Predictive Risk Thresholds for Survival Protection of Farmed Abalone, *Haliotis diversicolor supertexta,* Exposed to Waterborne Zinc

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ABSTRACT: Using a probabilistic risk-based framework, we have developed a simple predictive risk threshold model for protecting the survival of farmed abalone, *Haliotis diversicolor supertexta,* exposed to waterborne zinc (Zn). Probabilistic techniques using Monte Carlo analysis propagate parameter uncertainty/variability throughout the model, providing decision makers with a credible range of information and increased flexibility in establishing a specific Zn level in aquacultural ecosystems. We coupled a first-order two-compartment bioaccumulation model with a reconstructed dose–response profile based on a three-parameter Hill equation model to form a probabilistic risk model in order to determine the risk quotient associated with a 10% probability of exceeding the abalone 5% effect concentration (EC_5) at site-specific abalone farms. Sensitivity analysis revealed that waterborne Zn concentration (C_w) and algae bioconcentration factor (BCF_a) have a significant effect on Zn levels in abalone. Using multiple nonlinear regression analysis with C_w and BCF_a as the parameters, a predictive risk threshold equation that can be used in a variety of site-specific conditions was developed for protecting the survival of farmed abalone. We believe this probabilistic framework provides an effective method for conceptualizing a public policy decision vis-à-vis the establishment of a specific acceptable risk threshold for aquacultural water quality management. Environ Toxicol 20: 202–211, 2005.

Keywords: abalone; probabilistic; risk; toxicity threshold; zinc

INTRODUCTION

Zinc (Zn) is an essential micronutrient found at high levels (50–120 $\mu g\ g^{-1}$ dry wt) in the tissues of gastropod mollusks (Lin and Liao, 1999; Richardson, 2001; Wang and Ke, 2002). Zinc is available to abalone both from the dissolved phase (e.g., gill uptake) and from diet (e.g., algae ingestion). If waterborne Zn levels are elevated, toxicity can occur, which has severe effects on the health of abalone, resulting in reduced market prices and the closure of abalone farms (Hahn, 1989; Conroy et al., 1996; Knauer et al., 1997). Previous investigations indicated that Zn has been detected in many rivers in that average Zn concentrations in aquaculture waters were reported to range from 60 to 130 ng mL^{-1} in different areas of Taiwan (Lin and Liao, 1999; Liao et al., 2002a). Because few previous studies have evaluated Zn toxicity to *H. diversicolor supertexta*, the mechanisms of how Zn threatens survival and inhibits growth remain unknown.

In Taiwan abalone, *Haliotis diversicolor supertexta*, are appreciated for their delicacy and thus have a high market value. This makes *H. diversicolor supertexta* commercially important to Taiwan's aquaculture and the aquaculture of *H. diversicolor supertexta* a promising business (http://www.fa.gov.tw, 2001). However, the coastal regions of Taiwan where the abalone farms situated are subjected to polluted discharges from rivers.

Zinc was chosen for investigating for practical as well as theoretical reasons, with the availability of a reasonable

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amount of suitable information the primary consideration. Generally, the prerequisites for data suitability that we require are exposure and whole-body Zn levels measured by accepted analytical techniques. We consider experimental exposure data as acceptable only when whole-body concentration data are available and when the duration of exposure is at least 14 days. Our previously published Zn-abalone database conforms to this principle (Lin and Liao, 1999, 2001; Liao et al., 2002a, 2002b). On the other hand, Zn was chosen in this study because it is representative of metals that are of general concern about protecting the environment. Future studies can investigate metals that span the continuum from nutritionally essential to nonessential, such as cadmium or copper.

Janssen et al. (2000) and Bergman and Dorward-King (1997) pointed out that neither total nor dissolved aqueous metal concentrations are good predictors of metal bioavailability and toxicity and are inadequate for the accurate assessment of the potential impact of metals on the ecological quality of ecosystems. Rather than develop a single-value waterborne metal concentration for establishing water quality criteria, it is better to derive a predictive risk threshold model that explicitly incorporates the factors controlling bioavailability and bioaccumulation in aquacultural ecosystems.

In the present work, we have developed a systematic and quantitative risk-based framework that takes into account site-specific water quality characteristics in order to derive the risk thresholds for protecting the survival of farmed abalone. A major complication in deriving a risk threshold for aquacultural species is the high degree of uncertainty resulting from the lack of dose–response information and the large environmental variability in exposure among individuals (Liao et al., 2003; Liao and Ling, 2004). A better approach would be to explicitly model the uncertainties inherent in the risk (toxicity) threshold model for aquatic species in that the output would be a distribution of possible risk (toxicity) criteria for protecting the survival of abalone from which the level of conservatism can be predicted. For example, we can choose an appropriate risk criterion value based on a 10% probability of exceeding the effect concentration affecting 10% (EC_{10}) of sensitive aquatic species as suggested by the U.S. EPA (1995). Suggestions have been made that the EC_5 would be more protective of ecosystem structure and function than would the EC_{10} or EC_{50} (van der Hoeven, 1997; van der Hoeven et al., 1997; Moore and Caux, 1997). Because *H. diversicolor supertexta* is commercially important in Taiwan aquaculture and is sold at high market prices, we chose EC_5 as the threshold of Zn toxicity for the mortality end point to derive the risk thresholds.

Our aim is to present a probabilistic risk-based approach for deriving a predictive risk threshold for protection of the survival of farmed abalone exposed to waterborne Zn. We have demonstrated the utility of this approach by applying it to real abalone farms using the methodology for water quality criteria derivation developed by the U.S. EPA (1995). The methodology that we adopted is an analysis tool that couples probabilistic submodels of the bioaccumulation process with the dose–response relationship in order to arrive at a probabilistic risk model for determining a suitable Zn risk threshold for risk managers who prefer that a risk threshold have a higher or lower level of protection.

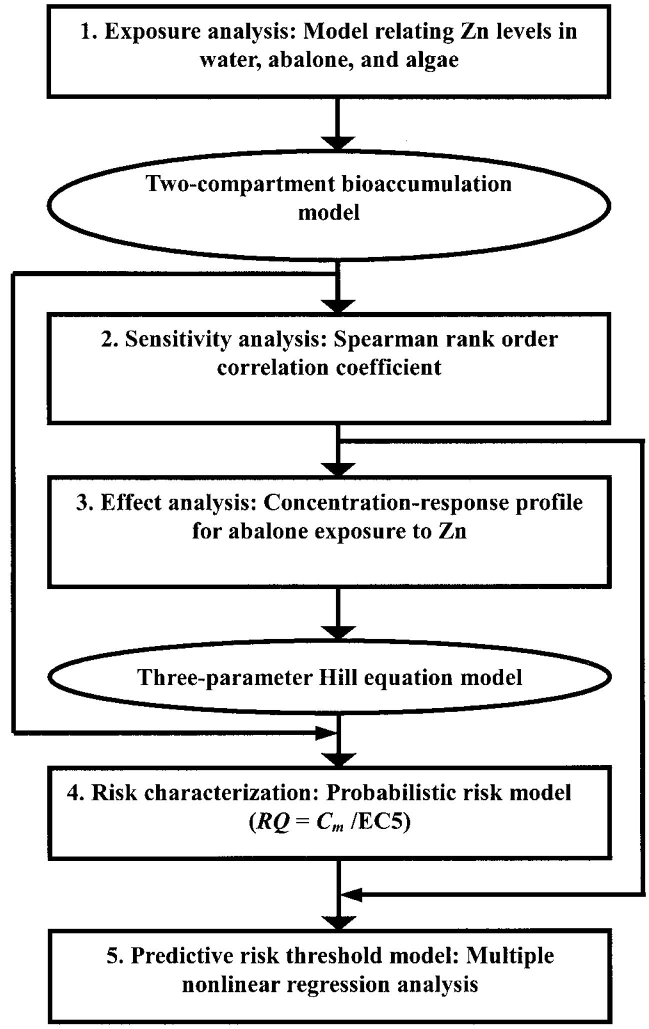

Fig. 1. A conceptual algorithm describing the approach phases of a probabilistic risk-based model to derive a predictive risk threshold model for the survival protection of farmed abalone (*H. diversicolor supertexta*) exposed to waterborne Zn.

MATERIALS AND METHODS

Our probabilistic risk-based approach is divided into five phases (Fig. 1), which are described in the subsequent sections.

Exposure Analysis

The major Zn exposure data was obtained from the previous studies conducted by Lin and Liao (1999) and Liao et al. (2002a, 2002b, 2004). They chose three appropriate management practices on abalone farms for three different study sites: Toucheng, Kouhu, and Anping, in the northern, central, and southern regions of Taiwan, respectively. They measured Zn concentrations in pond water, algae, and the soft tissue of abalone and conducted laboratory exposure experiments in order to estimate essential biokinetic and physiological parameters.

Zinc is accumulated in abalone both by dietary (i.e., red algae, *G. tenuistipitata* var. *liui*) and nondietary (i.e., water source) routes. If the dissolved Zn concentration in water is assumed to be constant, whereas the Zn concentration in algae is assumed to vary with time, the temporal change of Zn concentration in abalone could be modeled using a first-order two-compartment bioaccumulation model as

$$\frac{dC_m(t)}{dt} = \alpha f C_a(t) + k_1 C_w - (k_2 + g)C_m(t), \qquad (1)$$

where $C_m(t)$ is the time-dependent Zn concentration in abalone at time of day t (μg g^{-1} dry wt), C_w is the dissolved Zn concentration in water (ng mL^{-1}), $C_a(t)$ is the time-dependent Zn concentration in algae at time of day t (μg g^{-1} dry wt), α is the assimilation efficiency of abalone (%), f is the abalone grazing rate (g g^{-1} d^{-1}), k_1 is the abalone uptake rate of Zn (mL g^{-1} d^{-1}), k_2 is the abalone depuration rate (d^{-1}), and g is the abalone growth rate (d^{-1}). Assuming that the initial Zn concentration is equal to zero in algae, $C_a(t)$ in Eq. (1) can be expressed as (Liao et al., 2004)

$$C_a(t) = \mathrm{BCF}_a C_w \left(1 - e^{-(k_{2a}+g_a)t}\right), \qquad (2)$$

where $\mathrm{BCF}_a = k_{1a}/(k_{2a} + g_a)$ is the bioconcentration factor of *G. tenuistipitata* var. *liui* for Zn (mL g^{-1}), k_{2a} is the algae depuration rate of Zn (d^{-1}), k_{1a} is the algae uptake rate of Zn (mL g^{-1} d^{-1}), and g_a is the algae growth rate (d^{-1}).

Eq. (1) is solved by substituting Eq. (2) into Eq. (1) (Gross-Sorokin et al., 2003), obtaining

$$C_m(t) = \left[\frac{\alpha f G_a}{B_a - B_m}\right]\left(e^{-B_m t} - e^{-B_a t}\right) + \left[\frac{\alpha f G_a}{B_m}\right]\left(1 - e^{-B_m t}\right) + G_m\left(1 - e^{-B_m t}\right), \qquad (3)$$

where $G_m = \mathrm{BCF}_m C_w$, $G_a = \mathrm{BCF}_a C_w$, $B_m = k_2 + g$, and $B_a = k_{2a} + g_a$, in that $\mathrm{BCF}_m = k_1/(k_2 + g)$ is the bioconcentration factor of abalone for Zn (mL g^{-1}).

Eq. (1) describes the gain and loss of Zn accumulation in abalone featuring constant biokinetic and physiological rates and a constant water concentration. The major processes in Eqs. (1) and (2) were: (i) the exchange of Zn between abalone and dissolved Zn was modeled as a first-order process, with additional Zn accumulation from ingested algae; (ii) abalone ingested only algae and neglected other suspended particles, bacteria, and detritus uptake; (iii) tissue concentration of Zn per unit biomass of abalone increased as a result of direct uptake from water and through assimilation of contaminated algae; and (iv) tissue concentration tended to decrease as a result of elimination from the whole body and of growth dilution. The input variables including biokinetic parameters (k_2, k_{2a}, f, g, g_a, α, BCF_a, BCF_m) and geochemical variable of C_w were treated probabilistically in estimating the Zn levels in abalone.

We performed a sensitivity analysis in order to identify the most significant parameters that influence the level of Zn accumulation in abalone. We assessed the sensitivity of each variable relative to each other by calculating Spearman rank correlation coefficients between each input and output during simulations and then estimating each input contribution to the output variance by squaring the output variance and normalizing to 100% (Zar, 1999). The results of the sensitivity analysis can be used to derive a predictive risk threshold model based on the combinations of the two most sensitive parameters that have significant influence on the Zn level in abalone.

Effect Analysis

The mortality responses in relation to Zn whole-body burden in abalone were fitted using an empirical three-parameter Hill equation model (Lalonde, 1992; Bourne, 1995) based on published acute toxicity data and the relationship, previously established by Liao et al. (2002b), between Zn tissue residues and effects on mortality in abalone. In fitting the Hill model to the observed mortality for specific-interval acute toxicity data, the dose–response profile can be expressed as (Liao et al., 2002b),

$$M = \frac{100 \times C_w^{3.70}}{(\text{24-h } \mathrm{LC}_{50})^{3.70} + C_w^{3.70}}, \qquad (4)$$

where M is mortality (%), the exponent 3.70 is an average value of the fitted Hill coefficient, and the 24-h LC_{50} is the 24-h median lethal concentration (mg L^{-1}).

We appropriately transformed Eq. (4) to a tissue residue–response relationship using the Hill model framework to predict the response as (Liao et al., 2002b),

$$M = \frac{100 \times C_m^{3.70}}{(C_L 50)^{3.70} + C_m^{3.70}} = \frac{100 \times C_m^{3.70}}{(\mathrm{BCF}_m \cdot \mathrm{LC}_{50}(\infty))^{3.70} + C_m^{3.70}}, \qquad (5)$$

where C_L50 is the internal effect concentration at the site of action that cause 50% mortality (μg g^{-1} dry wt), and

$LC_{50}(\infty)$ is the incipient value of LC_{50} (mg L^{-1}). We treated BCF_m and $LC_{50}(\infty)$ in Eq. (5) probabilistically. Applying the Hill model, the cumulative distribution function (cdf) of predicted mortality function for a given tissue Zn concentration, $F(M/C)$, could be expressed symbolically as a conditional cdf,

$$F(M \mid C) = \Phi\left(\frac{100 \times C^{3.70}}{(\mathrm{BCF}_m \cdot \mathrm{LC}_{50}(\infty))^{3.70} + C^{3.70}}\right), \quad (6)$$

where C is the given Zn concentration and $\Phi(\bullet)$ is the cumulative standard normal distribution. We used Eq. (6) to estimate the distribution of EC_5.

Risk Characterization

We used a probabilistic risk model to estimate risk thresholds for the survival protection of farmed abalone exposed to waterborne Zn for different combinations of the Zn bioaccumulation parameters identified in sensitivity analysis as having a significant influence on Zn levels in abalone. We employed a risk quotient equation to estimate risk as

$$RQ = \frac{C_m}{\mathrm{EC5}}, \quad (7)$$

where RQ is the risk quotient (unitless), C_m is the Zn concentration in abalone tissue (μg g^{-1} dry wt), and EC_5 is the effect concentration that produces 5% mortality in abalone. If C_m were equal to EC_5, then the RQ would be 1.0. Thus, for RQ values greater than 1.0, some potential threat to survival can be inferred. RQ values less than 1.0 indicate that the potential threat to survival is low. C_m and EC_5 were treated probabilistically in Eq. (7).

Derivation of Predictive Risk Threshold Model

For each combination of the two most significant biokinetic/geochemical parameters identified from the sensitivity analysis in the calculation of Zn level in abalone in Eq. (3), a risk threshold could be determined that corresponded with a 10% probability of exposure exceeding the EC_5 for farmed abalone. We used the Statistica® software package (StatSoft, Tulsa, OK, USA) to perform multiple nonlinear regression to derive a predictive risk threshold equation for abalone exposed to waterborne Zn that can be used for any user-specified combination of those significant parameters. The final multiple regression model predicting risk threshold from biokinetic/geochemical variables was the best combination of significant independent variables ($p \leq 0.05$), that is, the combination producing the highest r^2 value.

Model Parameterization

Parameterization of the model involved selecting data sets and deriving input distributions. The current literature was reviewed in order to develop probability distributions for the random variables in the adopted bioaccumulation and dose–response models. The source data of the input variables included in Eqs. (3) and (5) were obtained from published studies by Chen (1984, 1989), Lee et al. (1996), Chen and Lee (1999), Lin and Liao (1999), and Liao et al. (2002a, 2002b, 2003). Data were sorted according to reported statistical measure, for example, mean, standard deviation, and standard error. Multiple sources of variability and uncertainty need to be considered during distribution development for model input variables from measured values. Therefore, data were log-transformed when necessary to meet the assumptions of statistical normality. We used the Statistica® software package to analyze data and distribution parameters. We used chi-square (χ^2) and Kolmogorov–Smirnov (K-S) statistics to optimize the goodness-of-fit of distributions (Zar, 1999). The implemented parameter probability distributions are summarized in Table I and will be described in subsequent sections.

Biokinetic Parameters (k_2, k_{2a}, α, f, g, g_a, BCF_a, BCF_m)

Distributions were fitted to polled lab- and field-derived biokinetic data (k_2, k_{2a}, BCF_a, BCF_m) obtained from different sources, and the selected lognormal distributions had acceptable χ^2 fit and K-S fit in that optimizations using either statistic yielded a geometric mean (gm) and geometric standard deviation (gsd) (Table I). We used a beta distribution to describe assimilation efficiency of abalone (α) because it is bounded by 0 and 1 (Table I). A normal distribution was determined to provide the best fit for parameters f, g, and g_a (Table I).

Geochemical Parameter (C_w)

Distributions of waterborne Zn concentrations in the abalone pond (C_w) were fitted to the polled field observations obtained from the three assigned abalone farm locations, and the selected lognormal distributions had optimal K-S and χ^2 goodness-of-fit (Table I).

Dose–Response Parameters: [$LC_{50}(\infty)$]

In applying dose–response relationships derived from the experimental study, it is necessary to 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 for this uncertainty, we constructed distributions for the input variables BCF_m and $LC_{50}(\infty)$ of the Hill dose–response function in Eq. (5). We

TABLE I. Distributions of model input parameters used in Monte Carlo simulations

Parameters	Uncertainty/ Variability	Distribution
Field-derived biokinetic parameters[a]		
BCF_a (mL g^{-1})	U	LN(635.362, 8.656)[b]
BCF_m (mL g^{-1})	U	LN(264.053, 1.928)
Lab-derived biokinetic parameters		
k_2 (d^{-1})[a]	U	LN(0.390, 4.746)
k_{2a} (d^{-1})[a]	U	LN(0.556, 1.535)
f (g g^{-1} d^{-1})[c]	V	N(0.250, 0.050)[d]
g (d^{-1})[e]	V	N(0.004, 0.00012)
g_a (d^{-1})[f]	V	N(0.038, 0.013)
α (%)[g]	V	B(3.02, 4.06)[h]
Geochemical parameter[a]		
C_w (μg mL^{-1})	U	
Toucheng		LN(0.127, 1.31)
Kouhu		LN(0.055, 1.70)
Anping		LN(0.059, 1.77)
Dose–response parameter[i]		
$LC_{50}(\infty)$ (mg L^{-1})	U	N(1.080, 0.127)

[a] Adapted from Lin and Liao (1999).
[b] LN(gm, gsd) represents lognormal distribution with geometric mean (gm) and geometric standard deviation (gsd).
[c] Adapted from Chen and Lee (1999).
[d] *N*(m, sd) represents normal distribution with mean (m) and standard deviation (sd).
[e] Adapted from Liao et al. (2004) in that shell length range of 2–3.5 cm.
[f] Adapted from Lee et al. (1996).
[g] Adapted from Chen and Lee (1999).
[h] B(α, β) represents beta distribution with alpha and beta in that scale = 1.
[i] Adapted from Liao et al. (2002b).

determined normal distributions of $LC_{50}(\infty)$ (Table I) and incorporated these distributions into the Monte Carlo simulation in order to obtain the 2.5th and 97.5th percentiles as the 95% confidence interval (CI) of the reconstructed dose–response profile. Uncertainty and/or variability was not considered for the reported Hill coefficient. This is unfortunate but unavoidable because the Hill coefficient was reported in the published study only as an average value.

Probabilistic Risk Model Parameter (EC_5)

We parameterized a lognormal distribution for EC_5 because that variable was right-skewed with a lower bound of 0 and no upper bound. We incorporated the distributions into the Monte Carlo simulation in order to obtain the 2.5th and 97.5th percentiles as the 95% CI for EC_5.

Monte Carlo Analysis

Uncertainty arises from estimation of both exposure and effects. To quantify this uncertainty and its impact on the estimation of expected risk, we implemented a Monte Carlo simulation that included input distributions for the parameters of the derived dose–response function as well as for estimated exposure parameters. To test the convergence and stability of the numerical output, we performed independent runs of 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 showed that running 5000 iterations was sufficient to ensure the stability of results. The Monte Carlo simulation was implemented with Crystal Ball® software (Version 2000.2, Decisioneering, Inc., Denver, CO, USA).

RESULTS

Results of Sensitivity Analyses and Validation

A comparison with the field observations showed that the median estimates of Zn in abalone generally were below the measured Zn values [Fig. 2(A)] of abalone subjected to the site-specific Zn concentrations in pond water [Fig. 2(B)]. Three field observation data sets for selected abalone farms of Zn in abalone were all within the predicted 25th- to 75th-percentile range [Fig. 2(A)]. The relative skewness and spread in modeled output varied between water and abalone; distributions of Zn levels in abalone were more highly skewed, with a long tail at the higher concentration present at the Toucheng abalone farm, indicating estimated abalone Zn concentration had a higher uncertainty as quan-

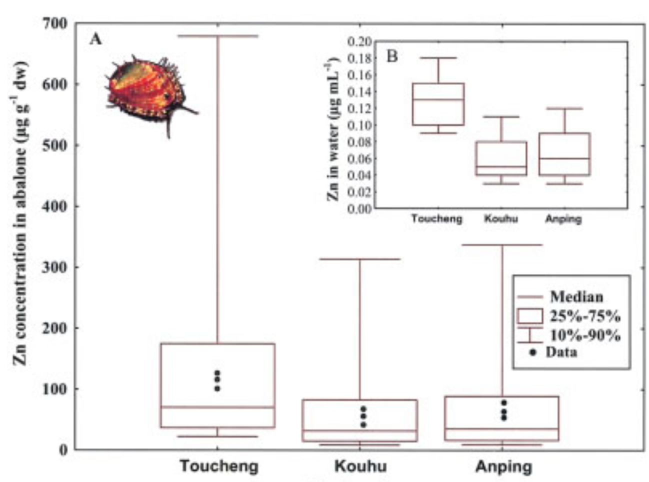

Fig. 2. Box-and-whisker plot representations of distribution of Zn in (A) abalone and (B) water at three selected abalone farms. Box-and-whisker plots are used to represent the uncertainty in Zn level estimates. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tified by the variance [i.e., output variability: geometric standard deviation; Fig. 2(A)]. Thus, applying the Monte Carlo technique to the proposed first-order two-compartment bioaccumulation model generated probabilistic estimates of Zn concentrations in abalone that were favorably consistent with field data. Relative to minimum and maximum field data, however, the lower and upper probabilistic percentile predictions were more conservative. This is evidence that the probabilistic framework regarding the distributional parameters and assumptions is appropriate for estimating bioaccumulation of Zn in abalone.

The results of the sensitivity analyses are shown in the form of a tornado plot illustrating the Spearman rank-order correlation coefficients (Fig. 3). Sensitivity analyses revealed that simulated Zn concentrations in abalone were most sensitive to the algae bioconcentration factor, BCF_a (59%), and Zn waterborne concentration, C_w (43%), at the Kouhu and Anping abalone farms, whereas they were most sensitive to the BCF_a (65%) and the abalone depuration rate constant, k_2 (−43%), at the Toucheng abalone farm (Fig. 3). The most important factor in the overall Zn abalone tissue concentration was the BCF_a, which showed considerable environmental variability, as indicated in Table I, in that the lognormal distribution of BCF_a [i.e., LN(635.36 mL g^{-1}, 8.66)] has a large geometric standard deviation, 8.66. Because the depuration rate constant, k_2, is a lab-derived parameter and is not easily estimated compared with the field-derived parameters of BCF_a and C_w, we determine a C_w–BCF_a combination as the most significant independent variable in abalone at the Zn level.

Risk Estimates in Abalone Farms

The Hill equation model and a 5000-iteration run of the Monte Carlo simulation provided an adequate fit to the data [χ^2 goodness-of-fit, $P > 0.5$; Fig. 4(A)]. In the present work, we employed the more restricted regulatory endpoint EC_5 as a surrogate threshold in probabilistic risk assessment. The EC_5 calculated from the fitted dose–response profile [Fig. 4(A)] was 126.65 μg g^{-1} dry wt of whole-body abalone, with a 95% CI of 33.21–487.63 μg g^{-1} dry wt. We appropriately log-transformed the EC_5 value and the results in a lognormal distribution with a geometric mean of 127.24 μg g^{-1} dry wt and a geometric standard deviation of 1.98, that is, LN(127.24 μg g^{-1} dry wt, 1.98) [Fig. 4(B)].

We applied the probabilistic risk model in Eq. (7) to three selected abalone farms, resulting in a probability of exceeding EC_5 of 36%, 21%, and 20%, respectively, for the Toucheng, Kouhu, and Anping abalone farms, subjected to $RQ = 1$ [Fig. 5(A)]. The risk curves shown in Figure 5(A) indicate the estimated probabilistic of effects of differing magnitudes for abalone for each selected abalone farm location. The plotted probabilities, calculated from the outcome of the Monte Carlo simulation followed a probabilistic risk model describing the exceedance cdfs [Fig. 5(A)] associated with a particular degree of effect [Fig. 4(A)], taking into account the uncertainty in estimating risk derived from variability and the uncertainty in the model parameters.

Figure 5(B) shows that for the Kouhu and Anping abalone farms, a 75% probability or less of experiencing a RQ less than 1 for abalone exposed to waterborne Zn, indicating that these probability distributions are fairly acceptable. In contrast, the 75th-percentile RQ was larger than 1 for the Toucheng abalone farm, indicating a conservative

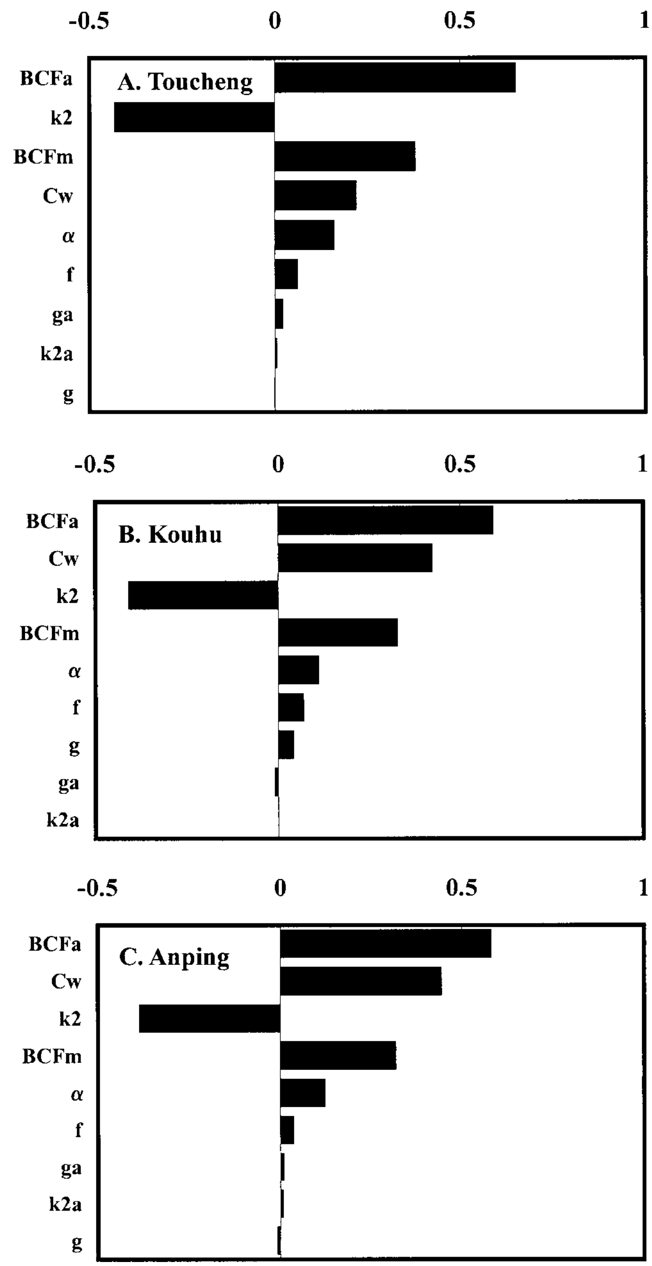

Fig. 3. Sensitivity analysis of the Zn level in abalone at three abalone farms: (A) Toucheng, (B) Kouhu, and (C) Anping.

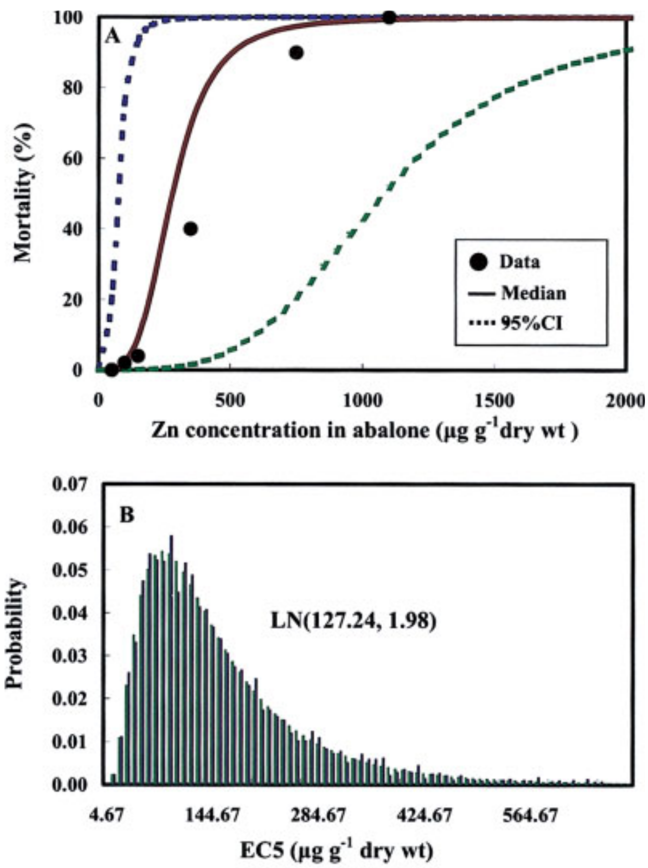

Fig. 4. (A) Reconstructed concentration–response profile with 95% CI showing the relationships between abalone mortality and Zn level in abalone. (B) Probability density distribution of effect concentration causing 5% mortality of abalone (EC_5) in that EC_5 has a lognormal distribution with a geometric mean of 127.24 μg g^{-1} dry wt and a geometric standard deviation of 1.98. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

potential for threatening abalone survival was inferred under the restricted EC_5 value.

Site-Specific Predictive Risk Threshold Equation

Risk thresholds were derived for each of 121 combinations of C_w and BCF_a (i.e., 11 × 11 C_w–BCF_a combinations; Table II). The C_w values considered ranged from 0.05 to 0.15 ng ml^{-1} in increments of 0.01, whereas the BCF_a values considered ranged from 300 to 800 mL g^{-1} in increments of 50 (Table II). The list of possible independent variables included C_w, BCF_a, $C_w \times BCF_a$, $\log_{10} C_w$, $\log_{10} BCF_a$, C_w^2, and BCF_a^2. For each C_w–BCF_a combination, different values were entered for risk threshold in an iterative series of analyses until the probability of $RQ > 1$ (which equals the probability that exposure would exceed the EC_5 for abalone) was 10 ± 0.3%, as suggested by Moore et al. (2003). Then multiple nonlinear regression analysis was performed to derive the risk thresholds equation, which can be used for any user-specified C_w–BCF_a combination. The multiple regression analysis applied to determine the predictive risk threshold equation for the survival protection of abalone exposed to waterborne Zn indicated that adding input variables to the best four-variable (i.e., BCF_a, $\log_{10} C_w$, $\log_{10} BCF_a$, and $C_w \times BCF_a$) model produced no additional benefit of improving the r^2 value.

Thus, the predictive risk threshold equation for the protection of abalone survival exposed to waterborne Zn can be expressed as a best four-variable regression model with an r^2 of 0.993,

$$\log_{10} RT = -0.625 + 0.0004\text{BCF}_a + 0.653\log_{10}\text{BCF}_a + 1.899\log_{10} C_w - 0.0043(C_w \cdot \text{BCF}_a), \quad (8)$$

where RT is the risk threshold for protection of the survival of abalone exposed to waterborne Zn. The graphical repre-

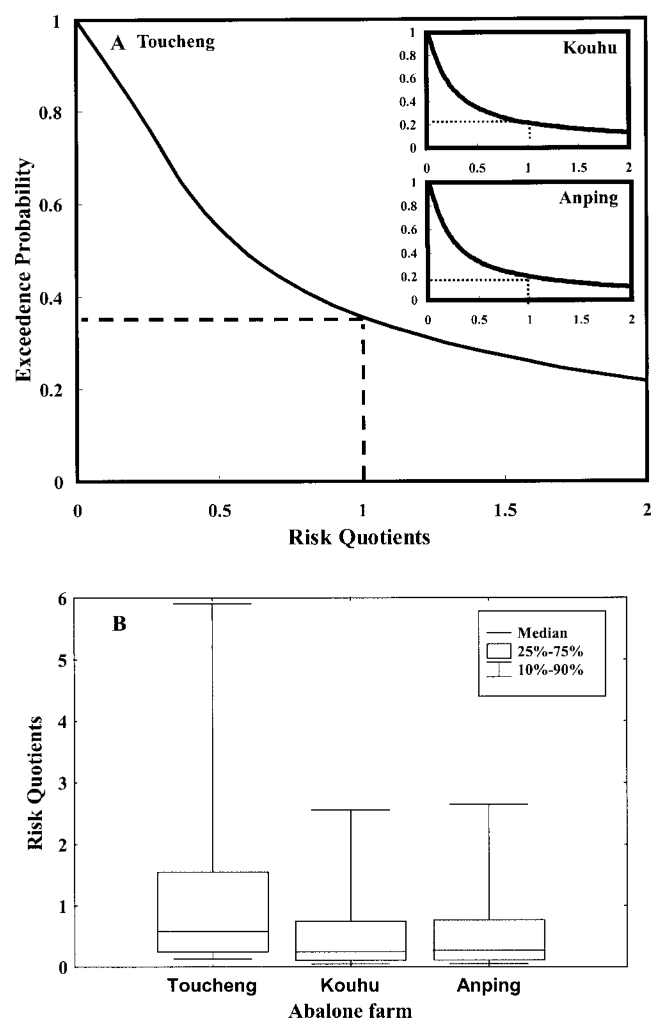

Fig. 5. (A) Risk quotient distribution and (B) box-and-whisker plots of risk quotient for three selected abalone farms.

TABLE II. Predictive risk thresholds for the protection of survival of abalone exposed to waterborne Zn

BCF_a	Zn Concentration in Pond Water (μg mL^{-1})										
(mL g^{-1})	0.05	0.06	0.07	0.08	0.09	0.10	0.11	0.12	0.13	0.14	0.15
300	0.0428	0.0534	0.0606	0.0888	0.104	0.1302	0.1442	0.1598	0.1952	0.2112	0.2356
350	0.0424	0.054	0.0792	0.0976	0.1292	0.1388	0.1596	0.1862	0.2136	0.219	0.2488
400	0.045	0.0632	0.0836	0.111	0.1348	0.139	0.1738	0.205	0.2162	0.247	0.251
450	0.0516	0.0712	0.0988	0.1202	0.139	0.1546	0.1908	0.2172	0.2364	0.2508	0.2742
500	0.0562	0.0822	0.1064	0.134	0.1594	0.173	0.207	0.2206	0.2498	0.2748	0.2684
550	0.0674	0.0856	0.104	0.145	0.1538	0.1808	0.1954	0.2446	0.2544	0.2882	0.3102
600	0.078	0.0992	0.1246	0.1452	0.1646	0.1984	0.2264	0.2376	0.2664	0.2988	0.3212
650	0.0758	0.1024	0.123	0.1462	0.1696	0.1932	0.2278	0.2456	0.289	0.3046	0.3178
700	0.0918	0.1156	0.1322	0.1786	0.1882	0.2132	0.258	0.265	0.3048	0.3188	0.3318
750	0.0882	0.1212	0.1484	0.1628	0.1932	0.2182	0.2522	0.2684	0.2966	0.3296	0.3392
800	0.0924	0.1168	0.146	0.1732	0.2096	0.2404	0.2632	0.2884	0.3034	0.3282	0.3712

sentation of Eq. (8) is shown in Figure 6, indicating surface response of the effect of water Zn concentration (C_w) and bioconcentration factor of algae (BCF_a) for Zn on risk thresholds for the protection of the survival of farmed abalone under $RQ = 1$.

DISCUSSION

Risk (Toxicity) Threshold for Farmed Abalone

In current ecological risk assessment or in developing the criteria for the protection of wildlife there is a tendency to rely on the deterministic approach of using a hazard quotient (HQ), by which an effect concentration or a statistically derived no-observed-effect concentration (NOEC) or a lowest-observed-effect concentration (LOEC) is divided by an exposure concentration to determine if an effect might be expected and subsequently to develop a toxicity threshold for each selected species of concern (Suter, 1995; U.S. EPA, 1995; Liao and Ling, 2004). One concern is that the NOEC or LOEC is not representative of a concentration at which no biologically significant effect is occurring. More complex estimates of risk from exposure to contaminants for aquatic communities involve the use of probabilistic ecological risk assessments (PERA) in that the methods usually rely on EC_{50} or LC_{50} estimates (Ferrari et al., 2004; Pennington et al., 2004). van der Hoeven et al. (1997) suggested that if an EC_x value is chosen to replace the NOEC, the preferred value of x should be 5% or 10%.

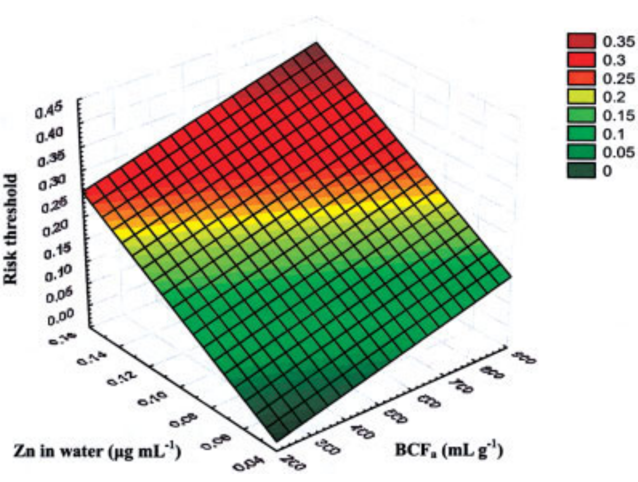

Fig. 6. Surface response plot of the algae bioconcentration factor for Zn (BCF_a) and Zn waterborne concentration (C_w) on risk threshold for the survival protection of farmed abalone exposed to waterborne Zn. The plot is the graphical representation of the nonlinear regression equation, Eq. (8). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In this work, a new method was developed to estimate the risk (toxicity) threshold for aquacultural animals. The method involved determining the EC_5 of the mortality end point from a reconstructed dose–response model for farmed abalone. This threshold is the risk, or can be appropriately transformed to a concentration following the proposed predictive risk threshold equation, at which no effects should be observed for the mortality end point above that response level. The thresholds and distributions then can be used as a surrogate for the NOEC or LOEC in risk assessment techniques, such as the HQ and PERA techniques. This new method of estimating risk (toxicity) thresholds not only is more realistic than the use of arbitrary uncertainty factors but also is more conservative than current probabilistic risk assessment methods. In our analysis, we used a model-based approach to reconstruct a dose–response curve [Fig. 4(A)] for farmed abalone in order to estimate the EC_5 distribution. The EC_5 is considered attractive because this parameter is a model-based value and the method is well established. We believe that this to be a substantial improvement over reliance on a single NOEC or LOEC for the development of aquacultural water quality management. In our work, the use of a probabilistic analysis allowed us to precisely state the level of protection that would be achieved if the risk thresholds listed in Table II were adopted. The method is easily adaptable if risk managers and the public desire a level of protection that is more relaxed or more stringent.

Model Validation

Care should be taken when using this kind of empirical regression equation for predicting risk thresholds in aquacultural ecosystems: (1) the model should be used only for predictions in abalone pond water with water Zn concentration and algae bioconcentration factor of Zn in the range of values used to develop the models, and (2) it should be noted that in some combinations, the model will yield meaningless, negative values of risk thresholds. Future research is necessary to further elucidate the relations among water quality variables where they have been shown to have consistent effects on biovavilability, bioaccumulation, and/or toxicity (Morel and Hering, 1993; Markert, 1997), and in the long run, the water quality criteria should be expressed as functions that explicitly incorporate these variables. Furthermore, the influence of water chemistry on abalone Zn accumulation will not be precisely identical from one abalone farm to another. Variations in physical, geological, and biological/ecological factors prevent a precise replication of any particular regression relationship (Lin and Liao, 1999; Tsai et al., 2004).

The use of field-derived data in risk assessment is advantageous as it provides a more realistic estimate of toxicity as normal degradation and partitioning of toxicants can occur as compared to laboratory data, which can result in an overestimation of adverse impacts. By combining the field-derived parameters of Zn waterborne concentration and algae bioconcentration factor of Zn for establishing a predictive risk threshold equation and using a modified PERA or the estimated EC_5 distribution in the calculation of an *RQ*, the risk assessor can be more confident that the proposed empirical regression model can be a simple first tool for regulatory applications until future research further verifies the model. We believe that the nonlinear predictive risk threshold model with explicit threshold effect performs better than the *HQ* model. The applications of the proposed model to real abalone farms has provided the greatest support for a threshold relation among exceedence of metal criteria, the results of ambient bioassay, and aquacultural ecosystems.

Implications

In this work, we used the PERA concepts to develop risk threshold criteria in a more integrated and efficient process in that we used probabilistic methods to establish less subjective order-of-magnitude uncertainty factors in deriving risk thresholds from limited empirical data. We also used uncertainty analysis to estimate a concentration that would provide a specified level of protection (e.g., 10% probability of 95% aquacultural species survival) for high-market-prices aquacultural species. The results of field biomonitoring or field validation studies of proposed risk thresholds should be used as evidence of the appropriateness of the proposed risk thresholds.

We believe that a probabilistic risk-based framework—probability distributions and risk diagrams such as that shown in Figure 5—is an effective representation of state-of-the-art results of scientific assessments for aquacultural species exposed to waterborne contaminants and has the potential to be used in the establishment of water quality criteria. To our knowledge, this risk-based framework has not been addressed until now. Despite great uncertainty in many aspects of integrated assessment, for example, the problem of physical and chemical variables of water such as temperature, pH, turbidity, oxygen level, that may modify water–metal concentrations, cautious interpretation of observations obtained from optimized, controlled laboratory experiments can substantially reduce this 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 are assessments that do not attempt to present results in a 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 predictive risk threshold modeling technique offers a risk-management framework for discussion of the future in deriving ambient water quality criteria for aquacultural ecosystems.

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