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Risk-based approach to appraise valve closure in the clam *Corbicula fluminea* in response to waterborne metals

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A model was developed to link valve closure in clams to concentrations of metals in water.

Abstract

We developed a risk-based approach to assess how the valve closure behavior of Asiatic clam *Corbicula fluminea* responds to waterborne copper (Cu) and cadmium (Cd). We reanalyzed the valve closure response data from published literature to reconstruct the response time-dependent dose–response profiles based on an empirical three-parameter Hill equation model. We integrated probabilistic exposure profiles of measured environmental Cu and Cd concentrations in the western coastal areas of Taiwan with the reconstructed dose–response relationships at different integration times of response to quantitatively estimate the valve response risk. The risk assessment results implicate exposure to waterborne Cu and Cd may pose no significant risk to clam valve activity in the short-time response periods (e.g., <30 min), yet a relative high risk for valve closure response to waterborne Cu at response times greater than 120 min is alarming. We successfully linked reconstructed dose–response profiles and EC50-time relationships associated with the fitted daily valve opening/closing rhythm characterized by a three-parameter lognormal function to predict the time-varying bivalve closure rhythm response to waterborne metals. We parameterized the proposed predictive model that should encourage a risk-management framework for discussion of future design of biological monitoring systems.

Keywords: Valve closure; *Corbicula fluminea*; Risk assessment; Probabilistic; Cadmium; Copper

1. Introduction

In 1986, a mass mortality of oysters caused by high levels of copper pollution, known as the green oyster incident, occurred in the mariculture beds located along the western coastal areas of Taiwan (Lee et al., 1996). Since then, the pollution of various rivers and coastal areas by heavy metal has been received increasing attention in Taiwan. Jeng et al. (2000) carried out a 3-year Asia/Pacific Mussel Watch project (1995–1998) to investigate the heavy metal concentrations in various organisms collected from several coastal areas of Taiwan, and proposed that several species of clams are potential candidates for monitoring copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd) in the marine environment. Hung et al. (2001) further analyzed the correlations among the trace metal concentrations in bivalves, water and sediments collected in these areas, and indicated that good correlations were observed between bivalves and water for Zn, Cd, Pb, and Cu. Doherty et al. (1987) reported that the bioconcentration factors are especially higher for Cu and Cd of the Asiatic clam *Corbicula fluminea*. Therefore, monitoring of

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behavioral activities of the clam as well as their body residues can provide information regarding the metal levels in the environment.

For the water quality management in aquatic ecosystems, it is important to be able to predict the impact of chemical and toxic effects on aquatic species. Building such a predictive capability requires continuous, real-time monitoring for environmental toxicity. Conventional monitoring methods that are performed with discrete sampling followed by chemical analysis in the laboratory, however, are usually characterized as non-continuous processes, and the analytical results are available after a certain time interval (Borcherding and Wolf, 2001; Kramer and Foekema, 2001). Consequently, biological early warning systems, which are designed to detect developing toxicity by continuously tracking behavioral or physiological change of a tested organism, have been increasingly applied to water quality control during the last few decades (Borcherding and Jantz, 1997; van der Schalie et al., 2001).

Many aquatic organisms including fish, mussel, invertebrate, and algae are selected as biological early warning indicators because of their sensitive, immediate responses to the occurrence of contaminants at concentrations which could threat the organisms therein (Baldwin and Kramer, 1994; Sluyts et al., 1996; van der Schalie et al., 2001). One of the more successful biological early warning systems is the valvometric technique that employs bivalves as sentinel organisms for monitoring the concentration of selected pollutants in the environment (Curtis et al., 2000; Tran et al., 2003a,b).

Bivalves are commonly available organisms that are abundant in the freshwater as well as the marine environment. They have been suggested as ideal contamination indices in aquatic ecosystems because of their wide distribution, extensive population, sedentary nature and the ability to accumulate contaminants (Jeng et al., 2000; El-Shenawy, 2004). Moreover, they close their shells for an extended period of time as an escape behavior to exclude themselves from the outside environment when exposed to a contaminant (Wildridge et al., 1998; Kadar et al., 2001). Continuous monitoring of the frequency and duration of valve closure in bivalves can thus provide a reliable means for estimating the level of pollution in the environment (Sluyts et al., 1996; Heinonen et al., 2003).

In this present work, we develop a systematic and quantitative risk assessment framework, which is most needed to interpret the significance of the reported exposures. A major complication in predicting or estimating risks for aquacultural species is the high degree of uncertainty resulting from the lack of dose–response information and the large environmental variability in exposures among individuals. As a result, formal risk assessments are scarce regarding the aquacultural species. We focus on the risk of physiological and behavioral changes of clam exposed to waterborne Cu and Cd because evidence for this type of adverse effect has been presented in numerous studies (Doherty et al., 1987; Markich et al., 2000; Borcherding and Wolf, 2001; Kadar et al., 2001; Heinonen et al., 2001; Markich, 2003). Thus, knowledge of the potential risks associated with exposure to Cu and Cd is essential for the effective formulation of conservation and management plans.

The objectives of this study are twofold: (1) to conduct an environmental risk assessment based on the USEPA methodology and (2) to develop a mathematical model to better describe the concentration–time-response relationships for the clam exposed to waterborne Cu and Cd. Based on the pharmacodynamic concept, we reanalyze the valve closure response data of the Asiatic clam *C. fluminea* exposed to waterborne Cu and Cd proposed by Tran et al. (2003a,b) to reconstruct the temporal, concentration–response profiles. We use a probabilistic approach to address the uncertainties of Cu and Cd concentrations presented in clam farms along the western coastal areas of Taiwan. We combine the probabilistic exposure profiles with the concentration–response relationships at different integration times of response of 30, 60 and 120 min to arrive at the risk characterization that allows us to quantitatively estimate the valve closure response risk for clams exposed to waterborne Cu and Cd. Incorporating modeled daily opening/closing rhythm into dose–time-response profiles enables us to predict the time-varying bivalve closure response and to assess behavioral endpoint for clams exposed to waterborne metals.

2. Materials and methods

Our risk assessment approach is divided into 5 phases (Fig. 1) and is described in the subsequent sections.

2.1. Problem formulation

The problem formulation of this study is the phase where the assessment endpoint is defined, analyses for associating Cu/Cd contamination with the assessment endpoint are planned, and a conceptual model is developed (Fig. 1). The conceptual model is developed starting from the estimation of predicted environmental contamination of Cu/Cd in terms of measured environmental concentrations in clam farms in that the major database were adopted from the studies conducted by Jeng et al. (2000) and Hung et al. (2001).

We define the assessment endpoint for clam is the valve closure response risk. The conceptual model is based on a number of assumptions. These assumptions are necessary mainly because of lack of data, and to

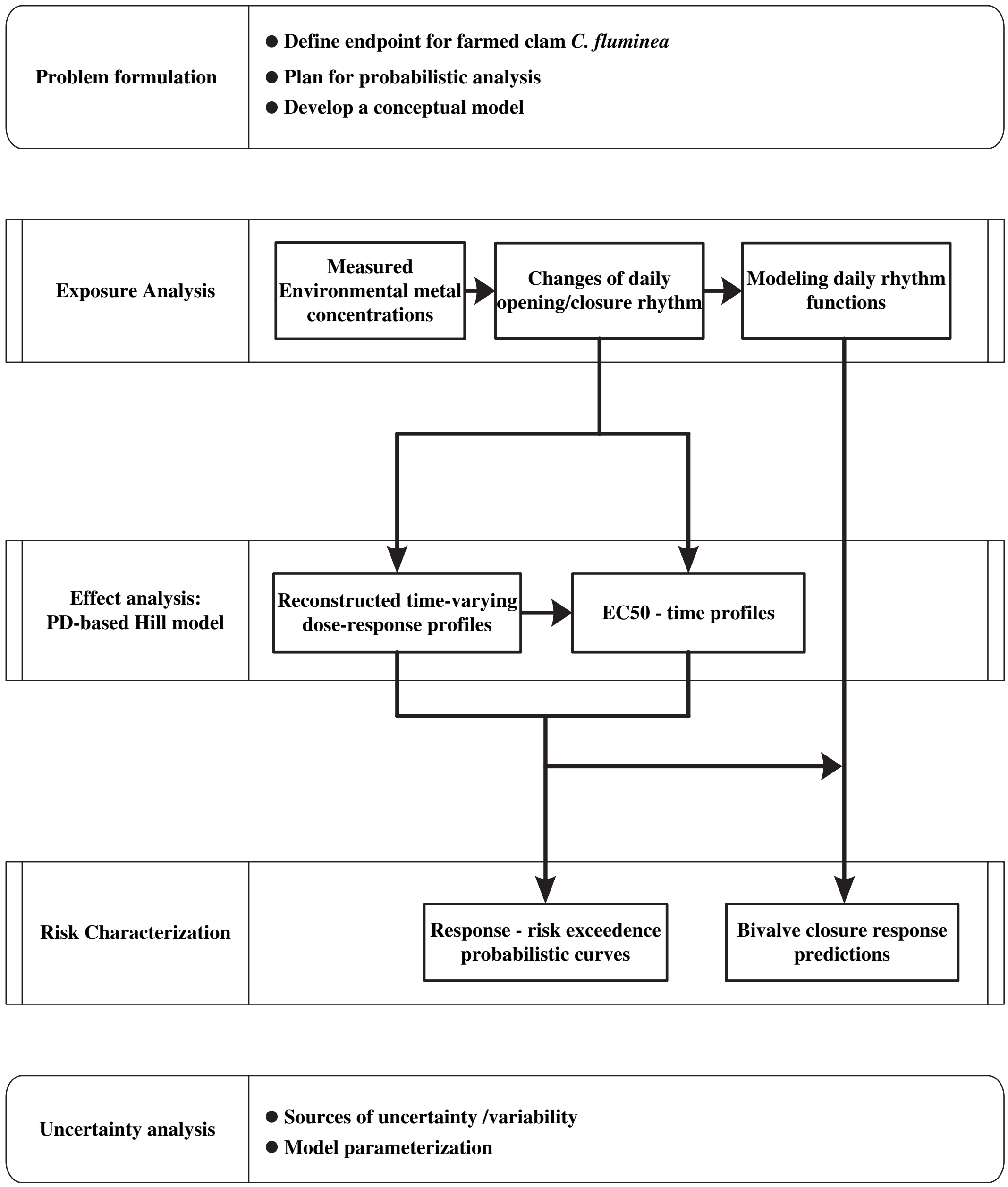

Fig. 1. A conceptual diagram showing a risk-based approach including problem formation, exposure analysis, effect analysis, risk characterization, and uncertainty analysis developed to assess *Corbicula fluminea* valve closure response risk exposed to waterborne metals.

keep the model simple yet reasonably functional. Most of the assumptions are stated in the parameter descriptions in the subsequent sections. The major assumptions are as follows. The pathway of Cu/Cd exposure of farmed clams is only via water ingestion. Farmed clams are contaminated with Cu/Cd through aquacultural water that is obtained from surface water along the western coastal areas of Taiwan.

2.2. Exposure analysis

Measured environmental metal concentrations for Cu and Cd were obtained from a large field study of effluent monitoring data collected along the western coastal areas of Taiwan (Jeng et al., 2000; Hung et al., 2001). We selected the major *C. fluminea* farming sites at Chunghua, Yunlin, Chaiyi, and Tainan located at the western coastal areas of Taiwan as our study clam farms. In these areas, since the land-subsidence caused by overusing groundwater for aquaculture was serious, the major farming strategies of *C. fluminea* are polyculture by mixing seawater and freshwater to reduce aquaculture freshwater demand and groundwater dependence. The selected clam farms had similar feeding strategies. We reanalyzed the measured environmental Cu/Cd concentrations through statistical tests. There are multiple sources of variability and uncertainty to be considered during distribution development for concentrations of Cu and Cd in water from measured values. Therefore, data were log-transformed when necessary to meet the assumptions of statistical tests. All statistical analyses were conducted using the Statistica® software package (StatSoft, Tulsa, OK, USA).

Thanks to the excellent recordings of typical daily valve opening/closing activities of *C. fluminea* from the previous researchers (Doherty et al., 1987; Tran et al., 2003a), distributions of the daily valve opening/closing rhythm in *C. fluminea* have a well-established sequence framework. Tran et al. (2003a) used 71 *C. fluminea* (average fresh weight without the shell was 0.76 ± 0.03 g) over a period of 50 d 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 distribution of daily valve opening/closing rhythm. We used the maximum-likelihood method to fit the distribution of the daily rhythm of clam valve opening/closing. We used TableCurve 2D (Version 5, AISN Software Inc., Mapleton, OR, USA) to optimal fit the distribution data of the opening/closing valve daily rhythm in *C. fluminea* obtained from Tran et al. (2003a). A value of $p < 0.05$ was judged significant.

2.3. Effect analysis

Time-varying percentage of response events (closure or brief opening followed by closure) in relation to occasional additions of Cd or Cu to the water were fitted using an empirical three-parameter Hill equation model (Lalonde, 1992; Bourne, 1995) based on the previously published data of % response versus μg L^{-1} Cd or Cu in water from Tran et al. (2003a,b). In fitting the Hill equation model to the observed data of % valve response as a function of Cd/Cu concentration in water, the dose–response profile can be expressed as,

$$R = \frac{R_{max} \times C_w^n}{K_{0.5}^n + C_w^n}, \tag{1}$$

where R is the measured response (% response), $K_{0.5}$ is the metal concentration yielding half of maximal response of R_{max} (μg L^{-1}), C_w is the metal concentration in water (μg L^{-1}), and the exponent n is a fitted average value or referred to as the Hill coefficient which is a measure of cooperativity. A value of $n > 1$ indicates positive cooperativity. The EC50 values were calculated as the dose where $R = 50\%$ response in that relation between EC50 values of *C. fluminea* to Cd or Cu concentrations and response time were adopted from Tran et al. (2003a,b) and incorporated into the reconstructed dose–response models to determine the effect profiles.

We reanalyzed the published data obtained from Tran et al. (2003a,b) regarding the effect concentrations of Cd and Cu causing 50% of total valve closure response of clam at different integration times of response of 30, 60 and 120 min. The Hill mathematical model was used because it allows for comparison of cooperativity among different response time periods that validates the observations of published studies and shows that bivalve closure response is dependent on the response time and metal concentration.

We treated EC50 values in Eq. (1) probabilistically and the cumulative distribution function (cdf) of predicted bivalve closure response function for a given metal concentration, $F(R|C)$, could be expressed symbolically as a conditional cdf,

$$F(R|C) = \Phi\left(\frac{R_{max} \times C^n}{(\mathrm{EC50})^n + C^n}\right), \tag{2}$$

where $\Phi()$ is the cumulative standard normal distribution.

2.4. Risk characterization

Risk characterization is the phase of risk assessment where the results of the exposure and quantitative effect assessments are integrated to provide an estimate of risk for the population under study. Risk at a specific metal concentration to bivalve closure response can be calculated as the proportion of clam expected to expose to that metal concentration multiplied by the conditional probability of bivalve closure response, given a certain concentration. This results in a joint probability function (JPF) or exceedence profile, which describes the probability of exceeding the concentration associated with a particular degree of effect. This can be expressed mathematically as

$$R(C) = F(C) \times F(R|C), \tag{3}$$

where $R(C)$ is the risk for a valve closure at concentration C, and $F(C)$ is the cdf of having metal concentration C in water.

A risk curve was generated from the cumulative distribution of simulation outcomes. Each point on the risk curve represents both the probability that the chosen proportion of clam will be affected and also the frequency with which that level of effect would be exceeded. The x-axis of the risk curve can be interpreted as a magnitude of effect (a percentage of the given clam expected to suffer the adverse effect), and the y-axis can be interpreted as the probability that an effect of at least that magnitude will occur. These probabilities are based on the current exposure data so at each point on the JPF we can also interpret as: under current conditions, x% of clam will be effected and that this proportion of clam would be affected by y% of the current observations.

2.5. Uncertainty analysis

2.5.1. Model parameterization

Parameterization of the model involved selecting data sets and deriving input distributions. Data were sorted by reported statistical measure, e.g., mean, standard deviation, standard error, etc. We used the chi-square (χ^2) and the Kolmogorov–Smirnov (K-S) statistics to optimize the goodness-of-fit of distributions. We employed Statistica® to analyze data and to estimate distribution parameters. The selected distribution type and parameters were based on statistical criteria and comparisons of distribution parameters.

2.5.2. Measured environmental metal concentration: C_w

Distributions of measured environmental water Cu and Cd concentrations (C_w) were fit to the polled field observations obtained from selected clam farms located at the western coastal Taiwan region. We determined that the lognormal distribution model fits the observed data of Cu concentrations in Chunghua, Yunlin, Chaiyi, and Tainan clam farms favorably. All variables modeled as the lognormal distributions from which geometric mean and geometric standard deviation for each variable was calculated (Table 1). The results of data reanalysis give the Cd distributions in Chaiyi and Tainan clam farms ranged from 0.3 to 0.5 and 0.5 to 0.7 ng mL^{-1}. We used uniform distributions with these ranges for Cd concentration distributions (Table 1).

2.5.3. Parameter in Hill equation model: EC50

In applying dose–response relationships derived from experimental studies adopted from Tran et al. (2003a,b), we must consider the limitations of the data and account for the inherent uncertainty that arises from a number of sources, including the limited number of observations and limited sample size within treatment sets. To account this uncertainty, we constructed distributions for the input variables of EC50 values of Hill model derived dose–response function in Eq. (1). We determined a normal distribution for EC50 values based on K-S statistics, and incorporated the distributions into the Monte Carlo simulation to obtain 2.5- and 97.5-percentiles as the 95% confidence interval for reconstructed dose–response profiles.

Table 1
Model input distributions of measured metal concentrations in selected clam farms

Study clam farm	Measured environmental metal concentration in water (ng mL^{-1})	Distribution
Copper concentration		
Chunghua	3.0 ± 0.8[a]	LN(2.90, 1.30)[c]
Yunlin	1.3 ± 0.1	LN(1.30, 1.08)
Chaiyi	6.3 ± 0.4	LN(5.54, 1.66)
Tainan	6.0 ± 1.7	LN(5.77, 1.32)
Cadmium concentration		
Chaiyi	0.3–0.5[b]	U(0.3, 0.5)[d]
Tainan	0.5–0.7	U(0.5, 0.7)

[a] Mean ± SD.
[b] Minimum–maximum.
[c] Lognormal distribution with geometric mean and geometric standard deviation.
[d] Minima and maxima of uniform distribution.

2.5.4. Monte Carlo analysis

In order to quantify this uncertainty and its impact on the estimation of expected risk, we implemented a Monte Carlo simulation that includes input distributions for the parameters of the derived dose–response function as well as for estimated exposure parameters. To test the convergence and the stability of the numerical output, we performed independent runs at 1000, 4000, 5000, and 10 000 iterations with each parameter sampled independently from the appropriate distribution at the start of each replicate. Largely because of limitations in the data used to derive model parameters, inputs were assumed to be independent. The result shows that 5000 iterations are sufficient to ensure the stability of results. We employed Crystal Ball® software (Version 2000.2, Decisioneering, Inc., Denver, Colorado, USA) to implement the Monte Carlo simulation.

3. Results

3.1. Exposure assessment

Fig. 2 shows the box plots of interquartile and 50th-percentile associated with whisker plots indicating 2.5- and 97.5-percentile of Cu and Cd concentrations in study clam farms in that the median Cu and Cd levels in water range from 1.3 to 5.74 and 0.4 to 0.6 ng mL^{-1}, respectively.

Optimal statistical models were selected on the basis of least squared criterion from a set of generalized linear and nonlinear autoregression models provided by Table Curve 2D package fitted to the daily rhythm distributions of valve opening/closing of *C. fluminea*. The optimal fitted nonlinear regression function was the three-parameter lognormal distribution, given by

$$f(t;a,b,c) = a\exp\left[-0.5\left(\frac{\ln\left(\frac{t}{b}\right)}{c}\right)^2\right] + d, \tag{4}$$

where a is the amplitude, b is the value of t at maximum, c can be derived from the area-under-curve (AUC) $= abc\sqrt{2\pi\exp(c^2)}$, and d is the displacement in y-axis. Here we denote the valve opening function as $\psi(t;\ a,b,c)$ and $\phi(t;\ a,b,c)$ for valve closing function.

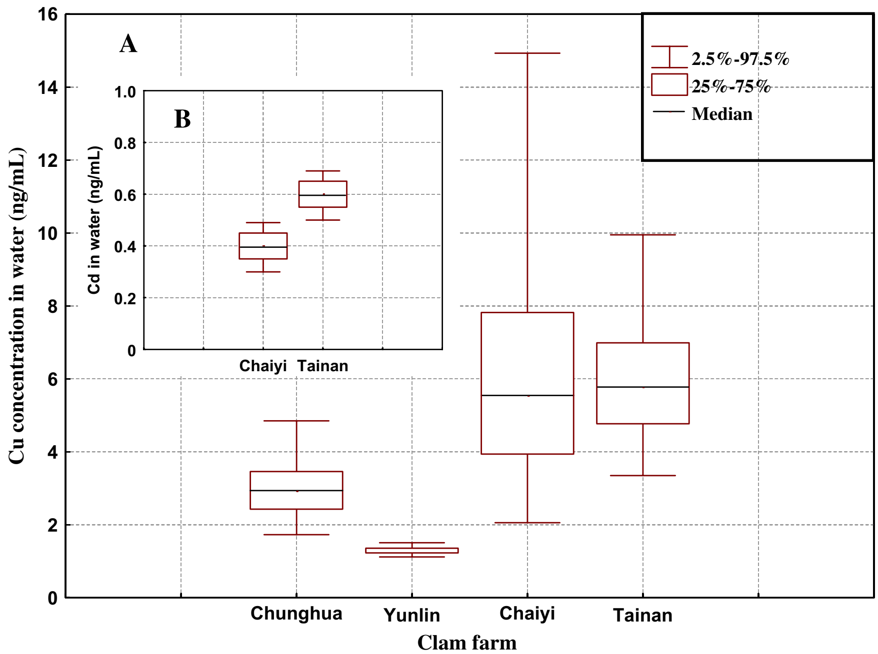

Fig. 2. Box and whisker plot representations of (A) water Cu concentrations at four selected clam farm locations and (B) water Cd concentrations at two selected clam farm locations.

Fig. 3 demonstrates the fitted three-parameter lognormal models for the observations of daily rhythm of valve opening and closing.

A bimodal distribution of the daily rhythm of valve opening/closing is separated at 7 AM that is based on the suggestion by Tran et al. (2003a). The fitted three-parameter lognormal models for daily rhythm of valve opening and closing have the forms, respectively, as

$$\psi(t)=\begin{cases}\psi_1(t)=6.4\exp\left[-0.5\left(\frac{\ln\left(\frac{t}{3.2}\right)}{0.47}\right)^2\right]+1.9, & 0\le t\le 7,\ r^2=0.96,\\ \psi_2(t)=16.8\exp\left[-0.5\left(\frac{\ln\left(\frac{t}{10.8}\right)}{0.24}\right)^2\right]+1.7, & 7<t\le 24,\ r^2=0.96,\end{cases} \tag{5}$$

$$\phi(t)=\begin{cases}\phi_1(t)=12.3\exp\left[-0.5\left(\frac{\ln\left(\frac{t}{4}\right)}{0.20}\right)^2\right]+3.8, & 0\le t\le 7,\ r^2=0.84,\\ \phi_2(t)=14.8\exp\left[-0.5\left(\frac{\ln\left(\frac{t}{18.2}\right)}{0.083}\right)^2\right]+3.6, & 7<t\le 24,\ r^2=0.92.\end{cases} \tag{6}$$

where $\psi(t)$ and $\phi(t)$ are the daily rhythm functions of valve opening and closing proportions at any given time t, respectively.

3.2. Effect analysis

As can be seen in Fig. 4, dose–response profiles were in a time-dependent fashion. The Hill model and a 5000 Monte Carlo simulation provided an adequate fit for the data (χ^2 goodness-of-fit, $P > 0.5$). The n and EC50 values clearly show that there were profound differences in sensitivity to Cu and Cd in different integration times of response. Regression lines from the nonlinear Hill three-parameter model transformations of percent valve closure response versus metal concentrations in water curves had good fit as judged by high r^2 values (0.991–0.997, $p < 0.05$). The Hill coefficient (n) for Cu concentration to valve response (1.27–1.71) was indicative of positive cooperativity. In the case of Cd-response profiles, $n = 1.43$ for response time less than 30 min, whereas for response time greater than 60 min, n values all less than 1.

Based on our fitted concentration–response model (Fig. 4), the estimated EC50 values in Cu-response profiles were 21.4, 10.9, and 8.1 ng mL^{-1} for valve response times of 30, 60, and 120 min, respectively; whereas in Cd-response relationships were 114.2, 42.72, and 25.91 ng mL^{-1}, respectively, for 30, 60, and

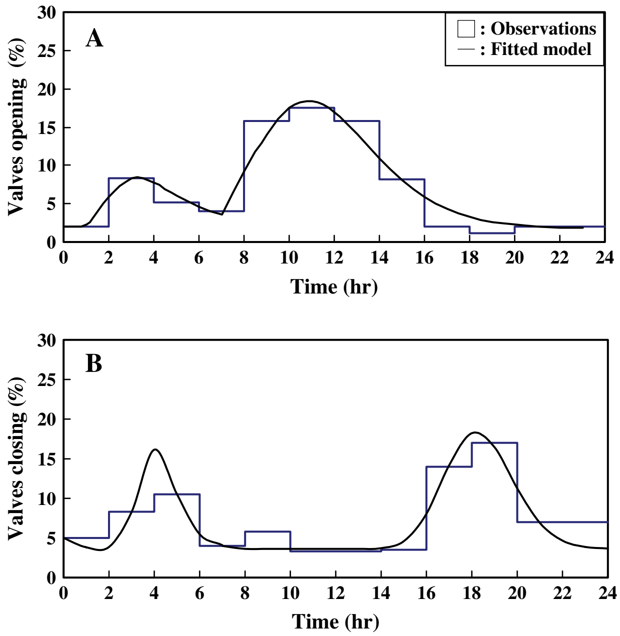

Fig. 3. Selected three-parameter lognormal functions fitted to observations of daily rhythm of *Corbicula fluminea* valve (A) opening and (B) closing histograms.

120 min. Therefore, low concentrations of Cu and Cd caused a significant change in the valve position, suggesting that valve position is suitable for a biologically sensitive endpoint.

3.3. Risk estimates

Risk curves shown in Fig. 5 indicate the estimated probabilistic of effects of differing magnitude for clam for each selected clam farm locations exposed to Cu and Cd in water. The plotted probabilities, calculated from the outcome of the Monte Carlo simulation followed a JPF shown in Eq. (3) describing the exceedence cdfs (Fig. 5) associated with a metal-specific dose–response relationship (Fig. 4), take into account the uncertainty in estimating risk derived from variability and uncertainty in model parameters.

Table 2 gives the probabilities that 10% or more of the valve opening response of clam is affected by waterborne Cu and Cd (risk = 0.1) for 30 and 120 min response times at different clam farms. Table 2 demonstrates that proportion of clam closing response affected by Cu and Cd in 30 min response time ranged from 1.0 to 23.1% and 0.04 to 0.07%, respectively, indicating that it poses no threat to valve response behavior. For 120 min response time, however, the proportions of clam response affected by Cu at Chaiyi (58.5%) and Tainan (50.7%) are relative high. The results of the risk assessment indicate that waterborne Cu and Cd concentrations pose no significant risk to clam valve activity in the short-time response periods (e.g., <30 min), yet a relative high risk for valve closure response to waterborne Cu concentration at response times greater than 120 min in Chaiyi and Tainan clam farms is alarming.

3.4. Bivalve closure response predictions

We link the reconstructed time-varying dose–response profiles (Fig. 4) and EC50-time relationships provided by published data associated with the fitted model of daily rhythm valve opening/closing (Fig. 3) to predict the bivalve closure rhythm response to waterborne Cu and Cd. The mathematical description using an analysis of the dose–response-type curve that integrates time of any detection mechanisms therefore can be expressed as,

$$\psi(t+\Delta t)=\psi(t,0)\times F(R|C_w)=\psi(t,0)[1-R(\Delta t,C_w)], \tag{7}$$

where $\psi(t+\Delta t)$ is the daily rhythm function of valve opening at any given incremental time Δt (h), $\psi(t,0)$ is the daily rhythm function of valve opening exposed to unpolluted water, $F(R|C_w)$ is the cdf of predicted bivalve opening response function for a given metal concentration C_w, and $R(\Delta t,C_w)$ is the Hill model-based dose–response function at any given Δt. The daily rhythm function of valve opening exposed to unpolluted water $\psi(t,0)$ has the same form as given in Eq. (5).

The Hill model-based dose–response function at any given Δt can be expressed as,

$$R(\Delta t,C_w)=\frac{100C_w^{n(\Delta t)}}{[\mathrm{EC50}(\Delta t)]^{n(\Delta t)}+C_w^{n(\Delta t)}}, \tag{8}$$

in that EC50(Δt) and $n(\Delta t)$ are time-dependent metal-specific functions. The function of EC50(Δt) for Cu can be adopted from Tran et al. (2003b) as,

$$\mathrm{EC50}(\Delta t)_{\mathrm{Cu}}=3.76\exp\left(\frac{91.9}{23.99+\Delta t}\right),\quad r^2=0.996. \tag{9}$$

We fitted a nonlinear regression to EC50-time relationships derived from Hill model (Fig. 4B) to obtain the function of EC50(Δt) for Cd,

$$\mathrm{EC50}(\Delta t)_{\mathrm{Cd}}=2.92+50.19\Delta t^{-1.212},\quad r^2=0.988. \tag{10}$$

The function of $n(\Delta t)$ for Cu and Cd can be obtained, respectively, by a nonlinear regression fitting the response time-dependent n values indicated in Fig. 4A and B, and results in

$$n(\Delta t)_{\mathrm{Cu}}=1.396\exp\left(-\frac{\Delta t}{0.379}\right)+1.284,\quad r^2=0.97, \tag{11}$$

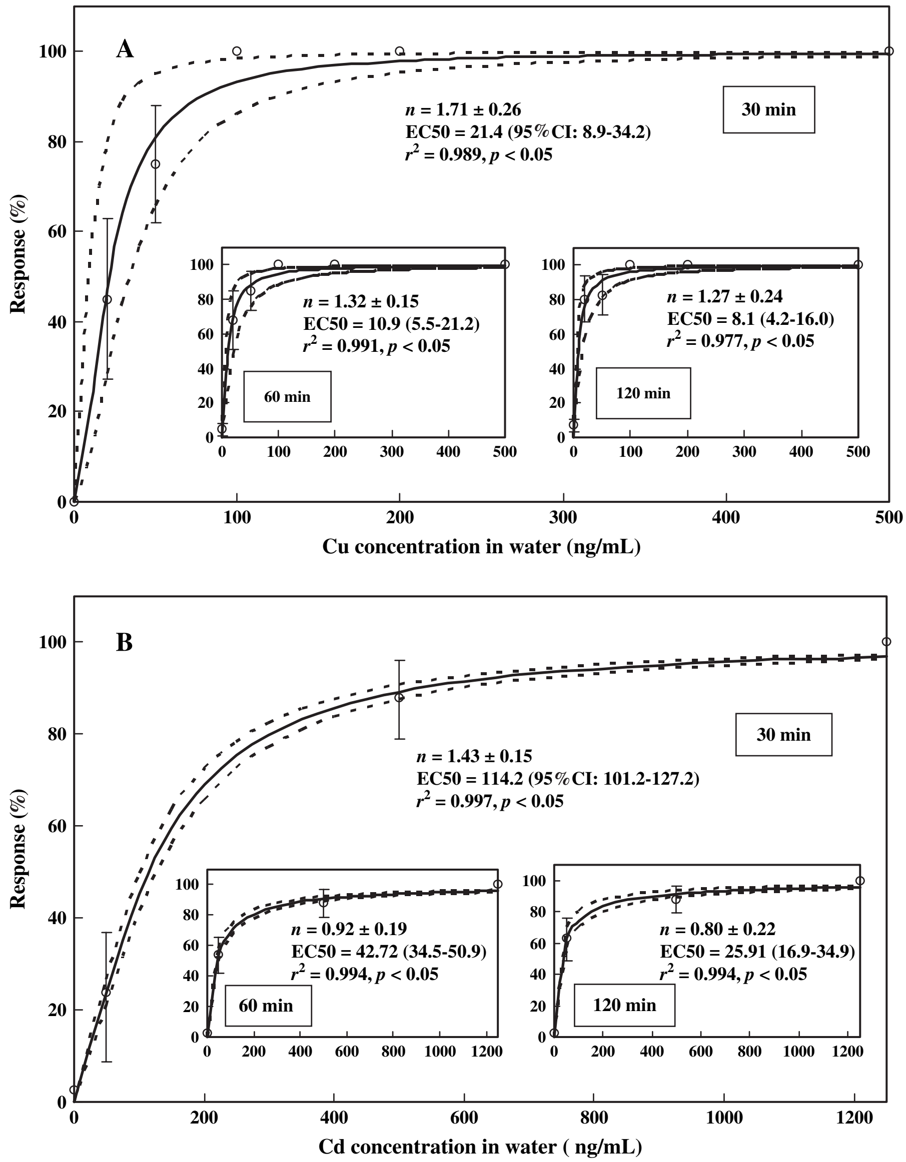

Fig. 4. Reconstructed dose–response profiles with 95% confidence interval for the percentage of valve response as a function of (A) Cu and (B) Cd concentrations characterized by a nonlinear three-parameter Hill equation model at different integration times of response of 30, 60, and 120 min. The measurements are shown with open circles. Error bars represent one standard deviation from the mean.

$$n(\Delta t)_{\mathrm{Cd}} = 1.45 \exp\left(-\frac{\Delta t}{1.21}\right) + 0.417, \quad r^2 = 0.94. \tag{12}$$

Fig. 6 demonstrates the simulations of daily rhythm of valve opening subjected to different waterborne Cu (20–100 ng mL^{-1}) and Cd (100–500 ng mL^{-1}) concentrations at different exposure time periods. The proposed simulation model can quantitatively describe the bivalve behavioral activity when clam exposed to metals. Moreover, the bivalve ability to close its shell as an alarm signal when exposed to a contaminant can be successfully tested in terms of the potential and the limitations of using bivalve as a rapid response and/or sensitive biosensor for different waterborne contaminants.

4. Discussion

A number of studies in modeling environmental fate, bioaccumulation, and toxicity have recognized the need for body burden–effect relationships and advocated a body burden-based approach in environmental toxicity and risk assessments (Bartell et al., 1988; McCarty and Mackay, 1993; Liao et al., 2002). As the body residue of xenobiotic metal elevated, *C. fluminea* reduces its rate of metabolism, heartbeat, and oxygen consumption with the onset of valve closure (Aunaas and Zachariassen, 1994; Curtis et al., 2000; Ortmann and Grieshaber, 2003). Consequently, the establishment of dose–response profile based on the internal body burden concept is necessary while assessing the potential

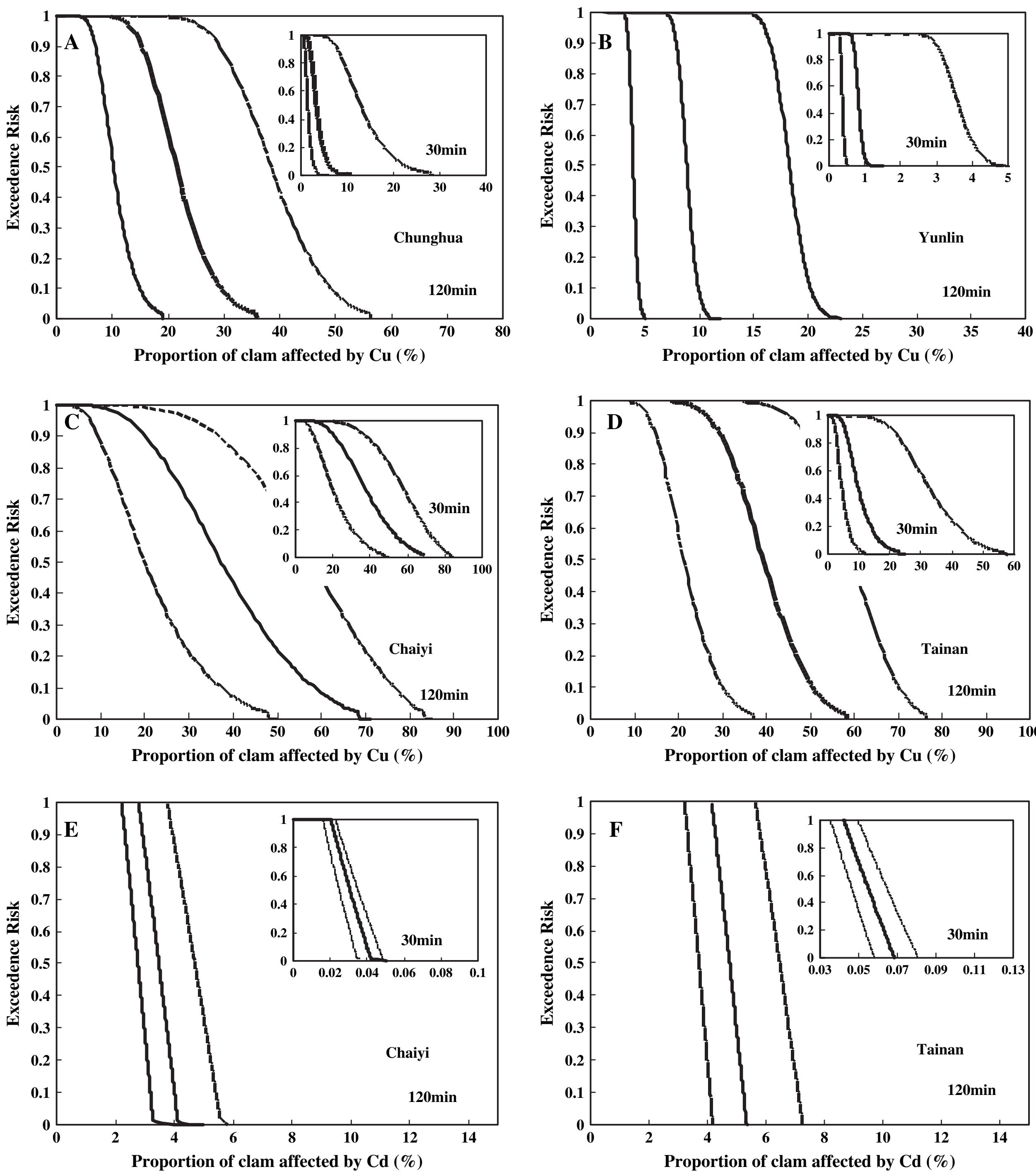

Fig. 5. Exceedence risk curves with 95% confidence interval at 30 and 120 min response times for selected clam farms at (A) Chunghua, (B) Yunlin, (C) Chaiyi, and (D) Tainan exposed to waterborne Cu, whereas (E) Chaiyi and (F) Tainan were exposed to waterborne Cd.

risk of *C. fluminea* exposed to metals. In the present study, however, valve response can only be expressed as a function of waterborne Cd/Cu concentration because of lack of body burden data in *C. fluminea*. Graney et al. (1983), Baudrimont et al. (1997), and El-Shenawy (2004) indicated that simple linear correlations exist between internal and external concentration while exposing clams to higher levels of waterborne metals such as Cd and Cu. Doherty et al. (1987) also pointed out that the valve closure responses of *C. fluminea* would not interfere with the bioaccumulation process in operation at low levels of Cd contaminations ($<0.1\ \mu g\ mL^{-1}$). In order to respond to the realistic situations, further research works will undoubtedly continue a trend

Table 2
The proportion of valve closure response of *C. fluminea* affected by waterborne Cu and Cd for exceedence risk is equal to 0.1 at selected clam farms

Response time	Chunghua	Yunlin	Chaiyi	Tainan
Proportion of clam valve response affected by Cu (%)				
30 min	5.5 (2.5–20.7)[a]	1.0 (0.4–4.2)	23.1 (11.9–57.4)	16.5 (8.1–46.9)
120 min	29.0 (14.8–48.9)	9.9 (4.4–20.2)	58.5 (37.2–76.5)	50.7 (30.2–70.3)
Proportion of clam valve response affected by Cd (%)				
30 min			0.04 (0.03–0.05)	0.07 (0.06–0.08)
120 min			4.0 (3.2–5.5)	5.2 (4.2–7.2)

[a] 95% confidence intervals were computed from 2.5 and 97.5-percentile of 5000 Monte Carlo simulations.

towards a better understanding regarding the relationships between external and internal metal concentrations at low exposure levels in clam farms.

It is generally recognized that an alarm must be set by a biological early warning system within 30 min after the addition of a toxicant (Borcherding and Jantz, 1997). The mathematical descriptions we developed in the present study, which combine the natural valve opening/closing rhythm and the valve behavior changes of *C. fluminea* exposed to various Cd/Cu contaminations, to arrive at an exhaustive comprehension of concentration–response relationships at any given time. We also show that the concentration-time–response profiles proposed here can be served as a powerful tool to quantitatively and continuously monitor the ambient water condition in which the clams farmed. A major

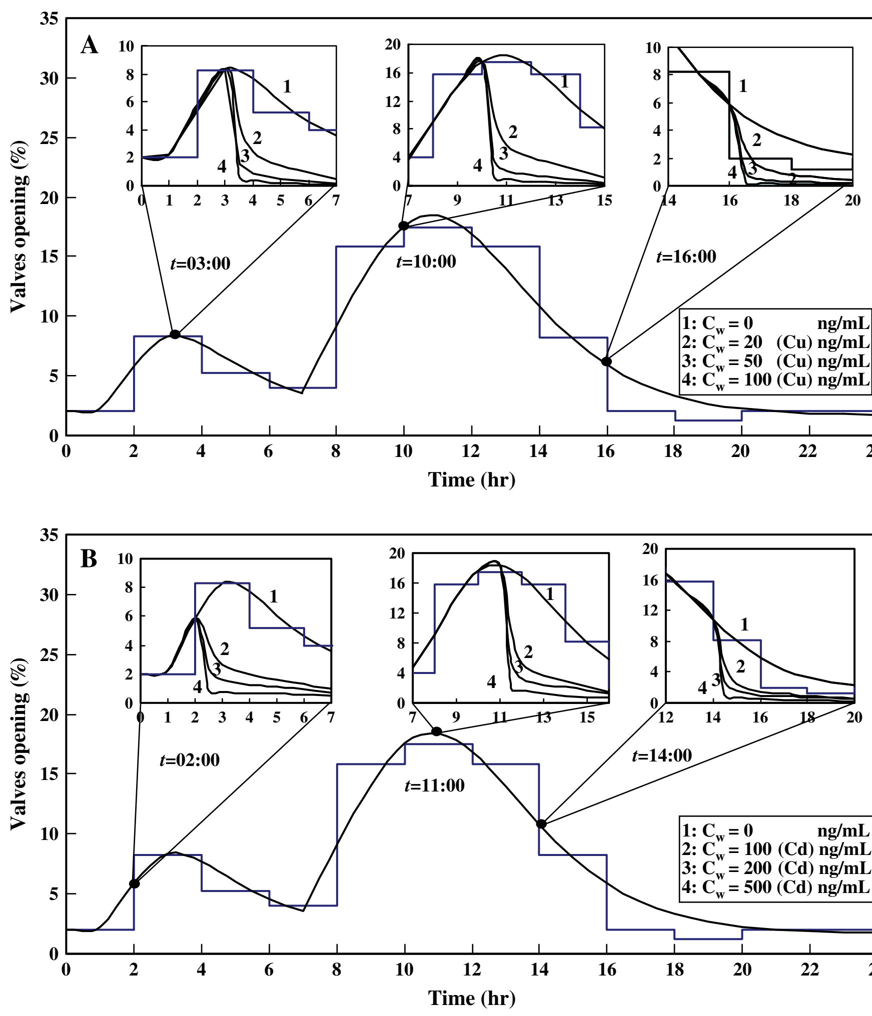

Fig. 6. Simulations of *Corbicula fluminea* valve daily opening rhythm subjected to different waterborne metal contaminations at various exposure time periods: (A) starting exposure times were 03:00, 10:00, and 16:00, respectively, subjected to waterborne Cu levels ranged from 20 to 100 ng mL^{-1} and (B) starting exposure times were 02:00, 11:00, and 14:00, respectively, subjected to waterborne Cd levels ranged from 100 to 500 ng mL^{-1}.

challenge in transferring laboratory data to the field is that organisms are simultaneously or sequentially exposed to multiple stressors in ecosystems (Eggen et al., 2004). We believe that the methodology established in this work can be extrapolated to other species and other contaminants to simultaneously monitor different types of contaminants with various kinds of aquatic organisms in the field.

We believe that a probabilistic risk-based framework – probability distributions and risk diagrams such as Fig. 5 – is an effective representation of state-of-the-art results of scientific assessments for bivalve closure response to waterborne contaminants and has potential for use in biological early warning systems design. To our knowledge, this risk-based framework has not been addressed until now. Despite great uncertainty in many aspects of integrated assessment, e.g., the problem of physical and chemical variables in water such as temperature, pH, turbidity, oxygen level, which may modify the daily rhythm of valve opening/closing (Sluyts et al., 1996; Simon and Garnier-Laplace, 2004; Fournier et al., 2004), cautious interpretation of observations obtained from optimized-controlled laboratory can substantially reduce the likelihood.

Although the suitability and effectiveness of techniques for presenting uncertain results is context-dependent, we believe that such probabilistic methods are more valuable for communicating an accurate view of current scientific knowledge to those seeking information for decision-making than assessments that do not attempt to present results in probabilistic framework. We suggest that our probabilistic framework and methods be taken seriously because they produce general conclusions that are more robust than estimates made with a limited set of scenarios or without probabilistic presentations of outcomes, and our bivalve closure response modeling technique offers a risk-management framework for discussion of future biomonitors design in biological early warning systems.

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