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A dynamic artificial clam (*Corbicula fluminea*) allows parsimony on-line measurement of waterborne metals

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A dynamic artificial clam allows on-line biomonitoring waterborne metal toxicity.

Abstract

We introduce a novel on-line biomonitoring system based on a valvometric conversion technique for clam *Corbicula fluminea*, allowing for rapid, continuous, and ecological relevant water quality control. Our model builds upon the basic principles of biological early warning system model in two ways. We first adopted a risk-based methodology to build a dynamic artificial clam for simulating how the bivalve closure rhythm in response to waterborne copper (Cu) and cadmium (Cd). Secondly, we integrated a probabilistic model associated with the time-varying dose–response relationships of valve closing behavior into the mechanisms of a dynamic artificial clam, allowing estimation of the time-varying waterborne Cu/Cd concentrations for on-line providing the outcomes of the toxicity detection technique. Measurements with Cu/Cd were performed and the calculated EC50 values were compared with published data for the valve movement test with *C. fluminea*. This proposed dynamic artificial clam provides a better quantitative understanding of on-line biomonitoring measurements of waterborne metals and may foster applications in clam farm management strategy and ecotoxicological risk assessment.

Keywords: Clam; *Corbicula fluminea*; Biological early warning system; Metal toxicity; Biomonitor; Valve movement

1. Introduction

In Taiwan brackish-water and freshwater bivalves (mainly *Corbicula*) are appreciated for their delicacy and are generally used as an ingredient of soup. Middle-aged and elderly people are attracted to the bivalve extract because they expect it to improve their liver function. Many food products and health drinks containing the brackish-water and freshwater bivalve extract are now available on the market with a widely varying ornithine (Uchisawa et al., 2004). Recently, it has been reported ornithine promotes the secretion of the growth hormone and builds muscle (Davenport et al., 1990; Bucci et al., 1990). Ornithine is thus attractive as an ingredient of dietary supplements. Wu and Shiau (2002) indicated that a freshwater clam or hard clam extract (or referred to as clam essence) contained more ornithine than that in a chicken or beef essence. This evidence makes brackish-water and freshwater *Corbicula* commercially important and has a high market value to Taiwan's aquaculture and the aquaculture of *Corbicula* a promising business.

The major *Corbicula* farming sites are clustered at Chunghua, Yunlin, Chaiyi, and Tainan located at the western coastal areas of Taiwan region. In these areas, since the land-subsidence caused by overusing groundwater for aquaculture was serious, the major farming strategies of *Corbicula* are ploy-culture by mixing seawater and freshwater to reduce

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aquaculture freshwater demand and groundwater dependence. However, the coastal regions of Taiwan where the clam farms situated are subjected to contaminated discharges from rivers. If waterborne toxicants are elevated, pollutant-induced changes in the mobility can occur, which present potential risk to the health of clam, resulting in reduced market prices and the closure of clam farms.

Continuous and rapid detection of environmental toxicity caused by waterborne metals is of great value for conserving aquacultural ecosystems and protecting species health. Over more than one decade, many researchers have been developed and used aquatic organisms as a biological early warning system (BEWS) to continuous automated monitoring of water supplies and effluents in that the fundamental components, design, and operating parameters of aquatic BEWS have been reviewed elsewhere (Gruber and Diamond, 1988; Kramer and Botterweg, 1991; Gerhardt et al., 1998; Van der Schalie et al., 2001). Bivalves were the sentinel organisms originally selected for BEWS, and they continuous to be a popular choice (Sloof et al., 1983; Doherty et al., 1987; Borcherding, 1994; Sluyts et al., 1996; Borcherding and Jantz, 1997; Curtis et al., 2000; Tran et al., 2003, 2004; El-Shenawy, 2004).

The principle of a BEWS is that test organisms react immediately to pollution peaks with changes in their physiology and behavior (Gruber and Diamond, 1988). These systems are used to protect freshwater ecosystems by giving early warning of toxic discharges, providing data about possible permit violations and the need for secondary treatment. They also allow for field validation of laboratory toxicity bioassays. Valve movement is a characteristic feature of many bivalves and part of their natural behavior and it cannot be neglected as an important physiological factor for their survival. Changes in the valve movement rhythm of bivalves can therefore be used as a suitable endpoint in ecotoxicological risk assessment.

There are various classical methods for waterborne metals detection. These include atomic absorption (AAS), frame (AFS) and emission (AES) spectroscopy, and inductively coupled plasma-mass spectrometry (ICP-MS). These methods are characterized by low detection limits, yet require expensive instrumentation and cannot be used for field measurements. Therefore, the need for a parsimonious, easy to handle, and highly sensitive detection method is obvious. A living organism can detect true real-time toxic reactions more easily. Therefore, our goal is to prove or develop a rapid and permanent working biosystem for the detection of toxic material that would use naturally occurring biosensors, making the system practical and cost-effective.

Recent technological advances have allowed the development of robust, relatively compact, low cost, rapid response biomonitors with sufficient sensitivity and specificity to quantify many waterborne metals in the aquatic ecosystems. BEWS detects developing toxicity by continuously tracking the physiological responses of whole organisms (Kramer and Botterweg, 1991). Because BEWS measures toxicity, they provide an important complement to available chemical monitoring technology. Biological measures of water quality may detect materials that analytical chemistry techniques cannot, because of inadequate detection limits or methodological limitations. More importantly, biological measures can detect unsuspected materials and evaluate the toxic action of mixture of multiple chemicals. BEWS is particularly useful for detecting intermittent toxic events in the environment. Continuous, real-time information on time-variable toxicity levels is important to environmental managers who need to understand point source and non-point source impacts in an aquatic ecosystem, and evaluate whether surface water is of suitable quality for use in aquacultural farms. Neither traditional toxicity tests nor chemical-specific sensors can provide comprehensive, real-time information on toxic events in an aquatic system.

Various protocols have been established using bivalves to monitor the impact of pollutants in aquatic ecosystems. Although lethality is often the endpoint, addressing sublethal toxicity (i.e., changes in reproductive output or changes in behavior) may be more useful, as mortality dose not always occur in organisms exposed to pollutants under natural settings (Weis et al., 2001; Perez and Wallace, 2004). Behavioral endpoints are particularly useful as: (1) they are relatively easy to assess, (2) they are ecologically relevant, (3) they can link toxicity at the biochemical/cellular level to impacts on populations and communities, and (4) they are useful indicators of sublethal exposure in both laboratory and field settings (Weis et al., 2001; Perez and Wallace, 2004).

Our present system uses sublethal changes in the daily valve opening/closing activities of *Corbicula fluminea* as a biological endpoint. The objective of the presented research was to develop an automated on-line biological monitor system as a bioassay tool to measure waterborne metal levels in clam farms in a real-time mode. We introduce a new on-line biomonitoring system based on a valvometric conversion technique for clam *C. fluminea*, allowing for rapid, continuous, and ecological relevant water quality control. We describe the biomonitoring strategies that we have developed and present representative aquacultural ambient water concentration to demonstrate their utility. Cadmium (Cd), a non-essential and priority pollutant and copper (Cu), a micronutrient for both plants and animals at low concentrations were chosen as model toxicants because it is toxic to *C. fluminea* at elevated concentrations and have been related to behavioral toxicity.

2. Materials and methods

2.1. System design algorithm

Our system design was mainly divided into two phases. In the first phase, we adopted a risk-based methodology to build a dynamic artificial clam for simulating the bivalve closure response rhythm in response to waterborne Cu and Cd. Secondly, by integrating a probabilistic model associated with the simulated data of valve opening/closing response behavior into the mechanisms of a dynamic artificial clam, allowing estimation of the time-varying waterborne metal concentration for on-line providing the outcomes of the toxicity detection technique. The constructed procedures of designing this system were interpreted in the subsequent sections.

2.2. A risk-based dynamic artificial clam response

Liao et al. (2005) have incorporated the reconstructed dose–time–response profiles into the fitted model of valve closure daily rhythm to successfully predict the time-varying bivalve closure response and assess behavioral endpoint for clams exposed to waterborne Cu/Cd. The mechanisms of a dynamic artificial clam respond to waterborne Cu/Cd were constructed followed a risk-based scheme proposed by Liao et al. (2005). Table 1 lists the essential mathematical expressions used to describe the dose–time–response relationships and daily rhythm of valve closing exposed to waterborne Cu/Cd.

We firstly used an empirical three-parameter Hill equation model (Table 1, Eq. (11)) based on the % response versus μg Cd/Cu L^{-1} in water to accordingly fit the observed data of the dose–response profiles published by Tran et al. (2003, 2004) at different integration times Δt of response (Fig. 1A). The dose–response model shown in Eq. (11) where R is the measured response (% response), EC50 is the metal concentration yielding half of maximal response of $R_{\max}$ (μg L^{-1}), C_w is the waterborne metal concentration (μg L^{-1}), and the exponent n is a fitted average Hill coefficient. The EC50 values were calculated as the effect concentration of Cd/Cu causing 50% of total valve closure response of clam at different integrated times of response of 10, 15, 30, 60, 120, and 300 min. A time-varying function of EC50(Δt) for Cu was adopted from Tran et al. (2004) (Table 1, Eq. (12)). The function EC50(Δt) for Cd (Table 1, Eq. (13)) was a fitted non-linear regression to EC50–time relationships derived from Hill model.

The computational relationship between the Hill model and different integrated times Δt of response was illustrated in Fig. 1A, B. The time-varying function of $n(\Delta t)$ for Cu/Cd was obtained by a non-linear regression fitting the response time-dependent n values (Table 1, Eqs. (14) and (15)) in that the computational relationship between the fitted n value of the Hill model and different integrated times Δt of response was shown in Fig. 1A, C. The Hill equation model was adopted because it validates the observations of published studies, indicating that bivalve closure response is dependent on the response time Δt and waterborne metal concentration C_w. The Hill model-based dose–response function at any integrated time Δt of response therefore could be obtained (Table 1, Eq. (16) and Fig. 1D) followed the algorithm shown in Fig. 1A–C.

We employed the reconstructed time-varying dose–response profiles (Fig. 1A) to derive the EC50–time relationships (Fig. 1B) obtained from published data and the fitted non-linear regressions of the response time-dependent n values (Fig. 1C) for obtaining the bivalve closure response featured as a surface response function of the response time Δt and waterborne metal concentration C_w (Fig. 1D). Fig. 1E shows the valve daily closing rhythm exposed to uncontaminated environment in that observations (Fig. 1E) reported by Tran et al. (2003) were fitted by a three-parameter lognormal model (Table 1, Eq. (17)) (Liao et al., 2005). The function $\phi(t)$ shown in Eq. (17) is the daily rhythm function of valve closing proportion at any given time t. The surface response function of bivalve closure response (Fig. 1D) incorporated with the fitted model of valve daily closing rhythm exposed to uncontaminated environment (Fig. 1E) was used to predict the bivalve closure response rhythm in response to waterborne Cu/Cd (Fig. 1F).

We modified the mathematical description for the bivalve closure rhythm in response to waterborne Cu/Cd (Table 1, Eq. (18)) where $\phi(t, C_w)$ is the daily

Table 1
The mathematical descriptions adopted from Liao et al. (2005) used to describe the dose–time–response profiles of valve movement and *C. fluminea* daily closure rhythm in response to waterborne Cu/Cd (see text for the meanings of symbols)

Mathematical Expressions	
Hill model-based dose–response model	
$R = \dfrac{R_{\max} C_w^n}{\mathrm{EC50}^n + C_w^n}$	(11)
Time-varying function EC50(Δt) for Cu[a]	
$\mathrm{EC50_{Cu}}(\Delta t) = 3.76 \exp\left(\dfrac{91.9}{23.99 + \Delta t}\right), \quad r^2 = 0.996$	(12)
Time-varying function EC50(Δt) for Cd	
$\mathrm{EC50_{Cd}}(\Delta t) = 2.92 + 50.19 \Delta t^{-1.212}, \quad r^2 = 0.988$	(13)
Time-varying function of $n(\Delta t)$ for Cu	
$n(\Delta t)_{\mathrm{Cu}} = 1.396 \exp\left(-\dfrac{\Delta t}{0.379}\right) + 1.284, \quad r^2 = 0.97$	(14)
Time-varying function of $n(\Delta t)$ for Cd	
$n(\Delta t)_{\mathrm{Cd}} = 1.45 \exp\left(-\dfrac{\Delta t}{1.21}\right) + 0.417, \quad r^2 = 0.94$	(15)
Hill model-based dose–response function at any integrated times Δt of response	
$R(\Delta t, C_w) = \dfrac{100 C_w^{n(\Delta t)}}{[\mathrm{EC50}(\Delta t)]^{n(\Delta t)} + C_w^{n(\Delta t)}}$	(16)
Fitted three-parameter lognormal models to valve daily closing rhythm function ($\phi(t,0)$) exposed to uncontaminated environment	
$\phi(t,0) = \begin{cases} \phi_1(t,0) = 12.3 \exp\left[-0.5\left(\dfrac{\ln(\frac{t}{4})}{0.2}\right)^2\right] + 3.8, \quad 0 \le t \le 7, \ r^2 = 0.84 \\ \phi_2(t,0) = 14.8 \exp\left[-0.5\left(\dfrac{\ln(\frac{t}{18.2})}{0.083}\right)^2\right] + 3.6, \quad 7 < t \le 24, \ r^2 = 0.92 \end{cases}$	(17)
Bivalve closure response rhythm in response to waterborne Cu/Cd	
$\phi(t, C_w) = \phi(t,0) + R(\Delta t, C_w)[1 - \phi(t,0)]$	(18)

[a] Adopted from Tran et al. (2004).

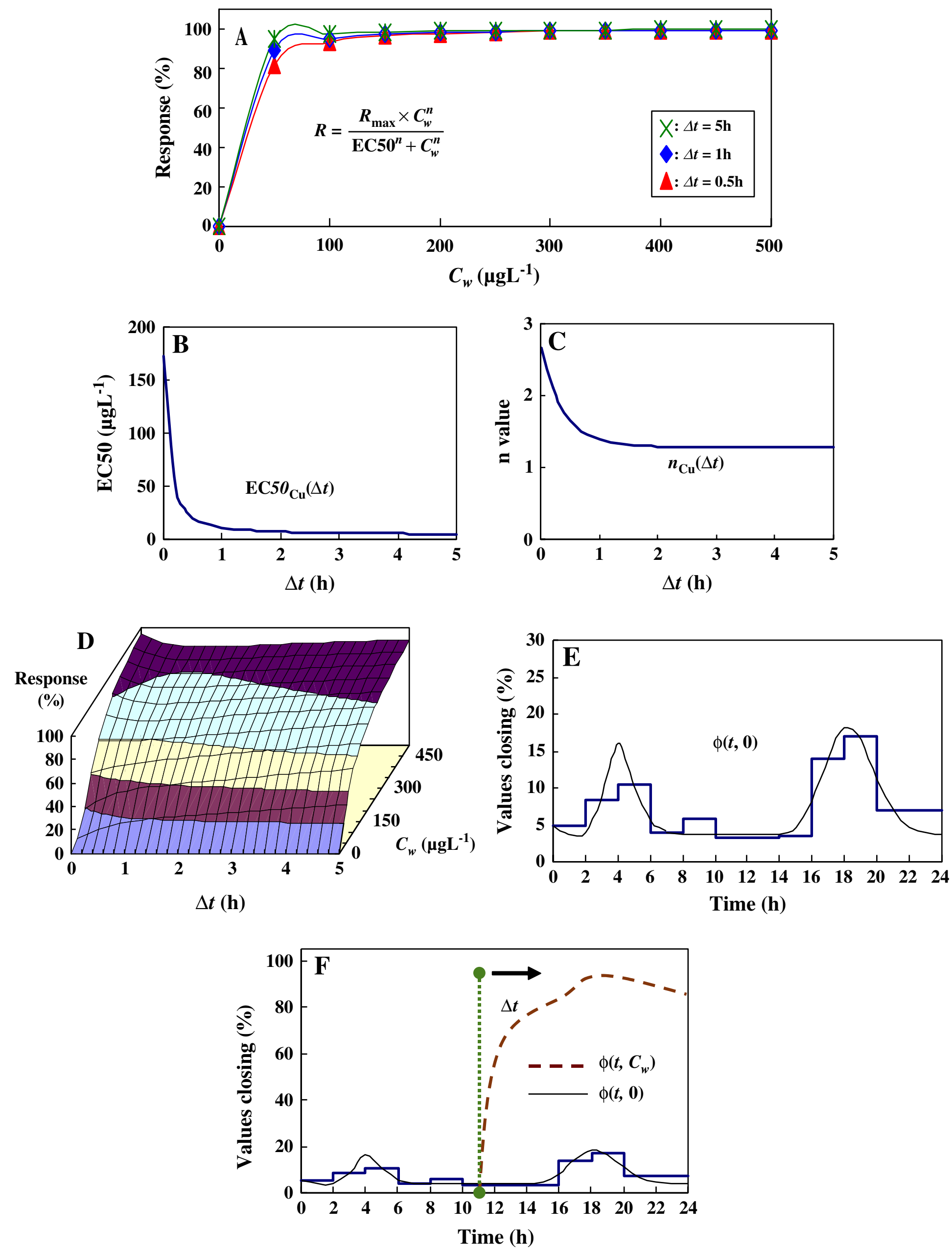

Fig. 1. A conceptual flowchart showing a risk-based methodology of designing a dynamic artificial clam consists of (A) the observed data of the Cu concentration–response profiles published by Tran et al. (2003, 2004) at different integration times Δt of response, (B) a Cu–EC50–time relationship was derived from Hill model to obtain the EC50(Δt), (C) the function of $n_{Cu}(\Delta t)$ was obtained from Hill model, respectively, by a non-linear regression fitting the response time-dependent n values, (D) the bivalve closure response is a surface function of the response time Δt and waterborne metal(Cu) concentration C_w, (E) the valve daily closing rhythm exposed to uncontaminated waterborne and using a three-parameter lognormal models to best fit the published data, and (F) the simulated *C. fluminea* valve closure response rhythm in response to waterborne metal.

rhythm function of valve closing at any given time t (h) and waterborne Cu/Cd concentration C_w ($\mu g\,L^{-1}$), $\phi(t,0)$ is the daily rhythm function of valve closing exposed to uncontaminated water and $\phi(t,0)$ has the same form as $\phi(t)$. We assumed that waterborne metal concentration (C_w) is a time-varying function,

$$C_w(t) = f(t)u(t-a), \quad u(t-a) = \begin{cases} 0 & t < a \\ 1 & t \geq a \end{cases}, \tag{1}$$

where $C_w(t)$ is a time-varying function of waterborne metal concentration, $f(t)$ is a function at any given time t, a is the occurred time of pollution, and $u(t-a)$ is a step function. When the detection time t is earlier than the occurred time a, the integrated time Δt of response is regarded as zero,

$$\Delta t = t - a, \quad \text{if } t < a \text{ then } \Delta t = 0. \tag{2}$$

We integrated exposure profiles of measured environmental metal concentrations and modeling bivalve daily rhythm function with effect analysis (time-varying dose–response profiles and EC50–time profiles) to develop the mechanism of a dynamic artificial clam for quantifying *C. fluminea* valve closure response rhythm in response to waterborne Cu/Cd.

Our system design processes were implemented and illustrated by employing a LabVIEW graphic control program language (Version 7.0, NI Inc., North

Mopac Expressway, Austin, USA) in a personal computer to dynamically on-line simulate the clam daily opening rhythm in response to waterborne metals in various time-varying scenarios. The constructed framework and block diagram of a dynamic artificial clam was shown in Fig. 2 featuring three functions of dose–response, clam valve daily rhythm in response to metals, and clam valve daily rhythm, respectively. The input and output blocks (Fig. 2) of a dynamic artificial clam were, respectively, represented as the time-varying Cu/Cd concentration in water ($C_w(t)$) and bivalve daily closure rhythm ($\phi(t, C_w(t))$) in response to waterborne Cu/Cd. The mechanistic model of a dynamic artificial clam consists of the time–dose–response profiles of *C. fluminea* valve for Cu/Cd ($R(\Delta t, C_w)$), the fitted daily rhythm function of valve closing exposed to uncontaminated water ($\phi(t, 0)$), and the integrated mechanism of clam daily rhythm.

2.3. Toxicity detection mechanism of dynamic artificial clam

Fig. 3 shows how we construct the framework and mechanisms of toxicity detection using a dynamic artificial clam-based methodology. Thanks to the previous excellent research work (Tran et al., 2003; Ortmann and Grieshaber, 2003), we further employed the recordings of typical daily valve opening/closing activities of *C. fluminea* to build a visual simulation concerning the detective mechanisms for waterborne metals and the clam daily closing rhythm exposed in the contaminated and uncontaminated aquatic environments.

Tran et al. (2003) used 71 *C. fluminea* over a period of 50 days at water temperature of 15 ± 0.5 °C with pH ranged from 7.8 to 8.0 and fed continuously with a unicellular algae *Scenedesmus subspicatus* to determine the daily valve opening/closing rhythm. We obtained the average percentage of *C. fluminea* daily opening time for 30 days of $44.3 \pm 8.5\%$ (mean ± SD) that we converted into a 24 h-based value of 10.656 ± 2.04 h. We showed that normal distribution best fits the observed data of clam daily opening time (Fig. 3A, B). We implemented a Monte Carlo simulation to estimate the opening time of each clam and to further describe the various periods among these clams using the mathematical expressions. We employed a Crystal Ball® software (Version 2000.2, Decisioneering, Inc., Denver, Colorado, USA) to implement the Monte Carlo simulation. The mathematical descriptions of these procedures concerning how to construct the clam daily rhythm function were interpreted as follows.

We used a Monte Carlo simulation technique to obtain the probabilistic distribution of *C. fluminea* opening time. We divided 15 bins in the Monte Carlo simulation for each interval time (Fig. 3B): $\Delta t_i = (15.79 - 5.56)/15 = 0.682$ h $= 40.92$ min. The occurring probability of opening time can be expressed as,

$$P(t_i) = \frac{n_{t_i}}{N}, \quad \sum_{i=1}^{15} n_{t_i} = N, \quad i = 1, \ldots, 15, \tag{3}$$

where $P(t_i)$ is the occurring probability of opening time t_i, N is the total number of bivalves and n_{t_i} is the number under the condition of opening time t_i. We used Eq. (3) to estimate accordingly the number of bivalves under the various opening times.

In view of a periodical step function, the daily rhythm function of a bivalve could be represented as an analogous mathematical pattern. We define and analyze the daily rhythm of one bivalve using the following mathematical expressions,

$$\begin{aligned} &f_j(t) = f_j(t+T) = \left[u(t-a_j) - u(t-b_j)\right], \; j = 1, \ldots, N, \; 0 \le t \le 24\,\text{h}, \\ &t_i = b_j - a_j, \; u(t-a_j) = \begin{cases} 0 & t < a_j \\ 1 & t \ge a_j \end{cases}, \; u(t-b_j) = \begin{cases} 0 & t < b_j \\ 1 & t \ge b_j \end{cases}, \end{aligned} \tag{4}$$

where $f_j(t) = f_j(t+T) = \left[u(t-a_j) - u(t-b_j)\right]$ is a periodical step function, in which $u(t-a_j)$ and $u(t-b_j)$ are step functions, T is a 24-h period, a_j is the start time of opening, and b_j is the end time of opening.

The mathematical description of valve opening proportion can be expressed as (Fig. 3C),

$$P(\Delta T_k) = \frac{\sum_{j=1}^{N} f_j(t)\big|_{T_{k-1} \le t < T_k}}{N}, \quad k = 1, \ldots, 12, \tag{5}$$

where $P(\Delta T_k)$ is the proportion of valve opening in the interval time ΔT_k. In order to dynamically and vividly visualize the simulation of clam daily rhythm, we link Eqs. (3) and (4) associated with Eq. (5) and Fig. 3C to appropriately describe opening/closing behavioral periodical function of each clam (Fig. 3D) in the programs of a dynamic artificial clam.

By incorporating the surface response function ($R(\Delta t, C_w)$) of the response time Δt and waterborne metal concentration C_w with the fitted model of valve daily closing rhythm ($\phi(t, 0)$) exposed to uncontaminated environment, we simulate the bivalve closure response rhythm ($\phi(t, C_w)$) in response to waterborne Cu/Cd using the descriptive mathematical functions in the LabVIEW software. The difference between the bivalve rhythms exposed to uncontaminated and contaminated environments depends on the proportion of bivalve closure response. Thus, the waterborne metal concentration can be derived from the Hill equation model describing bivalve closure of *C. fluminea* in response to Cu/Cd as,

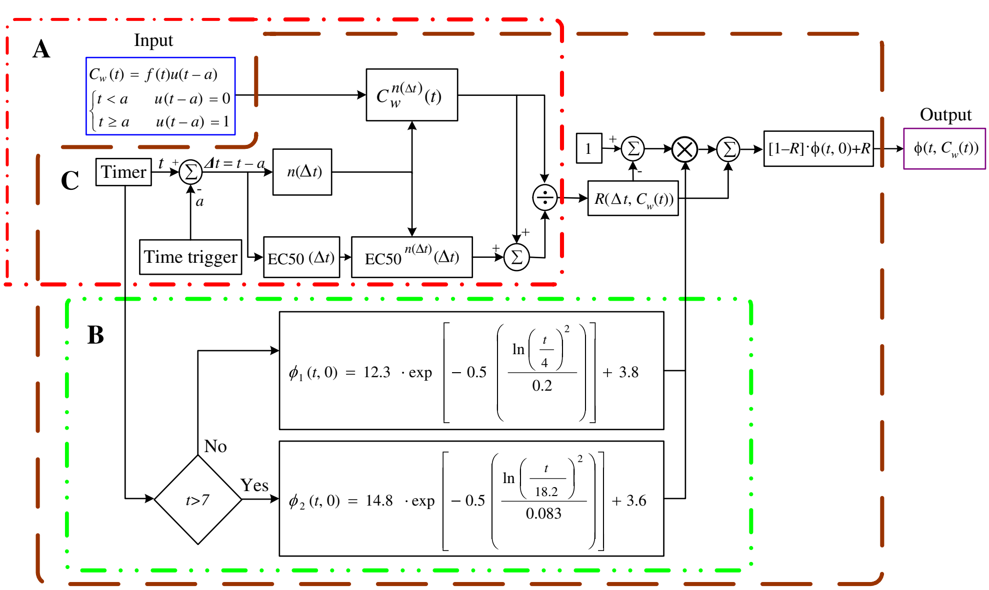

Fig. 2. The constructed framework of a dynamic artificial clam includes three major block diagrams: (A) the time–dose–response mechanism of valve movement of *C. fluminea* exposed to Cu/Cd, (B) the fitted daily rhythm function of valve closing exposed to uncontaminated water, and (C) the integrated mechanism of clam daily rhythm in response to waterborne Cu/Cd.

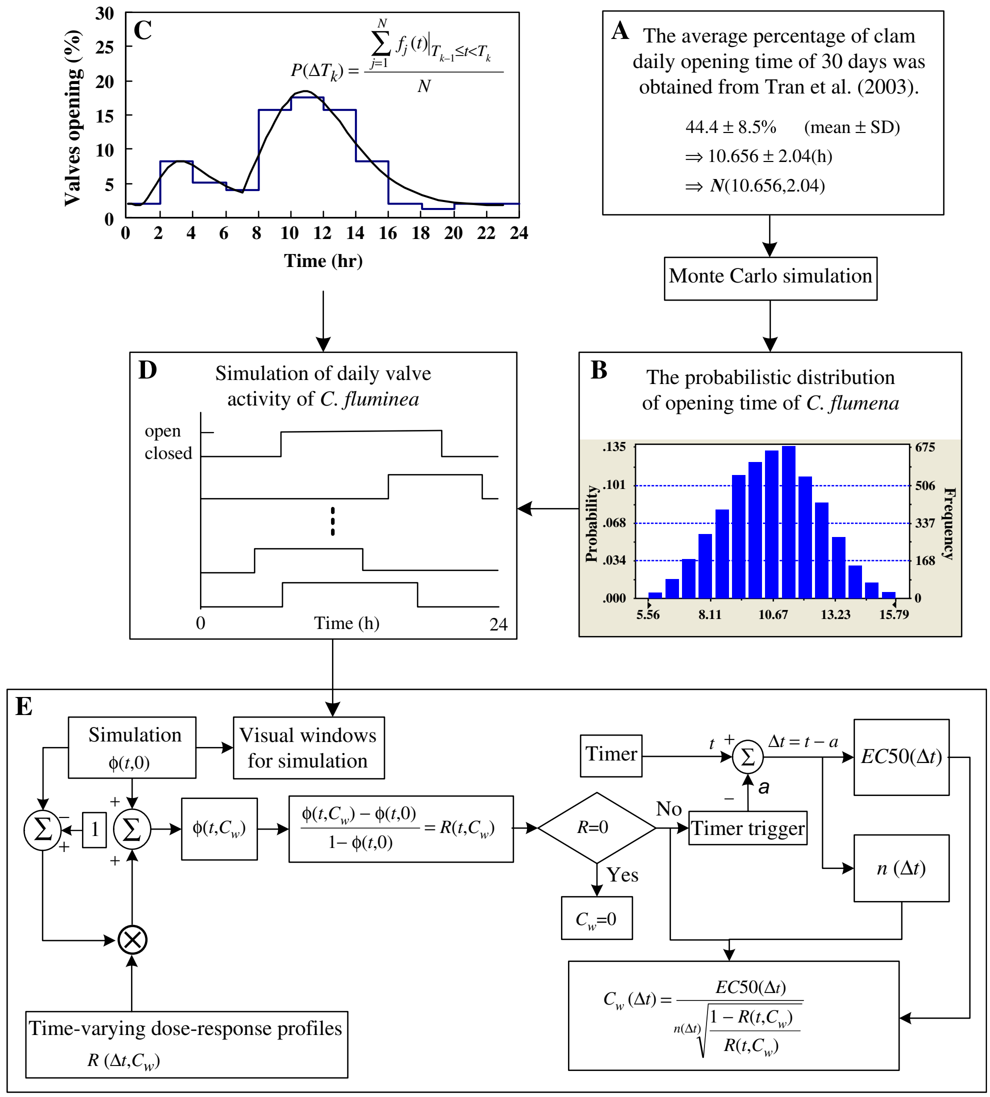

Fig. 3. A processing flowchart showing how to construct the framework and waterborne metals detection mechanisms using a dynamic artificial clam-based methodology: (A) the obtained average percentage of *C. fluminea* daily opening time for 30 days of $44.3 \pm 8.5\%$ (mean ± SD) was converted into a 24-h based value with a normal distribution N(10.656 h, 2.04 h), (B) a Monte Carlo simulation technique was used to obtain the probabilistic distribution of *C. fluminea* opening time, (C) valve daily opening rhythm ($\psi(t,0)$) exposed to uncontaminated environment showing the number of bivalves under the various opening times, (D) the simulation of daily valve activity of *C. fluminea* for representing opening/closing behavioral periodical step function of each bivalve in the programs of a dynamic artificial clam, and (E) detection mechanisms of a dynamic artificial clam exposed to waterborne metals.

$$C_w(\Delta t) = \frac{\mathrm{EC50}(\Delta t)}{\sqrt[n(\Delta t)]{\frac{1-R}{R}}}. \quad (6)$$

Thus by using a concept of a dynamic artificial clam, we integrate the simulated proportion of bivalve closure response exposed to contaminated environment and the time-dependent metal-specific functions EC50(Δt) and $n(\Delta t)$ for Cu/Cd into Eq. (6) to implement the detection mechanism of a dynamic artificial clam to waterborne metals (Fig. 3E).

3. Results

3.1. Bivalve monitoring interface design

We adopted the virtual instrumentation techniques to design and simulate a bivalve monitoring interface system for greatly reducing the costs, developed time and errors in implementation. Based on the mechanistic model of a dynamic artificial clam (Fig. 2), we integrated the virtual instrumentation techniques with a graphic control computational program language (LabVIEW) to create a human–machine monitoring interface (Fig. 4) featuring the bivalves daily closure rhythm in response to waterborne Cu/Cd. The display monitors were mainly divided into five panels (Fig. 4).

The basic input data and display information in monitor 1 (Fig. 4A) include the ratio of actual and simulation times, the options of waterborne metals (i.e., Cu or Cd), the time lag of pollution, a steady-state concentration of waterborne metal, and the present and accumulative response times. The profile of time-varying concentration of waterborne metals (Cu/Cd) was shown in the display monitor 2 (Fig. 4B). The profile of time-varying function of EC50(Δt) and $n(\Delta t)$ were, respectively, shown in the display monitors 3 and 4 (Fig. 4C). The display monitor 5 (Fig. 4D) shows the Hill model-based dose–response function ($R(\Delta t, C_w)$) at any given time Δt. The display monitor 6 (Fig. 4E) shows the daily rhythm variation of clam closing in response to waterborne Cu/Cd at any given time t (i.e., $\phi(t, C_w)$), indicating the statuses of valve closing exposed to contaminated and uncontaminated environment, respectively.

3.2. System testing

A system testing based on the designed windows of a bivalve monitoring interface was performed and illustrated in Figs. 4 and 5. We can, respectively, enter essential parameters including waterborne metal treated, various time constant settings, and a steady-state or a peak concentration of waterborne metal assigning, into the data/information display window (Figs. 4A and 5A). Waterborne metal concentrations were assumed as time-varying forms (Eqs. (1) and (2)), e.g.,

$$C_w(t, \Delta t) = C_w(\infty)\left[1 - \exp\left(-\frac{\Delta t}{\tau}\right)\right]u(t-a), \quad (7)$$

$$C_w(t, \Delta t) = C_{wp}\exp\left(-\frac{\Delta t - \alpha}{\beta}\right) \times \left[1 - \exp\left(-\frac{\Delta t - \alpha}{\beta}\right)\right]u(t-a), \quad (8)$$

where $C_w(t, \Delta t)$ is the time-varying waterborne metal concentration, $C_w(\infty)$ is the equilibrium water concentration, t is the time of a 24-h period, Δt is the response time after the contamination occurred, τ is a time constant of exponential function, C_{wp} is the peak value of waterborne metal concentration, α is the occurring lag time, and β is the reaching time of occurring peak value after contamination occurred.

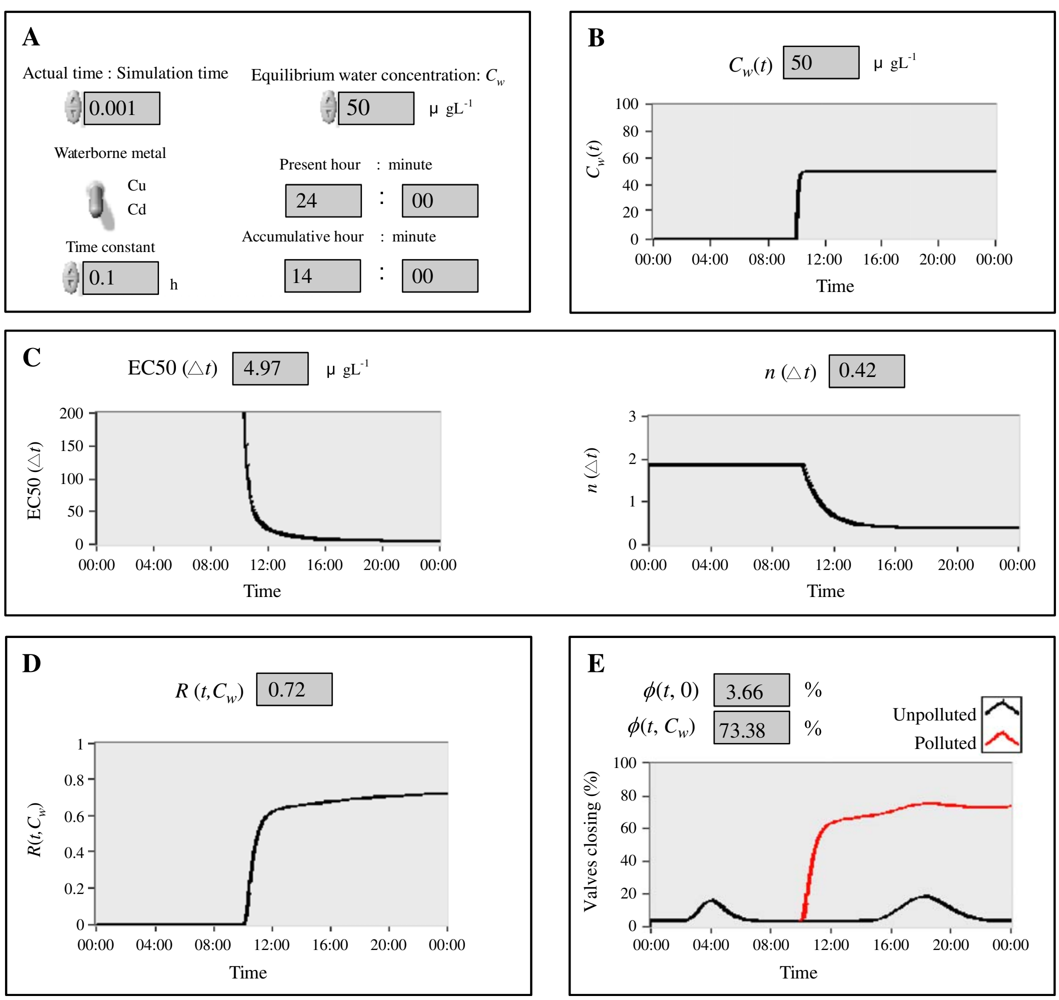

Fig. 4. A human–machine monitoring interface illustrating the function of a system testing with respect to the bivalve daily closure rhythm in response to waterborne Cu/Cd. The windows of a bivalve monitoring interface contains five panels: (A) input data and display information including (i) the ratio of simulation time (actual time: simulation time = 1 s: 1 ms), (ii) the options of waterborne metals (Cd), (iii) the time lag of pollution (time constant: 0.1 h), (iv) a steady-state concentration of waterborne metal ($C_w = 50$ μg Cd L^{-1}), and (v) the present and accumulative response times (present hour: minute and accumulative hour: minute), (B) the profile of time-varying concentration of waterborne Cd $C_w(t)|_{Cd} = 50(1 - \exp(-(t-10)/0.1)u(t-10))$, (C) the profile of time-varying function of EC50(Δt) and $n(\Delta t)$ for Cd, (D) the profile of the Hill model-based dose–response function ($R(\Delta t, C_w)$) at any given time Δt, and (E) the daily rhythm variation of clam closing in response to waterborne Cd at any given time t.

The input time-varying functions of waterborne Cd and Cu concentrations were expressed, respectively, as

$$C_w(t,\Delta t) = 50\left[1-\exp\left(-\frac{t-10}{0.1}\right)\right]u(t-10), \quad (9)$$

$$C_w(t,\Delta t) = 4\times 100\exp\left(-\frac{(t-10)-1}{3}\right)\times\left[1-\exp\left(-\frac{(t-10)-1}{3}\right)\right]u(t-10), \quad (10)$$

that were illustrated in the display windows shown in Figs. 4B and 5B, respectively. Subsequently, the variation of time-varying waterborne Cd/Cu concentrations and the variation of time-varying function of EC50(Δt) and $n(\Delta t)$ were accordingly illustrated in the display windows (Figs. 4C and 5C). The variation of time-varying dose–response function ($R(\Delta t, C_w)$) at any given time Δt was illustrated in the display windows shown in Figs. 4D and 5D. The daily rhythm variation of clam closing respond to waterborne Cu/Cd at any given time t (i.e., $\phi(t, C_w)$) can be simulated and illustrated in the display monitor shown in Figs. 4E and 5E, respectively.

3.3. Data interpretation

An integrated BEWS (Fig. 6) based on Fig. 3 is synthesized to simulate and demonstrate the outcomes and specificity with respect to the proposed detective mechanism. Fig. 6 demonstrates that we used the daily rhythm of 32 bivalves exposed to waterborne Cd as a simulated representation to interpret how to obtain the estimates of waterborne metal. The bivalve

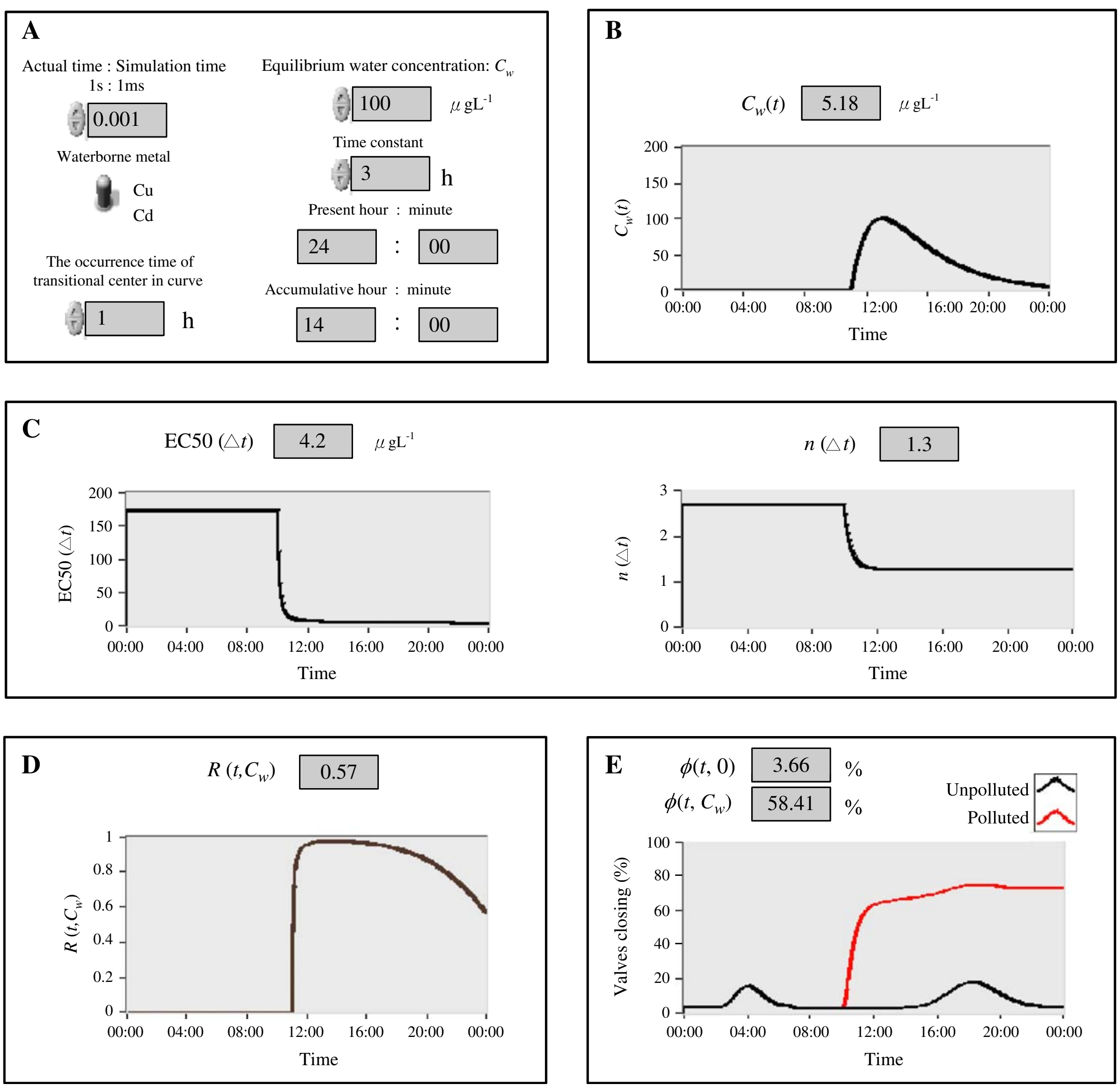

Fig. 5. Performance and results of the system testing based on the designed windows of a bivalve monitoring interface: (A) input essential parameters including waterborne metal treated (Cu), various time constant settings (time constant: 3 h), and a peak concentration of waterborne metal assigning (C_w: 100 μg Cu L^{-1}), were, respectively, keyed into the data/information display window, (B) the input time-varying functions of waterborne Cu concentration were expressed as $C_w(t,\Delta t) = 4\times 100\exp(-((t-10)-1)/3)[1-\exp(-((t-10)-1)/3)]u(t-10)$, (C) the profile of time-varying function of EC50(Δt) and $n(\Delta t)$ for Cu, (D) the profile of time-varying dose–response function ($R(\Delta t, C_w)$) at any given time Δt, and (E) the daily rhythm variation of clam closing respond to waterborne Cu at any given time t.

monitoring display windows (Fig. 6) were mainly divided into six panels for a dynamic artificial clam: (a) introduction panel: featuring operational procedures and basic assumptions (Fig. 6A), (b) data entry panel: basic data and information setting (Fig. 6B), (c) clam daily rhythm $\phi(t, C_w)$ display panel (Fig. 6C), (d) time-closing response $R(t, C_w)$ profile panel (Fig. 6D), (e) time-varying waterborne metals $C_w(t)$ display panel (Fig. 6E), and (f) simulated visual window of bivalve closure behavioral response (Fig. 6F).

By employing the simulated bivalve closure rhythm in response to Cd (Fig. 6C), we can evaluate the profile of time-closing response $R(t, C_w)$ (Fig. 6D) and incorporate the simulated proportion of bivalve closure response exposed to Cd and the time-dependent metal-specific functions $EC50_{Cd}(\Delta t)$ and $n(\Delta t)_{Cd}$ into Eq. (6) to estimate the profile of time-varying waterborne Cd (Fig. 6E).

4. Discussion

4.1. Operational and practical considerations for an artificial clam

4.1.1. Suitability of environmental conditions

Ortmann and Grieshaber (2003) pointed out that the freshwater *C. fluminea* acclimated at cold water temperatures during winter changed their valve movements and closure rhythm to depress the metabolic rate for reducing their heat dissipation and oxygen consumption. Fournier et al. (2004) studied the concentration–response profiles and sensitivity threshold for the valve closure of *C. fluminea* exposed to uranium during a 5-h period in the synthetic water at temperature 20 °C with two pH values (5.5 and 6.5), indicating the total uranium concentration, integration time of response and pH were the most significant influence parameters. Sluyts et al. (1996), Markich et al. (2003), and Tran et al. (2003) indicated that some physical and chemical variables in water such as temperature, pH, dissolved organic carbon, turbidity, dissolved oxygen level, which also affected the behavior and activity of the valve opening/closing to a greater or lesser extent.

Thus in the present operational and realistic constraining conditions, we have to consider the interactive effects of the dose–response and valve daily rhythm of *C. fluminea* exposed to various water temperatures and pH values to accurately implement the mechanisms of an artificial clam. Water temperature and pH value have to be taken into account in setting the monitoring parameters. Seasonal variations have shown the need to set different parameters related to the seasonal conditions (Ortmann and Grieshaber, 2003). In future work, we will incorporate the compensations of time-varying water temperature and pH values into the detection mechanisms of a dynamic artificial clam to precisely and completely respond to the practical statuses of suitability of environmental conditions for avoiding false-alarm detection of instrumental setting.

The most suitable farming conditions for freshwater clams *C. fluminea* in Taiwan were under the range values of water temperature 15–25 °C, salinity 0–2 ppt, pH 7.0–8.5, and D.O. 3–4 ppm (http://shell.sinica.edu.tw/english/program.php). In this research, we adopted the published experimental data of *C. fluminea* closure daily rhythm and dose–response profiles from Tran et al. (2003, 2004) based on the laboratory-acclimated bivalves to construct the mechanisms of a dynamic artificial clam. According to the published data, the experimental conditions of mean water temperature of 15 ± 0.5 °C with pH ranged from 7.8 to 8.0 that were different from the present farming environment in Taiwan region. Despite the uncertainty in many aspects in the problem of physical and chemical variables in water such as temperature, pH, oxygen level, which may modify the water metal concentrations, caution interpretation of observations obtained from the present dynamic artificial clam system can substantially reduce the likelihood.

4.1.2. Rapidity and sensitivity of response to environmental toxicants

Sensitivity and response time are correlated. Response time tends to vary inversely with toxicity (Gruber and Diamond, 1988). To obtain a rapid response time, a dynamic artificial clam uses either a physiological or a behavioral function of bivalves to monitor the fast changes. In this study we incorporate the closure daily rhythm of bivalves exposed to waterborne Cu/Cd into the bivalve concentration–response profiles to monitor the waterborne Cu/Cd. Based on the valve closure behavioral function depended on response time, concentration of waterborne Cd/Cu, and the developed detection mechanism of the proposed dynamic artificial clam, waterborne Cd above 50 $\mu g\, L^{-1}$ and Cu above 20 $\mu g\, L^{-1}$ can be, respectively, detected within less than 1 and 0.5 h (Tran et al., 2003, 2004).

Our present detection mechanisms of the dynamic artificial clam demonstrate that the detected threshold concentrations of dissolved Cd and Cu were estimated to be 16 and 4 $\mu g\, L^{-1}$, respectively, within 5 h of integration time after contaminant Cd/Cu occurred (Tran et al., 2003, 2004). Thus, the detection mechanisms of a dynamic artificial clam can only be implemented in such situations, indicating our proposed artificial clam is suitable in aquatic environments where at least 5 h transient rises above this value are expected. The other further limitations must be taken into consideration, i.e., the uncertainty/variability problems provoked by spatial and local conditions, e.g., photoperiod, light intensity and tropic additions, which may affect the daily rhythm activity and the sensitivity of the signal by exciting false responses (Englund and Heino, 1994; Higgins, 1980; McCorckle et al., 1979).

These limitations were not specific yet they seemed more suitable in the use of biosensors and cases in which pollution was acute. To our knowledge, the novel methodology of the detection mechanism based on a dynamic artificial clam has not been proposed until now. We believe that the detection mechanism designed by employing a dynamic artificial clam is an effective and measurable means to estimate waterborne metals by using the bivalves as biosensors for on-line biomonitoring the degree of pollution.

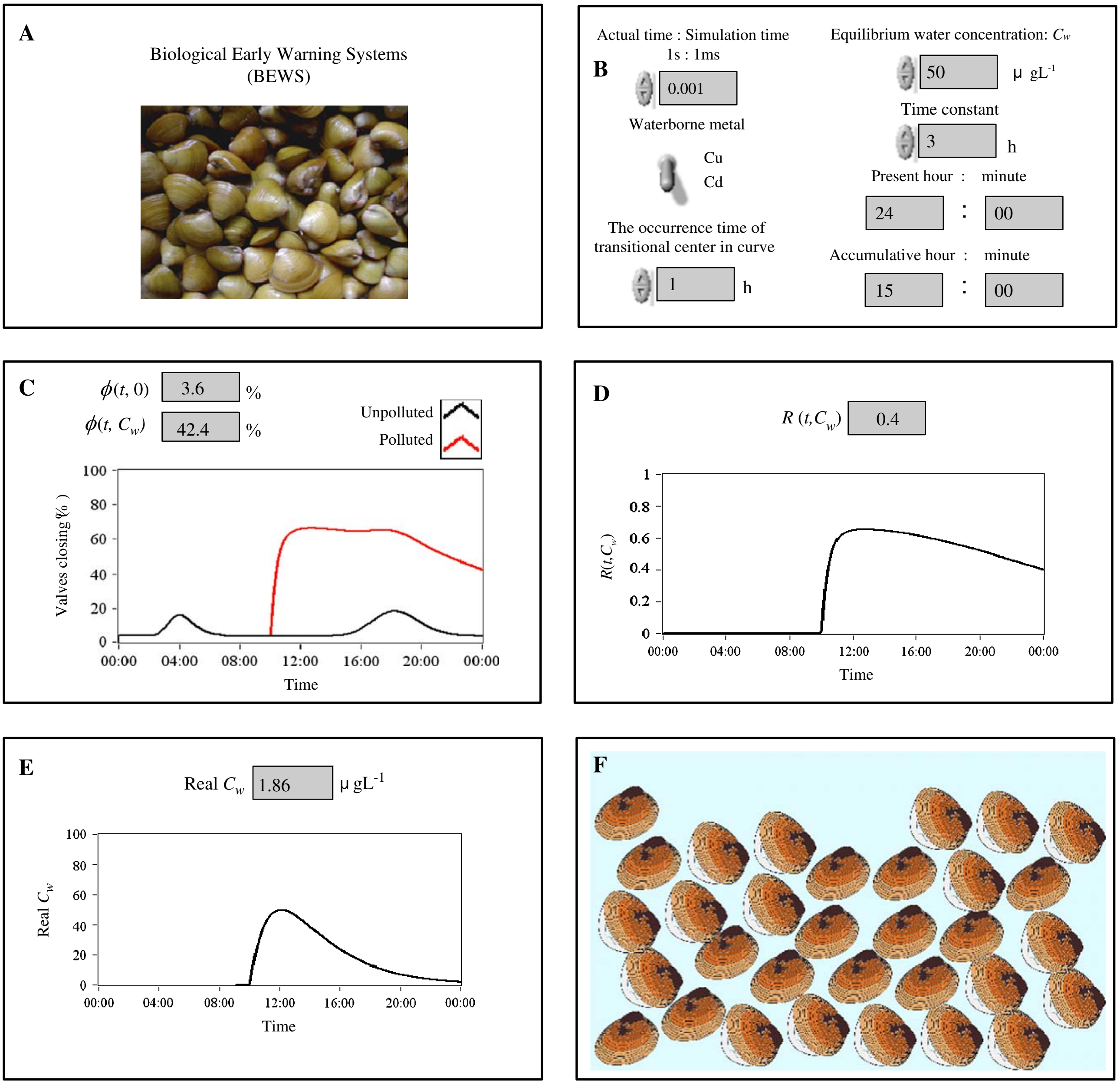

Fig. 6. Bivalve monitoring display windows mainly consists of (A) introduction panel: concerning operational procedures and basic assumptions, (B) data entry panel: basic data and information setting, (C) clam daily rhythm $\phi(t, C_w)$ display panel, (D) time-closing response $R(t, C_w)$ profile panel, (E) detected time-varying waterborne metals $C_w(t)$ display panel, and (F) simulated visual window of bivalve closure behavioral response.

4.2. Potential applications

4.2.1. Surrogate response signal

The freshwater clams *C. flumenia* are filter-feeder animals. They extend siphon from their bivalve shells to filtrate waterborne plankton or organic matter for uptake. Siphon extension related to the magnitude of shell gape (%) that was proportioned to the valve position as well as percentage of the shell span. When contaminants were discharged to waterborne, the bivalves exposed to contaminated aquatic environment reduced filtrating-uptake activity by closing their shells to escape toxicant damage and exclude themselves from the outside contaminated environment for maintaining their biotic faculty and increasing their survivability (Wildridge et al., 1998; Kadar et al., 2001). Based on the valve position, we can select two different monitoring responses with respect to behavioral activities of the clam as triggering alarms, i.e., closure (C) and filtration decreasing (D) alarms.

The C-alarm means that some bivalves isolate from the contaminated environment for a long time, i.e., occurs when a given number of bivalves at the same time show closure state. If the bivalve closes its shells lower than the set percentage of valve position, a timer is triggered, and if bivalve re-opens the shell to a degree higher than the set percentage, the timer is reset. When a given number of bivalves keep their closure state for a given number of minutes, the

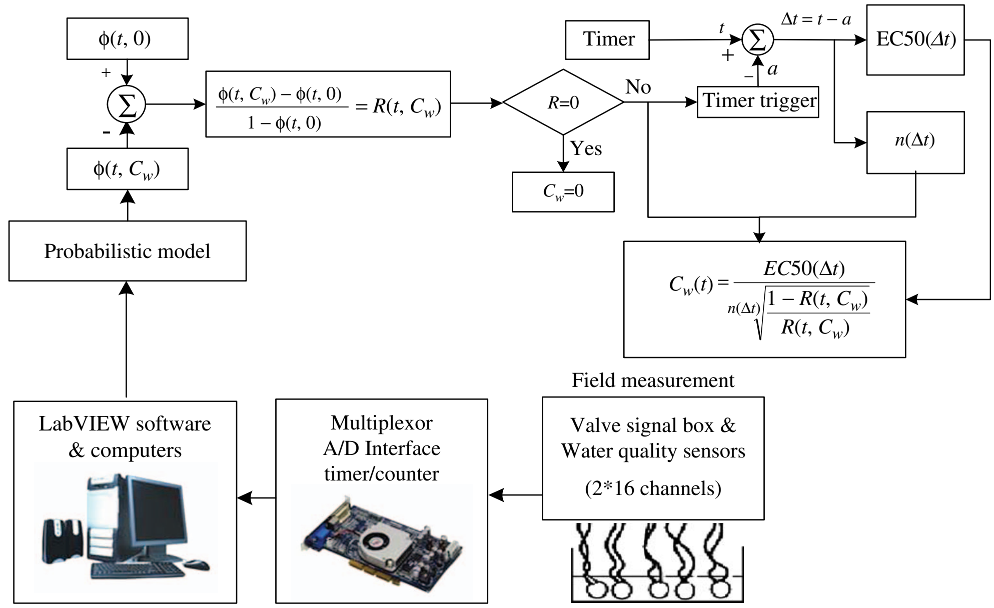

Fig. 7. The conceptual framework showing a field measuring mechanism of on-line waterborne metals detection implemented by employing a dynamic artificial clam-based methodology to monitor water quality in farmed freshwater clam *C. flumenia* ponds. A probabilistic-based model and auxiliary water quality sensors (e.g., temperature, pH, conductivity and dissolved oxygen) will be employed to more accurately observe and evaluate the monitoring of time-varying closure daily rhythm of bivalves in field for reliably providing a minimum false-alarm estimation to waterborne metals.

alarm threshold is triggered. In future research, we need to be able to set a proportion of clams in closure state and a closure time before alarm detection to avoid the occurrence of false alarm.

Decreasing average valve position (D-alarm) depends on the decreasing filtration activity, and could reveal the present level of pollution in the water. In the future work we can also consider both a more sensitive level and reliable range alarm signal to decrease the probability of false alarm.

4.2.2. System false-alarm rate

For any type of toxicity detection mechanism, false alarm is one important issue which needs to be especially addressed. The occurrence of false alarms may be provoked by equipment malfunctions or changes in water quality conditions. For instance, when precipitation causes simultaneous changes in temperature, dissolved oxygen, pH, whereas the suspended particulates in surface water containing toxic chemicals are washed into the aquatic environment. In this case, careful evaluation of response patterns is quite critical to identify the cause of alarm, and it is essential and auxiliary to monitor the common water quality parameters such as temperature, dissolved oxygen, pH and conductivity for reducing the occurrence probability of false alarms (Van der Schalie et al., 2001). We will employ a probabilistic-based model and auxiliary water quality sensors to carefully proceed and evaluate the monitoring of time-varying closure daily rhythm of bivalves in field (Fig. 7) for providing an accurate estimate of waterborne metals. When the accurate estimated metal concentration exceeds the threshold concentration (e.g., the effect concentration causing 10% of total valve closure response of clam at integrated response times of 10 min: EC10($\Delta t = 10$ min)) and sustains at a given number of minutes, the alarm threshold is triggered. We may also use a probabilistic technique, e.g., the Bayes identity to evaluate the false-alarm rate to show the relationships between failure-to-predict probability and the probability of alerts. Therefore, more parameters could be added to improve the performance further.

Aquacultural water quality problems are characterized by complex pollutant and pollutant precursor concentrations. The use of novel biomonitoring strategy, including those exploiting simulation techniques, to better characterize aquacultural ambient pollutant distributions and quantify sources fluxes is required to understand and address the water quality problems they create. This is particularly true in the aquacultural ecosystems, where the temporally and spatially undersampled data from conventional fixed water quality measurement sites is either limited or unavailable.

References

Borcherding, J., 1994. The "Dreissena-Monitor" – improved evaluation of dynamic limits for the establishment of alarm-thresholds during toxicity tests and for continuous water control. In: Hill, I.R., Heimbach, F. (Eds.), Freshwater Field Tests for Hazard Assessment of Chemicals. Lewis Publishers, Boca Raton, FL, pp. 477–484.

Borcherding, J., Jantz, B., 1997. Valve movement response of the mussel *Dreissena polymorpha* – the influence of pH and turbidity on the acute toxicity of pentachlorophenol under laboratory and field conditions. Ecotoxicology 6, 153–165.

Bucci, L., Hickson, J.F., Pivarnik, J.M., Wolinsky, I., McMahon, J.C., Turner, S.D., 1990. Ornithine ingestion and growth-hormone release in body builders. Nutrition Research 10, 239–245.

Curtis, T.M., Williamson, R., Depledge, M.H., 2000. Simultaneous, long-term monitoring of valve and cardiac activity in the blue mussel *Mytilus edulis* exposed to copper. Marine Biology 136, 837–846.

Doherty, F.G., Cherry, D.S., Cairns Jr., J., 1987. Valve closure responses of the Asiatic clam *Corbicula fluminea* exposed to cadmium and zinc. Hydrobiologia 153, 159–167.

Davenport, G.M., Boling, J.A., Schillo, K.K., Aaron, D.K., 1990. Nitrogen-metabolism and somatotropin secretion in lambs receiving arginine and ornithine via abomasal infusion. Journal of Animal Science 68, 222–232.

Englund, V., Heino, M., 1994. Valve movement of *Anodonta anatina* and *Unio tumidus* (Bivalvia, Unionidae) in a eutrophic lake. Annales Zoologici Fennici 31, 257–262.

El-Shenawy, N.S., 2004. Heavy-metal and microbial depuration of the clam *Ruditapes decussatus* and its effect on bivalve behavior and physiology. Environmental Toxicology 19, 143–153.

Fournier, E., Tran, D., Denison, F., Massabuau, J.-C., Garnier-Laplace, J., 2004. Valve closure response to uranium exposure for a freshwater bivalve (*Corbicula fluminea*): quantification of the influence of pH. Environmental Toxicology and Chemistry 23, 1108–1114.

Gerhardt, A., Carlsson, A., Ressemann, C., Stich, K.P., 1998. New online bio-monitoring system for *Gammarus pulex* (L.) (Crustacea): in situ test below a copper effluent in south Sweden. Environmental Science and Technology 32, 150–156.

Gruber, D., Diamond, J., 1988. Automated Biomonitoring Living Sensors as Environmental Monitors. Ellis Horwood, Chichester, UK.

Higgins, P.J.J., 1980. Effects of food availability on the valve movement and feeding behaviour of juvenile *Crassostrea virginica* (Gmelin). I. Valve movement and periodic activity. Journal of Experimental Marine Biology and Ecology 45, 229–244.

Kadar, E., Salanki, J., Jugdaohsingh, R., Powell, J.J., McCrohan, C.R., White, K.N., 2001. Avoidance responses to aluminium in the freshwater bivalve *Anodonta cygnea*. Aquatic Toxicology 55, 137–148.

Kramer, K.J.M., Botterweg, J., 1991. Aquatic biological early warning systems: an overview. In: Jeffrey, D.J., Madden, B. (Eds.), Bioindicators and Environmental Management. Academic Press, London, UK, pp. 95–126.

Liao, C.M., Jou, L.J., Chen, B.C., 2005. Risk-based approach to appraise valve closure in the clam *Corbicula fluminea* in response to waterborne metals. Environmental Pollution 135, 41–52.

Markich, S.J., Brown, P.L., Jeffree, R.A., Lim, R.P., 2003. The effects of pH and dissolved organic carbon on the toxicity of cadmium and copper to a freshwater bivalve: further support for the extended free ion activity model. Archives of Environmental Contamination and Toxicology 45, 479–491.

McCorckle, S., Shirley, T.C., Dietz, T.H., 1979. Rhythm of activity and oxygen consumption in the common pond clam *Ligumia substrata* (Say). Canadian Journal of Zoology 57, 1960–1964.

Ortmann, C., Grieshaber, M.K., 2003. Energy metabolism and valve closure behaviour in the Asian clam *Corbicula fluminea*. Journal of Experimental Biology 206, 4167–4178.

Perez, M.H., Wallace, W.G., 2004. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Archives of Environmental Contamination and Toxicology 46, 81–89.

Sloof, W., de Zwart, D., Marquenie, J.M., 1983. Detection limits of a biological monitoring system for chemical water pollution based on mussel activity. Bulletin of Environmental Contamination and Toxicology 30, 400–405.

Sluyts, H., van Hoof, F., Cornet, A., Paulussen, J., 1996. A dynamic new alarm system for use in biological early warning systems. Environmental Toxicology and Chemistry 15, 1317–1323.

Tran, D., Ciret, P., Ciutat, A., Durrieu, G., Massabuau, J.C., 2003. Estimation of potential and limits of bivalve closure response to detect contaminants: application to cadmium. Environmental Toxicology and Chemistry 22, 914–920.

Tran, D., Fournier, E., Durrieu, G., Massabuau, J.C., 2004. Copper detection in the Asiatic clam *Corbicula fluminea*: optimum valve closure response. Aquatic Toxicology 65, 317–327.

Uchisawa, H., Sato, A., Ichita, J., Matsue, H., Ono, T., 2004. Influence of low-temperature processing of the brackish-water bivalve, *Corbicula japonica*, on the ornithine content of its extract. Bioscience, Biotechnology, and Biochemistry 68, 1228–1234.

Van der Schalie, W.H., Shedd, T.R., Knechtges, P.L., Widder, M.W., 2001. Using higher organisms in biological early warning systems for real-time toxicity detection. Biosensors and Bioelectronics 16, 457–465.

Wildridge, P.J., Werner, R.G., Doherty, F.G., Neuhauser, E.F., 1998. Acute effects of potassium on filtration rates of adult zebra mussels, *Dreissena polymorpha*. Journal of Great Lakes Research 24, 629–636.

Wu, H.C., Shiau, C.Y., 2002. Proximate composition, free amino acids and peptides contents in commercial chicken and other meat essences. Journal of Food and Drug Analysis 10, 170–177.

Weis, J.S., Samson, J., Zhou, T., Skurnick, J., Weis, P., 2001. Prey capture ability of mummichogs (*Fundulus heteroclitus*) as a behavioral biomarker for contaminants in estuarine systems. Canadian Journal of Fisheries and Aquatic Sciences 58, 1442–1452.