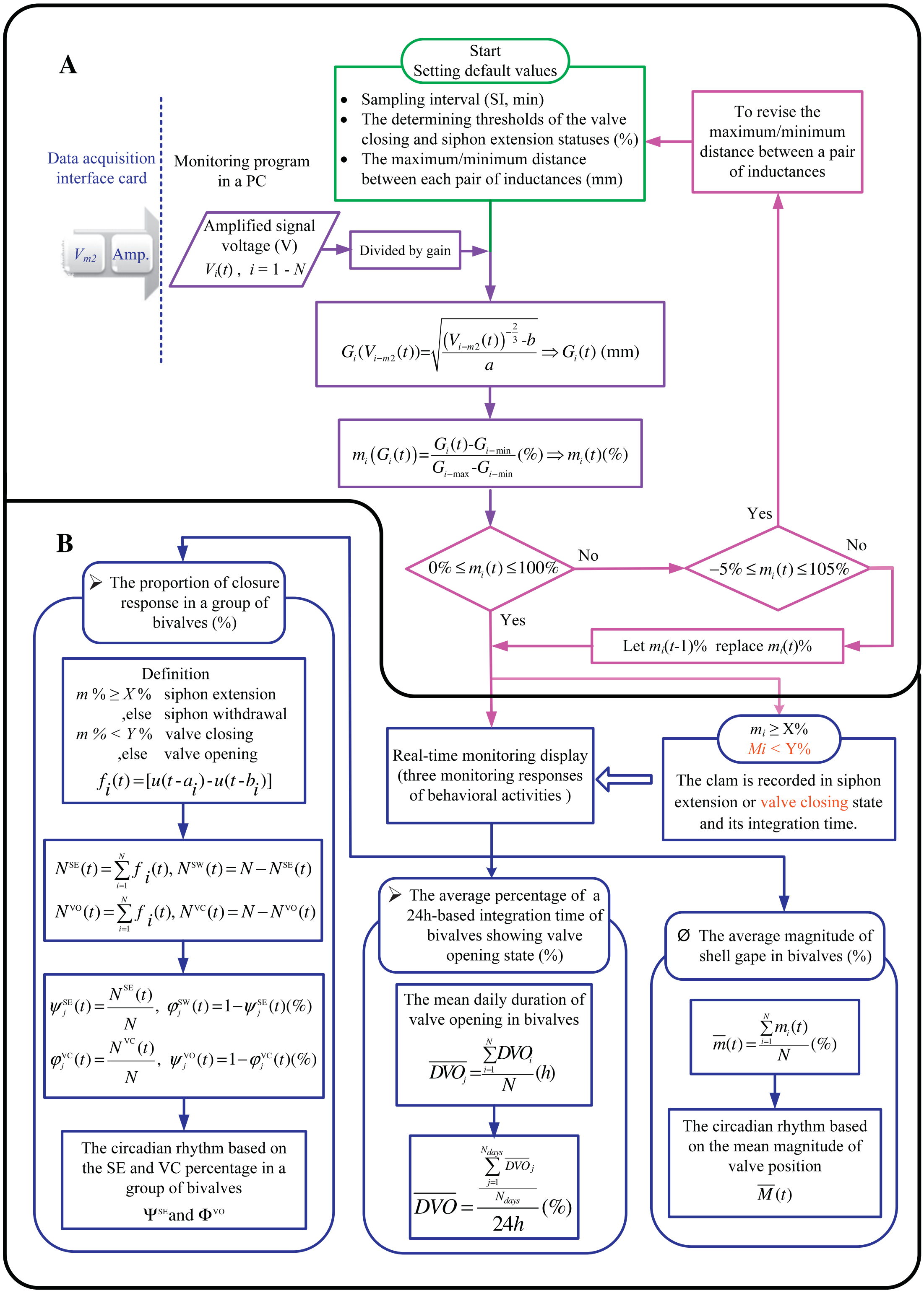

Fig. 4. Compiler of bivalve behavioral monitoring programs consists of: (A) the computational mechanism of the openness of valve opening activities and (B) the quantitative formulas of behavioral rhythm in a group of clams. (See text for detail description of symbols).

(3) The average percentage of daily valve opening/closing time in a group of clams: The average duration ($\overline{DVO_j}$ and $\overline{DVC_j}$) kept in the valve opening/closing states in a group of clams can be calculated by the following expressions:

$$\overline{DVO_j} = \frac{\sum_{i=1}^{N} DVO_i}{N}, \tag{15a}$$

$$\overline{DVO_j} = \frac{\sum_{i=1}^{N} DVC_i}{N}, \tag{15b}$$

where DVO_i and DVC_i are the daily total time spent in the valve opening and closing statuses of each clam, respectively. Tran et al. (2003) used a 24 h-based value to estimate the average percentage of clam daily opening time in 15 clams during 1, 3, 8, 20 and

30 days, respectively. Thus, the 24 h-based average percentages ($\overline{DVO}$ and $\overline{DVC}$) of daily valve opening/closing time in a group of clams can be expressed as follows:

$$\overline{DVO} = \frac{\frac{\sum_{j=1}^{N_{days}} \overline{DVO_j}}{N_{days}}}{24h}, \tag{16a}$$

$$\overline{DVC} = \frac{\frac{\sum_{j=1}^{N_{days}} \overline{DVC_j}}{N_{days}}}{24h}. \tag{16b}$$

2.6. Experiments of bivalve behavioral observations

One hundred tested *C. fluminea* were sampled from clam farms located in Hualien in northeastern Taiwan. After acclimation of four weeks, we attached the inductance-based valvometry sensor to the according shell of each clam, and as a result, the tested clams with a valvometry sensor needed to spend at least 14 days to acclimatize themselves to stress in the valvometry apparatus before the observation started.

The tested clams were settled in tanks (the dimension of fiberglass aquarium: $63 \times 33 \times 40$ cm^3, 60-L water) with a flow-through (1.7 ml s^{-1}) circulation system. The tested specimens were continuously fed during the acclimation period with the cultured algae *Platymonas sp.* using a water pump. To reduce the effect of water level and water flow on valve activities, we used the ultrasonic sensor and float switch to accordingly monitor and control the variation of water level in observation aquariums (see Fig. A2 in Supporting information). Acclimated water conditions were as follows: temperature = 25.23 ± 1.17 °C, pH = 8.26 ± 0.06, DO = 8.55 ± 0.20 mg/L (near-saturation), salinity = 2‰, turbidity = 2.32 NTU and electrical conductivity = 236.28 ± 29.55 μs/cm. Water ionic compositions were (in mg/L) Ca^{2+} = 22.12, Mg^{2+} = 5.52, Na^{+} = 6.59, K^{+} = 1.7, Zn^{2+} = 0.02, $CO_3^{-2} = 44.08$, $NH_4^{+} = 0.12$, Cl^{-} = 7.44, $SO_4^{-2} = 26.2$, NO_2^{-} = below limits and $NO_3^{-} = 0.0038$. No mortality was observed in our laboratory facilities during acclimation.

A clam can extend its siphon for filtering plankton such that the magnitude (%) of bivalve shell gape is in accordance with the degree of siphon extension. To obtain the threshold values of determining valve activities, during the initial 7 days the statuses of valve activities in 16 clams were observed and recorded by a visual inspection. At each sampling time (interval: 1 h), our developed real-time clam-based integrated behavioral monitoring system was performed to display the magnitudes (%) of bivalve shell gape. The valve activities can be identified by using three visual indicators: (1) siphon extension (SE), (2) siphon withdrawal (SW) (but valve opening [VO] is regarded as an unsteady state [US]), and (3) valve closing (VC).

Our valvometry sensor (with watertight and insulated coat) weighing 0.64 ± 0.01 g (mean ± sd) was restrained within 10% of the clam weight to minimize the bivalve stress from the valvometry sensor. Using the measuring methods of Archimedes' principle the weights of tested animals and valvometry sensors in water were determined (unit: g) as 2.35 ± 0.11 and 0.33 ± 0.01 (mean ± sd), respectively. To confirm the effect of the valvometry sensor on valve activity, we observed the difference in the valve activity between the clams with and without the valvometry sensor. After the acclimation period of 49 days, the experiment was performed on 36 clams over 14 days. Sixteen clams with the valvometry sensor were settled in two tanks ($n = 8$ per tank; the dimension of an aquarium containing 50-L water: $60 \times 30 \times 35$ cm^3) with limited range of free movement. Each observed clam has a 15×15 (cm^2) limited ranged to freely move in the substrate of sand. The distances among eight observed animals per tank are enough (Fig. 2E) to escape a possible false signal or interference from the electromagnetic field induced by the primary coils of neighbor animals. In addition, we also adopted an iron net around each clam to shield interference from the electromagnetic field induced by the primary coils of neighbor animals.

From the past visual inspection during a long period (>1 year), we found that the occurring time periods of the peak proportion of valve opening in clams were between 18 and 21 PM in a clam cycle rhythm. Similar observation and results in local-specific areas were presented by Tran et al. (2003) and Ortmann and Grieshaber (2003). To understand the influence of the valvometry sensor on siphon extension, we chose and regulated the working schedule of day ranged from 16:00 to 02:00 to manually observe the variation of the average proportion of valve opening in 20 clams without any sensor every 1 h for 50 d. Sixteen clams with sensors as a comparison group were synchronously and automatically measured by our developed valvometry system in the same observational environment.

By employing our developed valvometry system, the time-varying mean percentage of valve rhythm in 16 clams was automatically monitored and recorded every 5 min for 14 days. In the same experimental condition, twenty more clams without the valvometry sensor were observed by a visual observation to acquire their average proportion of siphon extension over 50 days from 16:00 to 02:00 (sampling time interval: 1 h) simultaneously. The visual observation was developed to escape involuntary contact for clams, which were adopted to evaluate the influence of the valvometry sensor on siphon extension. Based on the status of valve behavior of each clam, the values of 0 and 1 represent a clam lying in siphon retraction and extension, respectively. At each sampling time, the proportion of a given number of clams showing the siphon extension of twenty clams can be obtained. All clams in three tanks were free to move in a substrate of sand.

2.7. Statistical analyses

We used the function of nonlinear regression of the TableCurve 2D (Version 5, AISN Software Inc., Mapleton, OR, USA) package to perform all model fittings including expressions (3) and (5), and calibration curves of all differential amplifiers. By associating the virtual instrumental technology developed from the LabVIEW graphic control program language in a PC with the inductance-based valvometry apparatus, a real-time clam-based integrated behavioral monitoring system was developed to observe and acquire the experimental data of valve activities in clams. The Crystal Ball software (Version 2000.2, Decisioneering, Inc., Denver, CO, USA) was used to obtain the optimum distribution of sampling data. When the number of sampling data exceeded 40, the chi-square test was used to test the sampling data.

We used the Kolmogorov–Smirnov test to test the sampling data for the number of sampling data less than 40 (Zar, 2009). Statistical significance was judged by p values less than 0.05. We used the maximum-likelihood estimators to estimate the distribution of all observed data including the corresponding magnitudes (%) of bivalve shell gape at VC/SE states in clams. A Monte Carlo technique was performed to generate 2.5 and 97.5 percentiles as the 95% confidence interval (CI) for model parameters. We employed the Crystal Ball software to implement the Monte Carlo simulation.

3. Results

3.1. Windows of an online bivalve behavioral monitoring interface

Based on the computational mechanism of the bivalve behavioral monitoring system (Fig. 4), we integrated the instrumentation techniques with a LabVIEW graphic control computational

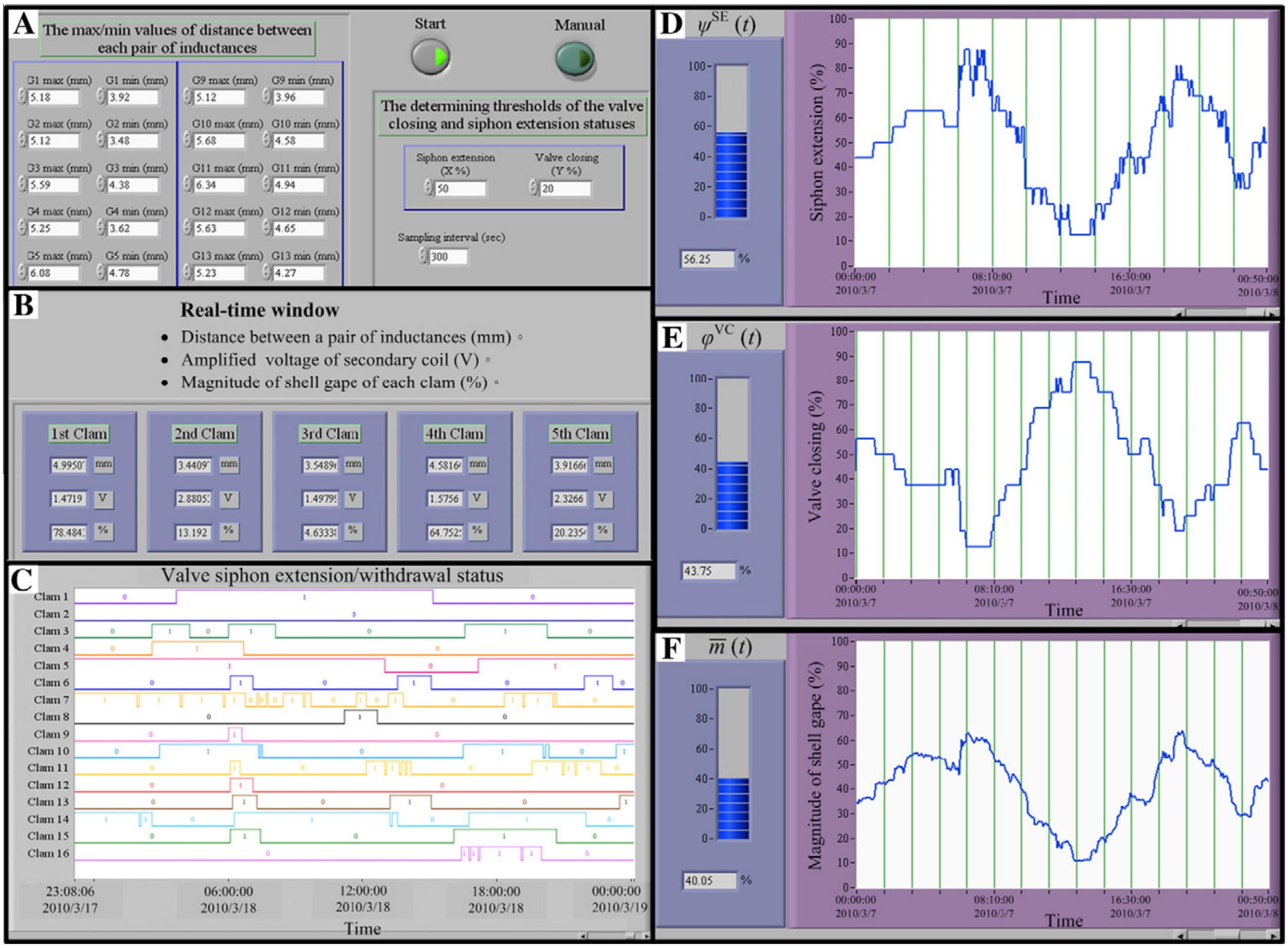

Fig. 5. Picture showing six real-time windows of a bivalve behavioral monitoring interface: (A) the maximum and minimum values (G_{i-max} and G_{i-min}) of distance (mm) between a pair of inductances glued on each bivalve, the determining thresholds (%) according to the magnitudes of shell gape of siphon extension (X) and valve closing (Y) statuses, and sampling interval (sec), (B) the real-time window including the time-varying distance ($G_i(t)$, mm) between each pair of inductances, amplified voltage ($V_i(t)$, V) of each secondary coil, and magnitude ($m_i(t)$, %) of shell gape of each clam, (C) the window representing a digitalized result of exhibiting valve siphon extension and withdrawal statuses in 16 clams, (D) the profile of time-varying proportion ($\Psi^{SE}(t)$) of a given number of clams showing siphon extension state at the same time to the total number of clams, (E) the profile of time-varying percentage ($\Phi^{VC}(t)$) of valve closing in 16 clams, and (F) the profile of the time-varying average magnitude ($\overline{m_j}(t)$) of shell gape in bivalves. (See text for detail descriptions).

program (Fig. 3) to create a human–machine monitoring interface (Fig. 5) featuring the real-time monitor of valve activities in 16 clams. The display monitors were mainly divided into six panels (Fig. 5).

The input default values and display information in window 1 (Fig. 5A) include the maximum and minimum values (G_{i-max} and G_{i-min}) of distance (mm) between a pair of inductances glued on each bivalve, the determining thresholds (X and Y, %) according to the magnitude of shell gape of siphon extension and valve closing statuses and sampling interval (s). Fig. 5B shows the real-time window 2 representing the time-varying distance ($G_i(t)$, mm) between each pair of inductances glued onto the valves, amplified voltage ($V_i(t)$, V) of each secondary coil, and magnitude ($m_i(t)$, %) of shell gape of each clam. The detailed recordings for $V_i(t)$, $G_i(t)$ and $m_i(t)$ during the observation period of 14 days from March 3 to 20, 2010 are shown in Fig. A3 in Supporting information.

By employing expression (9) to digitalize the valve rhythm (see Fig. A3C in Supporting information) of each clam, window 3 shown in Fig. 5C represents a converted result of exhibiting the valve siphon extension and withdrawal statuses in 16 clams. Based on the determining threshold of X, clams lying in siphon retraction and extension are correspondingly coded into the values of 0 and 1. Similarly, the valve closing/opening statuses in 16 clams during 14 days (from March 3 to 20, 2010) can be converted into a digitalized form based on the determining threshold of Y (see Fig. A4 in Supporting information). The profile shown in window 4 (Fig. 5D) reveals the time-varying proportion ($\Psi^{SE}(t)$) of a given number of clams showing siphon extension state at the same time to the total number of clams. Window 5 (Fig. 5E) shows the profile of time-varying percentage ($\Phi^{VC}(t)$) of valve closing in 16 clams. The profile of the time-varying average magnitude ($\overline{m_j}(t)$) of shell gape in bivalves is shown in window 5 (Fig. 5F). These parameters ($\Psi^{SE}(t)$ and $\Phi^{VC}(t)$) in Fig. 5D and 5E are practically the same for each other, only with opposite signs (a difference is of about 5%, i.e. within the measurement error). In our developed monitoring window, its simultaneous use is to provide optional information for observer. Although it is apparent redundancy of information, the redundancy of information may protect from errors, in particular, in high level noise conditions.

3.2. Threshold of determining valve closing and siphon extension status

The relationship between valve activities and the magnitude of bivalve shell gape is shown in Fig. 6A. From February 28 to March 6, 2010, we recorded all magnitudes of shell gape of 16 clams at VC

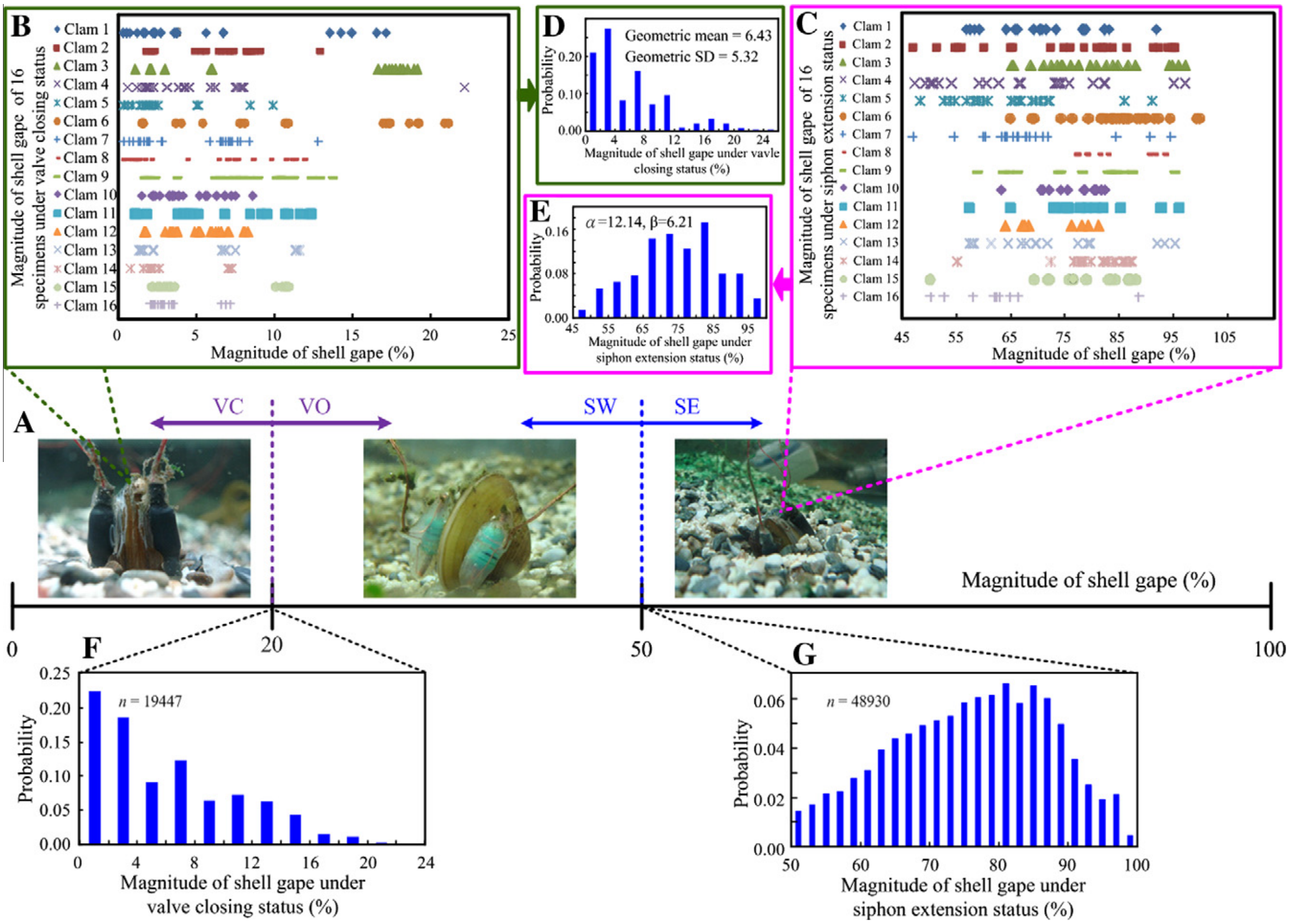

Fig. 6. (A) The according relationship between valve activities (VC, VC, SW and SE) and the magnitude (%) of shell gape of a clam. The recordings of all magnitudes (%) of shell gape of 16 clams in (B) valve closing and (C) siphon extension statuses using a manual visual observation. The probability distributions obtained from the visual observation data of the magnitude of shell gape of 16 clams in (D) VC and (E) SE statuses. The probability distributions obtained from the recordings of the on-line observation based on the magnitudes of shell gape of 16 clams determined in (F) VC and (G) SE states.

and SE (Fig. 6B and C) with visual observation. By using the linkage of these visual observations (SE, SW, VO and VC) and the corresponding magnitudes of bivalve shell gape, the threshold of determining valve activities can be obtain by statistical analysis of sampling data.

3.3. Observation of valve closing and siphon extension in bivalves

According to statistical analysis of sampling data ($n = 494$) shown in Fig. 6D, the VC magnitude of shell gape at 50% of total clams was 5.08% (95% CI: 0.30–20.06%) and the optimal distribution was the lognormal form. Based on the maximum-likelihood method, we used a Monte Carlo technique ($n = 10,000$) to obtain the probability distribution of the magnitudes of shell gape of clams at VC; the VC magnitude of shell gape at half of total clams is 5.15% (95% CI: 1.21–20.22%). The results of two statistical analyses showed that the magnitude of shell gape ranged from 0 to 20% can represent 97.5% of total clams at VC. Thus, we used the valve openness of 20% as the determining threshold of clams showing valve closing status, i.e., the clam is determined to be in a valve closing situation when $m_i(t) \leqslant 20\%$.

Based on statistical analysis of sampling data ($n = 334$) shown in Fig. 6E, the β form was tested as the optimal distribution and the SE magnitude of shell gape at 50% of total clams was 75.21% (95% CI: 49.26–96.06%). On the basis of the maximum-likelihood method, a Monte Carlo technique ($n = 10,000$) was performed to obtain the probability distribution of the magnitudes of shell gape of clams at SE. The SE magnitude of shell gape of half of total clams is 75.12% (95% CI: 49.71–96.09%). Both results of the above statistical analyses showed that the valve opening magnitude between 50% and 100% can reveal 97.5% of total clams at SE. Thus, the valve openness of 50% was used as the determining threshold of clams showing siphon extension status, i.e., if $m_i(t) \geqslant 50\%$, the status of the clam is viewed as SE.

3.4. Calibration of determining valve closing/siphon extension statuses

In light of the above results, the valve opening magnitudes of 20 and 50% were used as the threshold values of determining valve closing and siphon extension statuses to carry out the online observation of valve activities in 16 clams during 14 days (sampling interval: 5 min) using our improved valvometry system. Fig. 6F and 6G, respectively, show the probability distributions obtained from the online observation data of the magnitudes of shell gape of 16 clams determined in VC and SE states. The VC and SE magnitudes of shell gape at 50% of total clams were 4.97% (95% CI: 0.16–17.08%) and 76.89% (95% CI: 51.49–95.52%), respectively (Fig. 6F and G).

We compared the statistical profiles of the online observation data with other statistical profiles obtained from sampling data using the visual observation and a Monte Carlo analysis (Table 1), indicating that three statistical profiles have the near corresponding values for the valve opening magnitudes under VC and SE states in percentiles of 2.5, 50 and 97.5. Table 1 also demonstrates that the valve opening magnitudes of 20% and 50% in *C. fluminea* can be used as the undoubted threshold values of determining valve closing and siphon extension statuses in our improved valvometry determining system to perform the online observation of valve activities.

3.5. Influence between siphon extension of clams with and without sensors

By associating the visual observation with the improved valvometry apparatus, we looked for a possible influence between siphon extension of clams with and without sensors. Two groups of clams were settled in three tanks, respectively, with the same experimental conditions over 14 days. One group ($n = 20$) was used as the control group for an observation period of 50 days (from March 7 to April 25, 2010). The other ($n = 16$) was equipped with an inductance-based valvometry sensor glued onto their valves to compare them with the control group for a period of 14 days (from March 7 to 20, 2010). According to the visual observation, there was no significant difference in their siphon extension status among all individuals including free clams and the clams equipped with the valvometry sensor.

Fig. 7 shows that the experimental results enable us to confirm a negligible difference in valve activities between the two groups of clams, and also illustrates that the fluctuations in valve activities were near corresponding throughout the entire experiment. The difference in the peak proportion of siphon extension (at about 20:00) between the two groups of clams was 0.72% (with sensors: 45.98 ± 16% and without sensors: 46.70 ± 16.2%). Similarly, the difference in the lowest proportion of siphon extension (at about 01:00) was 0.72% (with sensors: 45.98 ± 16% and without sensors: 46.70 ± 16.2%). Thus, the valvometry sensors on clams in a free-range situation did not influence their free behavior and did not increase the probability of siphon withdrawal in such a spontaneous state.

Tran et al. (2003) first used an 18-day visual observation with a proportional index ranged from 0 to 1 at four specific times (10 a.m., 12 a.m., 2 p.m. and 4 p.m.) during the day to quantify valve opening activities in 28 clams in order to study the influence of a pair of impedance electrodes between clams with and without the devices. The valvometry technique was progressively innovated (Sow et al., 2011); as a result, we coupled such a visual observation with the auxiliary valvometry measuring system that allowed a long-term and tight observation on valve activities to acquire adequate information on behavioral rhythm of clams.

4. Discussion

Table 2 shows a comparison for two kinds of electromagnetic principle-based valvometry sensors. Nagai et al. (2006) and

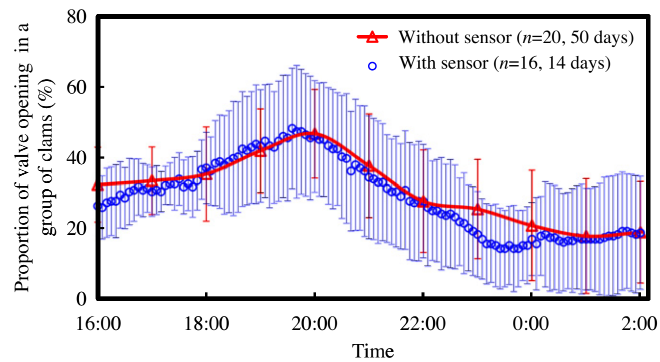

Fig. 7. Schematic showing the influence between behavioral rhythms of clams without and with sensors.

Gnyubkin (2009) used the output voltage (V_h) of the Hall element generated by changes in the external magnetic field (flux density) to form the Hall effect-based valvometry device to measure the valve movements of bivalves. Based on electromagnetic principles, the output voltage (V_h) of the Hall sensor is proportional to the inverse of the third power of the distance (L) between the Hall element and the magnet or V_h can be approximately regarded as the inverse proportional function of the quadratic power of L. V_h ranged from 4.377 to 5.452 mV (Nagai et al., 2006). This was a quite subtle signal, and as a result, the noise from environmental factors and signal processing should be taken into consideration.

Recently, the high-frequency noninvasive valvometry electric device with an unhindered condition has been widely used to measure valve activities (Chambon et al., 2007; Liao et al., 2009; Tran et al., 2010; Sow et al., 2011). Chambon et al. (2007) indicated that the strength of the electromagnetic field produced between the two coils decreases with the distance according to the transformation of D^{-1}, where D is the distance between the point of measurement and the center of the transmitting coil. Further, the measured signal voltage (V_D) produced from the transforming coil is calibrated by a 3-parameter model (Sow et al., 2011): $V_D = 151 \times D^{-0.35} - 1.48$. Based on the electromagnetic induction principles and AC circuit analyses in this study, a derived 2-parameter model (expression (5)) can be used to appropriately determine excitation frequency and potential according to the circuit parameters (resistance and inductance values) of a commercialized electric coil and to more precisely express the relationship between the measured signal voltage and valve gaping distance. We confirm that the measured signal voltage corresponds to the inverse of a third power function of valve gaping distance.

Recently, it has become more common to use a group of free-range bivalves as tested specimens to measure their valve movements to detect response from environmental stimulation (e.g.,

Table 1
A comparison for statistical profiles describing the magnitudes of shell gape of clams under SE and VC status.

Percentiles (%)	Magnitudes of shell gape (%)					
	Siphon extension				Valve closing	
	Sample data[a]	MCA[b]	Calibration[c]	Sample data[d]	MCA	Calibration[e]
2.5	49.26	49.86	51.49	0.30	1.21	0.16
50	75.21	75.12	76.89	5.08	5.15	4.97
97.5	96.06	96.12	95.52	20.06	20.22	17.08

[a] Using a visual observation of siphon extension to obtain the magnitudes of shell gape of 16 clams during 7 days ($n = 334$).
[b] Monte Carlo analysis ($n = 10{,}000$).
[c] Using our developed valvometry determining system to measure the magnitudes of shell gape of 16 clams during 14 days ($n = 19{,}447$).
[d] Using a visual observation of valve closing to obtain the magnitudes of shell gape of 16 clams during 7 days ($n = 494$).
[e] Using our developed valvometry determining system to measure the magnitudes of shell gape of 16 clams during 14 days ($n = 48{,}930$).

Please cite this article in press as: Jou, L.-J., et al. Synthesis and measurement of valve activities by an improved online clam-based behavioral monitoring system. Comput. Electron. Agric. (2012), http://dx.doi.org/10.1016/j.compag.2012.09.008

Table 2
A comparison for electromagnetic principle-based valvometry sensors.

Valvometry sensors	Target species	Glued valve position	Relationship[a]	Measured valve movements
Hall effect element and magnet	Akoya pearl oyster[b] Manila clam[c]	Vavle edge	Curve fitting[g]	AVO (mm) DVO (%, h day^{-1}) FVA (number h^{-1})
Hall effect transducer and magnet	Black Sea mussel[d] (Mediterranean)	Specific point	Not reported	AVO (mm)
A pair of electrodes (electric coils)	Oyster[e]	Unspecified	Calibration model[h]	AVO (mm, %) VO/VC velocity (mm s^{-1}) DVO (%, h.day^{-1}) FVA (number.time^{-1})[j] VC percentage (%)
A pair of inductances	Asiatic clam[f]	Specific range	Model fitting[i]	AVO (mm, %) VO/VC velocity (mm s^{-1}) DVO (%, h day^{-1}) FVA (number time^{-1}) VO/VC percentage (%)

[a] Between valve gaping (mm) and output voltage of a valvometry sensor (V).
[b] Adopted from Nagai et al. (2006).
[c] Adopted from Basti et al. (2009).
[d] Adopted from Gnyubkin (2010).
[e] Adopted from Tran et al. (2010) and Sow et al. (2011).
[f] This study.
[g] $V_h = 700473{\cdot}L^{-2} - 75.42$, $R^2 = 0.9998$ where V_h is the output voltage (μV) of a Hall element and L is the distance (mm) between a Hall element and a magnet.
[h] $V_D = 151.(D)^{-0.35} - 1.48$, $R^2 = 0.99$, P value < 0.001 where V_D is the measured signal (mV) produced from the transforming coil and D is the distance (mm) between the point of measurement and the center of the transmitting coil.
[i] $V_{im2}\ (V) = (a \cdot G^2 + b)^{-1.5}$, $a = 0.037579033$, $b = 0.1810587$ ($R^2 = 0.9971$).
[j] Number h^{-1} or number day^{-1}.

noxious dinoflagellates, illumination, toxicants, harmful algae, water quality, etc.) (Nagai et al., 2006; Basti et al., 2009; Gnyubkin, 2010; Sow et al., 2011). Basti et al. (2009) used a noninvasive Hall element sensor-automated data acquisition system developed by Nagai et al. (2006) to continuously measure the valve movement behavior of six to eight Manila clams *Ruditapes philippinarum* exposed to the toxic dinoflagellate *Heterocapsa circularisquama*. By quantifying three parameters—(1) the duration of valve opening (DVO, %), (2) the amplitude of valve opening (AVO, mm) and (3) the frequency of valve adductions (FVA, adductions per hour)—Basti et al. (2009) analyzed and evaluated toxic effects on the valve activities of these observed specimens under a mobile condition. According to the previous study adopted from Markich (1995), a sampling rate in the valvometry measuring system required at least two measurements per second to adequately and accurately measure and show the most subtle valve movement patterns (e.g., FVA).

Thus, we keyed in on 0.5 s as our sampling interval (i.e., sampling rate: 32 Hz) in our improved valvometry measuring system, which can observe the FVA pattern in valve activities (as shown in Fig. A3 in Supporting information). A valve position sensor based on a Hall-effect transducer attached to one shell valve and a permanent magnet glued to the other one was also used to measure the dynamic range of valve opening in 4–12 Mediterranean mussels *Mytilus galloprovincialis* under free-movement circumstances to characterize their reproducible valve movements in order to look for the influence of the light mode on the rhythmic parameters using a Fourier analysis (Gnyubkin, 2010). For obtaining behavioral responses in a group of immersed oysters in order to assess environmental impact from changes in water quality or harmful matters, a pair of small possible electrodes (coils) and wires were used as a valvometry sensor, so as not to interfere with the oysters' movements, to measure their valve activities in a spontaneous state (Tran et al., 2010; Sow et al., 2011).

In the present work and previous researches (Markich et al., 2000; Tran et al., 2003; Ortmann and Grieshaber, 2003; Basti et al., 2009; Sow et al., 2011), a high variability in valve activities (e.g., DVO, FVA, the valve gaping (mm) and the magnitude (%) of shell gape) between individuals was found. Sow et al. (2011) developed appropriate statistical modeling to approach and analyze the high-frequency valvometry data of 16 oysters living in the field station with valve activity measured over 183 days. Analogously, Gnyubkin (2009) used an analog monitoring mode (the summator/averager of voltages) to form the mean voltage of all working Hall sensors to use as a trigger of an early warning system to generate an alarm signal based on the determined high/low limits. In this work, we also made a screening of body size/weight of clams to choose tested specimens to decrease variability in valve movement between individuals (Markich, 2003). Synchronously, we used a computational mechanism (expression (8)) of the relative quantification to normalize the valve opening magnitude (%) of each clam without respect to the difference in body size among individuals. The statistical analysis of the valve movements in a group of clams was performed in all experiments to objectively assay valve activities and characterize the valve behavioral rhythm.

In the present work, we focused on synthesis and measurement of valve activities by an improved online clam-based behavioral monitoring system excluding the implementation of an early warning function to monitor water quality in an aquatic environment. By adding peripheral devices (e.g., a wireless emitter-receptor, a mobile communicator) and package development for assessing water quality (Sow et al., 2011), we believed that the bivalve species and our improved real-time valvometry measuring system could be efficiently used as a bivalve-based biomonitoring system. However, field experiments are necessary to provide further evidence to confirm the implementation of this monitor in a field where some factors (e.g., temperature, season, photoperiod, etc.) may disturb the valve movements.

5. Conclusions

For the improved real-time valvometry measuring system, we combined a free-range valvometry determining technique with the mini-inductance-based valvometry sensors glued on the valve to provide a low-stress determining condition for allowing tested clams in near unhindered and spontaneous situations that can promote the measuring precision of recording valve movements. We confirmed that this study provides a promising methodology to develop an improved online clam-based behavioral monitoring system based on a valvometric instrumental conversion technique combined with reliable statistical approaches for evaluating the valve behavioral reaction when exposed to a site-specific aquatic environment. In future work, we also confidently believe that such a valvometry monitoring system can be implemented and realized to determine valve activities in other bivalves (e.g., *D. polymorpha*, *M. edulis* and *V. angasi*) by performing the similar measuring protocol and following the basic sensing principles. Furthermore, this valvometry technique can be used to study the sensitivity of the valve-closure response following an addition of a contaminant in aquatic ecosystems.

Acknowledgements

This work was supported by the National Science Council of Republic of China under Grant NSC99-2313-B197-002.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.compag.2012.09.008.

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