^{65}Zn(II) accumulation in the soft tissue and shell of abalone *Haliotis diversicolor supertexta* via the alga *Gracilaria tenuistipitata* var. *liui* and the ambient water

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Abstract

This investigation analyzed the Zn-bioaccumulation kinetics in the abalone *Haliotis diversicolor supertexta* and in the red alga *Gracilaria tenuistipitata* var. *liui* for assessing bioconcentration and biomagnification in an aquacultural system. Laboratory exposure experiments estimated uptake and depuration rate constants (i.e., k_1 and k_2, respectively) of *H. diversicolor supertexta* via nondietary and dietary processes. Bioconcentration factor (BCF) and biomagnification factor (BMF) of *H. diversicolor supertexta* as well as BCF of *G. tenuistipitata* var. *liui* were determined. A simple first-order one-compartment model fitted the uptake and depuration characteristics of Zn-bioaccumulation and successfully determined k_1 and k_2. The resulting values of k_1 and k_2 of *H. diversicolor supertexta* were 101.4 ml g^{-1} d^{-1} and 0.611 d^{-1}, respectively, when the abalone were exposed to 1 μg ml^{-1} Zn seawater without the presence of *G. tenuistipitata* var. *liui*. When the abalone were fed with the algae, k_1 and k_2 values were estimated to be 114.5 g g^{-1} d^{-1} and 0.636 d^{-1}, respectively. BCF values for the alga and abalone were determined to be 170 and 180, respectively; and the BMF value was 1.06 for the abalone. Both field and laboratory data show that BMF values for Zn were about 1. Further more, the abalone in the tank without algae absorbed the same quantity of Zn as the abalone in the tank with alga. From these two findings we conclude that Zn in the abalone comes from the ambient water and not from the algae. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Abalone; Algae; Bioaccumulation; Bioconcentration; Biomagnification; Mollusc; Zn

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1. Introduction

Pollutants can be discharged into the ocean from polluted rivers, sewage outfalls or local industries. Many studies have demonstrated that contaminants affect marine organisms that live in polluted environments (Conroy et al., 1996). Some marine organisms have the ability to accumulate water-borne chemicals, and therefore they can be used to describe the environmental pollution or to monitor the level of contamination (Barron, 1990; Franke et al., 1994; Lee et al., 1996).

The accumulations of heavy metals in some marine organisms, such as algae and molluscs, have been suggested as indicators of heavy metal contamination in the water column (Zatta et al., 1992; Karez et al., 1994; Walsh et al., 1995; Han et al., 1997). Many algae and molluscs have wide distributions, extensive populations, sedentary nature and the ability to accumulate contaminants. Therefore, monitoring those bioaccumulators for heavy metals is useful as an ideal contamination index in the aquatic environment (Burdin and Bird, 1994; Walsh et al., 1994; Uno et al., 1997).

Abalone are common gastropod molluscs that inhabit the coastal reefs in tropical and subtropical areas (Hahn, 1989). The herbivorous gastropod, *Haliotis diversicolor supertexta*, is the most abundant abalone species in Taiwan. The aquaculture of *H. diversicolor supertexta* is one of the important aquatic products in Taiwan (Chen, 1984, 1989). *H. diversicolor supertexta* prefers red algae, *Gracilaria* spp., which yield the best growth of the abalone (Chen, 1989; Singhagraiwan and Doi, 1993). Because of economic considerations, the seaweed *Gracilaria tenuistipitata* var. *liui* has been selected to be the major forage for culturing *H. diversicolor supertexta*.

H. diversicolor supertexta is appreciated for its delicacy and high market value; therefore, the aquaculture of *H. diversicolor supertexta* and *G. tenuistipitata* var. *liui* is a promising business (Chen, 1989; Singhagraiwan and Doi, 1993). However, the coastal regions of Taiwan where the algal and abalone aquaculture facilities are located are subjected to polluted discharges from rivers. Previous investigations indicated that heavy metal contaminants, such as Zn, have been detected in many rivers in Taiwan. Heavy metal pollution can affect the abalone via the water, via the algae they eat or both may influence the abalone. These heavy metals may be important micronutrient for the algae and animals; but they are toxic at high concentrations and have severe effects on the health of organisms, which then become unsalable for human consumption (Hahn, 1989; Conroy et al., 1996; Knauer et al., 1997).

Bioaccumulation of heavy metals in animals occurs by two processes: bioconcentration and biomagnification. Bioconcentration occurs by means of passive diffusion of the heavy metals from the ambient water via the gills into the circulatory fluid and then deposition in the tissues of organisms. Biomagnification is the transfer of heavy metals from low trophic level biota to higher trophic level biota (Connell, 1998). Many studies have reported the bioconcentration and biomagnification of aquatic organisms, yet most of these studies were only concerned with one of these two processes. They rarely considered that the accumulation of heavy metals of organisms may occur in both nondietary and dietary processes. Thus, this study was designed to assess the bioaccumulation of ^{65}Zn(II) (Zn) in abalone *H. diversicolor supertexta* via dietary and nondietary routes of the alga *G. tenuistipitata* var. *liui* and ambient water, respectively.

The accumulation of heavy metals in molluscs has been mainly studied from the content of soft tissues (Lautie et al., 1988). Although Bertine and Goldberg (1972) and Walsh et al. (1995) noted that heavy metals can be accumulated in both soft tissue and calcareous shells of molluscs, the relationships between the concentrations of heavy metals in soft tissue and those in shell are poorly known. Chen (1989) reported that the shell coloration of *H. diversicolor supertexta* differs according to the algae eaten: it is green after eating Chlorophyceae and brown after eating Rhodophyceae. It seems conceivable that this abalone utilizes the deposition process of new shell material to relocate bioaccumulated recalcitrant chemicals from the metabolically active soft tissues to the relatively inert shell material. The exoskeleton can act as a receptor for unwanted chemicals, such as heavy metals.

The goals of this research were to analyze the Zn-bioaccumulation kinetics in both soft tissue and shell of *H. diversicolor supertexta* as well as in the algae and, if possible, to establish simple relationships of Zn concentrations between these organisms and their environment. Zn uptake and depuration were analyzed following short-term exposure experiments.

2. Materials and methods

2.1. Sampling and acclimation

The most important farming areas for the production of abalone *H. diversicolor supertexta* are in Toucheng on the north coast, Kouhu on the west coast and Anping on the south coast of Taiwan. All the abalone farms use sea water from polluted coastal areas. Therefore, we collected the samples of the abalone, the alga *G. tenuistipitata* var. *liui* and ambient water from 9 farms in the three locations mentioned above. Three abalone, three algae and three 500 ml water samples per site were collected. The abalone and algal samples initially were washed in sea water to remove epiphytes and kept at 4°C during transfer to the laboratory. The water samples were fixed by adding 5 ml 1N HNO$_3$.

H. diversicolor supertexta and *G. tenuistipitata* var. *liui* were collected from Toucheng for the laboratory exposure experiments, because this place was the most Zn contaminated area of the three sites. Abalone with a shell length of 4 cm were selected for the experiments. The algal samples selected were mature, whole and healthy. An amount of 200 abalone were transferred into 4 aquatic tanks of approximately 54 l volume, containing 50 l of artificial sea water. In order to imitate the environment of the abalone farms, the abalone were held in baskets. Each tank contained 10 baskets. Four abalone per basket were used for analysis. To be sure that at least 4 abalone would be alive at the end of the experiment, we put one extra abalone in each basket. The tanks were aerated to provide air and water movement. The temperature and salinity were maintained at 25°C and 35‰ under constant illumination (Yang and Ting, 1986). The abalone were fed daily with *G. tenuistipitata* var. *liui*. The abalone and algae were acclimatized for 2 weeks before they were exposed to the heavy metal Zn.

2.2. Exposure

In order to examine if, and in what way, (via the food chain and/or via the water) the abalone is affected by Zn pollution, we set up the experiment as follows. In two tanks Zn was added to the seawater; in one tank the abalone were fed with algae, in the other tank the abalone were kept without food. The Zn contamination level was determined by a preliminary experiment exposing abalone to different Zn concentrations (0.25, 0.5, 1, 2, 4 and 6 ppm). The tolerance (LT_{50}) of abalone at ≤ 1 ppm Zn was longer than 21 days. Therefore, the organisms were exposed to 1 $\mu g\ ml^{-1}$ Zn for 7 days in this experiment. The algae and the abalone were reared in the contaminated environment for 7 days uptake, then transferred to clean sea water and reared for 7 days of depuration.

To examine if starvation affects Zn depuration in abalone, the same procedure with abalone and algae was followed over 14 days using the other two tanks, but without Zn in the sea water.

Abalone, algae and water samples were collected at day 0, 1, 2, 4, and 7, starting from the day that the organisms were exposed to the contaminated sea water and from the day the organisms were transferred to clean sea water. Every time we took one basket along with 500 ml water out of each tank. From this basket four pieces of algae and four abalone were collected. Because preliminary observation showed that *H. diversicolor supertexta* only feeds at night, and has an empty gut in the evening, we collected the abalone at night to make sure the contents of gut would not influence the results.

The experiments in the four tanks, described above, were repeated again.

The water samples were fixed with 5 ml 1N HNO_3, and the samples of algae and abalone were stored in the dark at $-20°C$ until they were analyzed.

2.3. Analysis

The algae and shucked abalone were freeze-dried overnight, and then ground into a fine powder in a grinder (Tai-Hsiang S36-89). 500 mg portions of the ground samples were digested in 10 ml concentrated HNO_3 (65 wt.%) overnight at room temperature. The resulting solution was evaporated and redissolved in 0.1 N HCl (Karez et al., 1994). Zn concentration was determined by an atomic absorption spectrophotometer (Perkin Elmer 5000).

2.4. Calculation of BCF, BMF and rate constants

When steady-state chemical concentrations of tissue are attained, the equilibrium bioconcentration factor (BCF) of the abalone and algae can be calculated from the ratio of the chemical concentration in the biota to that in sea water; while the ratio of the chemical concentration in the abalone to that in the algae is used to calculate the equilibrium biomagnification factor (BMF). The BCF and BMF can also be calculated from the ratio of the uptake rate constant to the depuration rate constant as,

$$BCF = \frac{C_b}{C_w} = \frac{k_1}{k_2} \tag{1}$$

and

$$\mathrm{BMF} = \frac{C_{\mathrm{m}}}{C_{\mathrm{a}}} \tag{2}$$

where C_{b} (μg g^{-1}) is the chemical concentration in biota; C_{w} (μg ml^{-1}) is the chemical concentration in water; C_{m} (μg g^{-1}) is the chemical concentration in mollusc; C_{a} (μg g^{-1}) is the chemical concentration in algae; k_1 is the uptake rate constant (ml g^{-1} d^{-1} or g g^{-1} d^{-1}); and k_2 is the depuration rate constant (d^{-1}).

Eqs. (1) and (2) are based on a well-established model that is first-order one-compartment and that is used to estimate accumulated chemicals resulting from exposures to water-borne contaminants, at uptake phase,

$$\frac{\mathrm{d}C_{\mathrm{b}}}{\mathrm{d}t} = k_1 C_{\mathrm{w}} - k_2 C_{\mathrm{b}} \tag{3}$$

at depuration phase,

$$\frac{\mathrm{d}C_{\mathrm{b}}}{\mathrm{d}t} = -k_2 C_{\mathrm{b}} \tag{4}$$

The solution of Eq. (3) at the constant C_{w} is,

$$C_{\mathrm{b}}(t) = C_{\mathrm{b}}(t = 0) + C_{\mathrm{w}} \frac{k_1}{k_2} (1 - \mathrm{e}^{-k_2 t}) \tag{5}$$

As the first-order one-compartment model assumes that k_2 is not a function of tissue concentration, k_2 is often determined by depurating contaminated organisms in uncontaminated water and determining k_2 directly in that test organism. Therefore, after the algae and mollusc are transferred to clean water and diet tanks, respectively, the depuration rate constants (k_2) can be calculated by the linear regression of log-transformed tissue Zn concentrations on depuration time (days) as,

$$\ln C_{\mathrm{b}}(t) = \ln C_{\mathrm{b}}(t = T) - k_2 t \tag{6}$$

where T is the time when depuration begins. The k_1 and k_2 can also be estimated by fitting Eq. (5) to measured tissue Zn concentration data from the uptake experiments

Table 1
Zn concentration (mean $\pm$ S.E., $n = 9$), BCF and BMF in field samples of water, the algae *G. tenuistipitata* var. *liui* and the abalone *H. diversicolor supertexta* collected from Toucheng, Kouhu and Anping

Location	Water (ng ml^{-1})	Algae (μg g^{-1})	Abalone (μg g^{-1})	BCF[a]	BCF[b]	BMF[c]
Toucheng	131.04 $\pm$ 31.99	91.04 $\pm$ 33.10	111.00 $\pm$ 15.29	694.7	847.1	1.219
Kouhu	60.71 $\pm$ 21.60	25.44 $\pm$ 6.02	46.41 $\pm$ 7.69	419.0	764.5	1.824
Anping	69.59 $\pm$ 32.23	31.93 $\pm$ 12.85	49.77 $\pm$ 7.33	458.8	715.2	1.559
Average				524.2	775.6	1.534

[a] BCF of algae.
[b] BCF of abalone.
[c] BMF of abalone.

using an iterative, nonlinear, least-squares curve-fitting technique (SAS, Version 6.11). Variances in k_2 values derived from two methods were tested for homogeneity using an *F*-test. Values were then compared using *t*-test. The BCFs and BMFs were calculated from Eqs. (1) and (2).
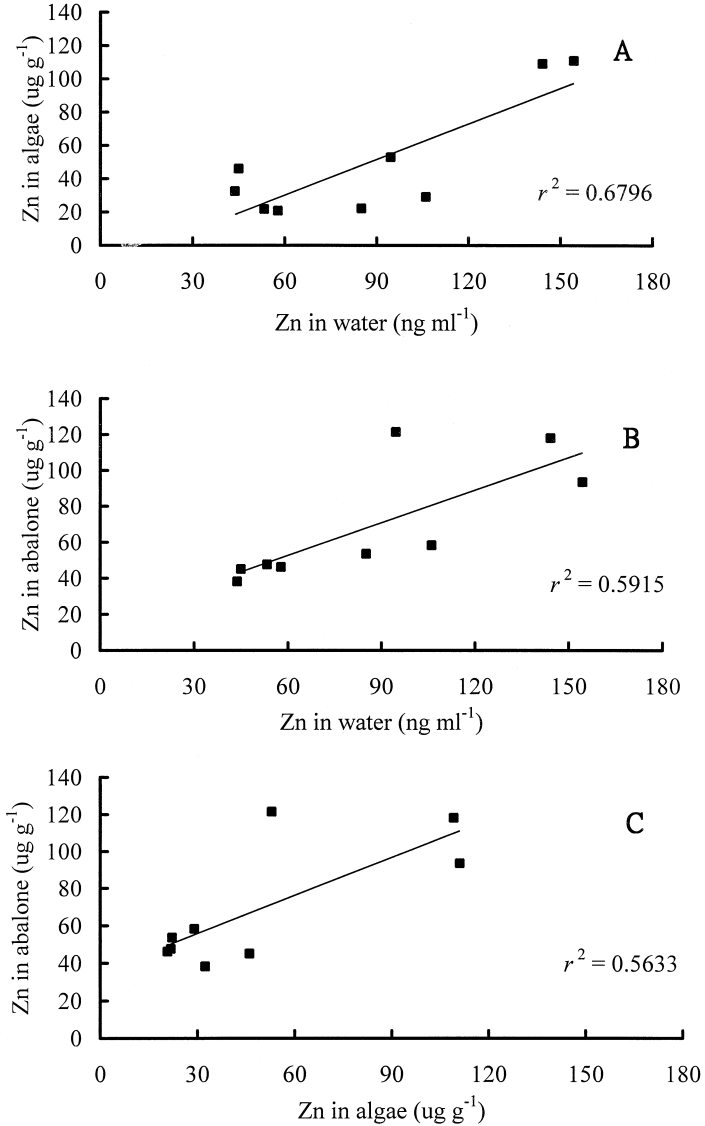

Fig. 1. Zn concentration in field samples of water, *G. tenuistipitata* var. *liui* and *H. diversicolor supertexta*: (A) Zn concentration in *G. tenuistipitata* var. *liui* as a function of Zn concentration in water; (B) Zn concentration in abalone as a function of Zn concentration in water; (C) Zn concentration in abalone as a function of Zn concentration in *G. tenuistipitata* var. *liui*.

3. Results

Field samples show that the level of Zn in the water of aquaria at Toucheng (131.04 ± 31.99 ng ml^{-1}) was higher than at Kouhu (60.71 ± 21.60 ng ml^{-1}) and Anping (69.59 ± 32.23 ng ml^{-1}) (Table 1). The algae and abalone from Toucheng also contained higher concentrations of Zn than those from Kouhu and Anping (Table 1).

As can be seen from Eq. (5), C_b is a linear function of C_w with all other parameters being constant within a given experiment. Consequently, model predictions for each of the experimental units analyzed in this study can be described by a straight line (Fig. 1). Variances of Zn concentrations in water, algae, and in the soft tissue and the shell of abalone were tested for homogeneity using F-test. Values of Zn concentration were then compared using the appropriate t-test. Fig. 1 shows the Zn concentrations in algae and abalone increased with that in water, while the Zn concentration in abalone increased with that in algae. The correlation of Zn concentrations in algae, abalone and water is significant ($r^2 = 0.68$, $F = 14.85$, $P < 0.05$ for algae and water; $r^2 = 0.59$, $F = 10.14$, $P < 0.05$ for abalone and water; $r^2 = 0.56$, $F = 9.03$, $P < 0.05$ for abalone and algae).
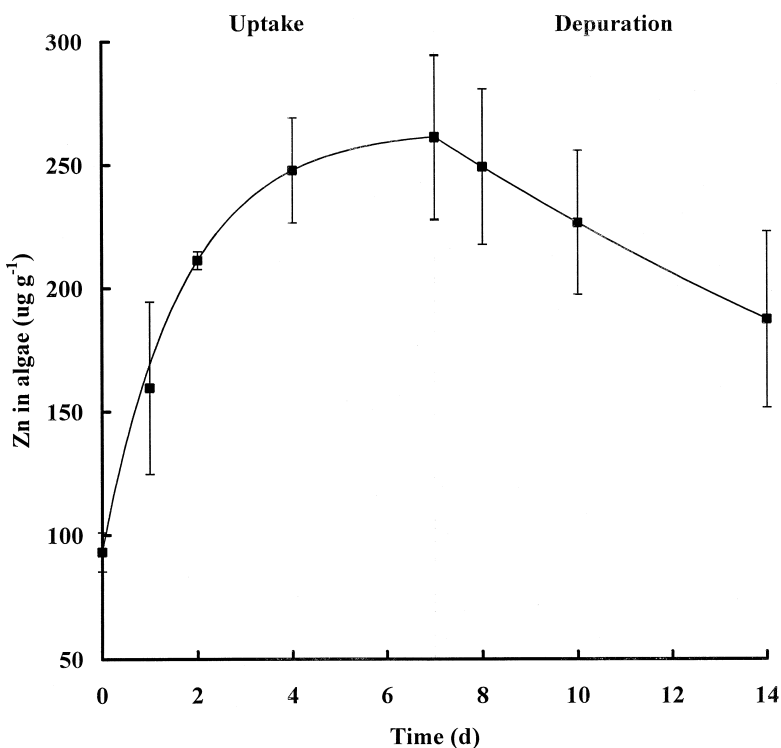

Fig. 2. Uptake and depuration of Zn by *G. tenuistipitata* var. *liui* during a 7-day exposure and then a 7-day depuration period. The measurements are shown with symbols and the model simulations are shown in solid line.

Fig. 2 shows a least-squares linear regression line plotted for the depuration experiment of Zn by algae as $\ln C(t) = 231.95 - 0.048t$, $(r^2 = 0.70)$; while the first-order one-compartment model curve fit to the uptake phase data using an iterative nonlinear technique was $C(t) = 163.92(1 - e^{-0.588t})$, $(r^2 = 0.98)$. The uptake/depuration experiment of Zn by abalone shown in Fig. 3 have linear regression equations of (1) food-exposed: $\ln C(t) = 226.18 - 0.035t$, $(r^2 = 0.70)$ and (2) water-exposed: $\ln C(t) = 190.03 - 0.036t$, $(r^2 = 0.67)$ for depuration phase; while for uptake phase the nonlinear regression equations are respectively as (1) food-exposed: $C(t) = 164.40(1 - e^{-0.636t})$, $(r^2 = 0.99)$ and (2) water-exposed: $C(t) = 13.01(1 - e^{-0.611t})$, $(r^2 = 0.98)$.

A simple first-order one-compartment model was thus successfully fitted by the nonlinear technique to the uptake curve of the 7-day exposure tissue Zn concentration data in that coefficients of determination (r^2 values) generally were high (> 0.95) (Figs. 2 and 3). Results suggest that the fitted first-order equation is an appropriate model for the data set. Estimates of k_2 (Table 2) were determined from the depuration-phase experiments (Figs. 2 and 3). All of these regressions were significant, with r^2
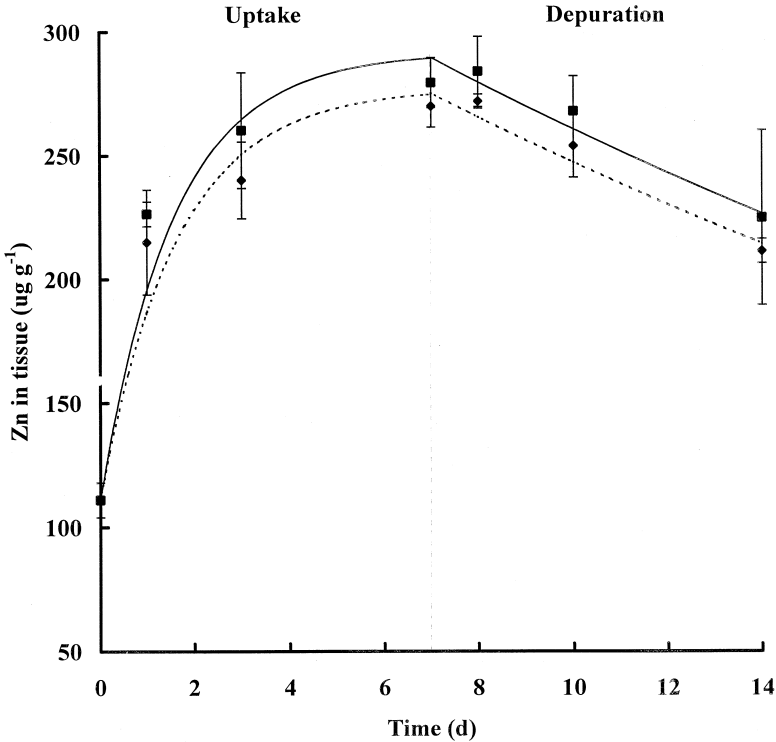

Fig. 3. Uptake and depuration of Zn by the soft tissue of *H. diversicolor supertexta* during a 7-day exposure and then a 7-day depuration period. The measurements are shown with symbols (■: fed with algae; ◆: kept without algae); and the model simulations are shown in lines (solid line: fed with algae; dotted line: kept without algae).

Table 2
The values (mean ± 95% confidence interval) of BCF, BMF, uptake rate constant (k_1) and depuration rate constant (k_2) of the algae *G. tenuistipitata* var. *liui* and the abalone *H. diversicolor supertexta* calculated from laboratory Zn exposure experiments

	k_1 (g g^{-1} d^{-1})	k_2 (d^{-1})	BCF	BMF
Algae	100.0 ± 22.8	0.588 ± 0.229	170	
Abalone				
Food-exposed	114.5 ± 23.4	0.636 ± 0.261	180	1.06
Water-exposed	101.4 ± 25.7	0.611 ± 0.534	166	0.92

values that ranged from 0.67–0.7. The k_2 values determined in depuration experiments were also statistically significant from their corresponding k_2 values derived from curve fitting the first-order one-compartment model to the uptake data.

Values of the bioconcentration and biomagnification factors estimated from these uptake and depuration experiments are listed in Table 2. The value of BCF of Zn in
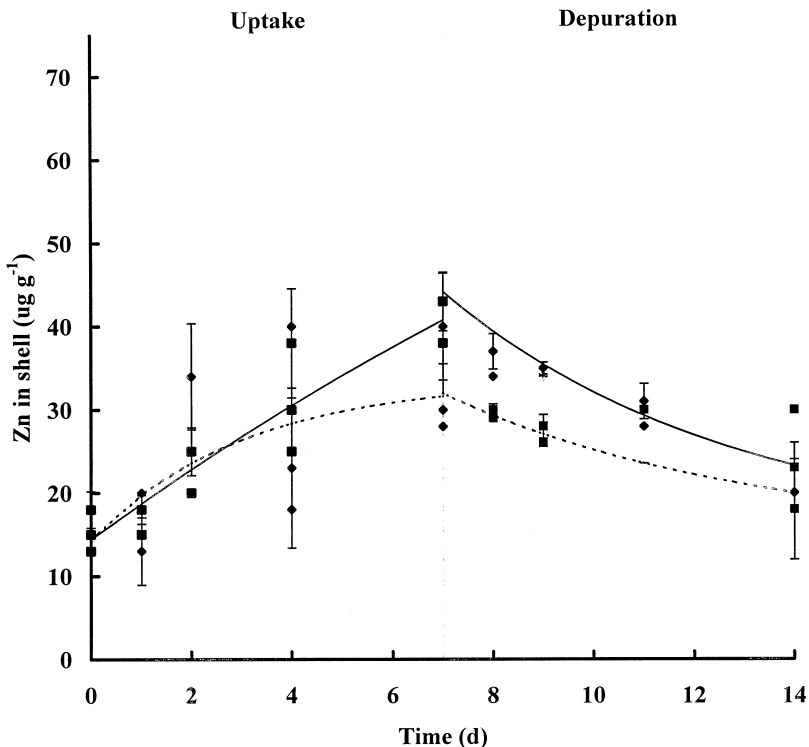

Fig. 4. Uptake and depuration of Zn by the shell of *H. diversicolor supertexta* during a 7-day exposure and then a 7-day depuration period. The measurements are shown with symbols (■: fed with algae; ♦: kept without algae); and the model simulations are shown in lines (solid line: fed with algae; dotted line: kept without algae).

algae was 170. The BCF value of abalone fed with algae was 180, while the value was 166 in the abalone kept without algae. The value of BMF of Zn in the abalone fed with algae was 1.06, while the value was 0.92 in the abalone kept without algae. BCF values estimated from laboratory exposure experiments were substantially lower than those calculated based on the field data (BCF $= 524.2 \pm 149.0$ for algae; BCF $= 775.6 \pm 66.6$ for abalone) (Table 1).

Analysis of variance revealed that the uptake rate constants of *H. diversicolor supertexta* fed with algae were not different from those of the abalone kept without algae ($F = 0.0000083$, $P > 0.05$ for soft tissue; $F = 0.012$, $P > 0.05$ for shell) (Figs. 3 and 4). In addition, there were no significant differences between water-exposed and food-exposed abalone concerning BCFs ($F = 0.539$, $P > 0.05$) and BMFs ($F = 0.08$, $P > 0.05$). The results indicate that uptake of Zn from food by the abalone is unimportant compared with uptake from water.

Variance analysis of Zn concentrations in soft tissue and shell of the abalone showed that the content of Zn in shell of *H. diversicolor supertexta* was less than that in soft tissue ($r^2 = 0.69$, $F = 29.59$, $P < 0.05$). The concentration of Zn in shell was proportional to that in tissue in that the ratio of Zn content in tissue to that in shell was 9.6:1 on a dry weight basis.

The results of the experiments without Zn showed that the Zn concentrations in the abalone fed with algae were not significantly different from those in the abalone kept without algae ($F = 1.87$, $P > 0.05$ for soft tissue; $F = 0.41$, $P > 0.05$ for shell). Thus the effect of starvation can be neglected during the experiment period.

4. Discussion

In this study, first-order one-compartment models successfully estimated the uptake and depuration rate constants. Results obtained indicate that *G. tenuistipitata* var. *liui* and *H. diversicolor supertexta* accumulated considerable amounts of Zn from sea water. The quantity of heavy metals in organisms clearly reflects the quantity of the heavy metals in the water in which the algae and abalone grow (Bertine and Goldberg, 1972). Based on these characteristics, the Zn concentrations in *G. tenuistipitata* var. *liui* and *H. diversicolor supertexta* indicate the Zn concentration in water. These two species can potentially be useful indicators for the bioaccumulation of pollutants in artificial and natural environments.

Both field and laboratory data show that BMFs for Zn were about 1. Further more, the abalone in the tank without algae absorbed the same quantity of Zn as the abalone in the tank with algae (Fig. 3). From these two findings we conclude that Zn in the abalone comes from the ambient water and not from the algae. A similar phenomenon was reported for BMF by Amiard-Triquet et al. (1987) where they demonstrated that the levels of Zn in algae-grazing molluscs, *Gibbula umbillicalis* and *Littorina littorea*, are not different from the Zn level in a brown alga, *Fucus serratus*, which is the food species of the molluscs. Consequently, concerning the aquaculture of abalone, it is important to control Zn concentration in the ambient water first.

Heavy metal concentration in algae has been reported mainly for temperate areas. Some of the algae, such as *Fucus* spp. and *Ascophyllum* spp., have been suggested as

indicators of heavy metal contamination in sea water (Seeliger and Edwards, 1977; Melhuus et al., 1978; Bryan, 1983; Soderlund et al., 1988). Burdin and Bird (1994) demonstrated that the living thalli of *Gracilaria tikvahiae* generally showed the greatest amounts of heavy metal accumulation compared to the other three red algae, *Agardhiella subulata*, *Chondrus crispus* and *Gelidium pusillum*. There is a relative lack of information for contaminated regions in tropical and subtropical areas (Karez et al., 1994). In the present study, the tropical/subtropical species *G. tenuistipitata* var. *liui* seems to be a useful bioindicator in sea water, due to the fact that the content of Zn in the algae is 524 times higher than the aquatic environment where the algae were sampled. An ideal bioindicator should be sedentary, abundant and have a long life. It should be also easy to collect, able to accumulate pollutants and provide sufficient tissue for contaminant analysis. *G. tenuistipitata* var. *liui* meets all of these conditions. Therefore, this red alga can be considered as an ideal species for environmental monitoring. In addition, this species is also an inexpensive material to grow, and could be used to remove heavy metals from polluted environments.

H. diversicolor supertexta could also be a good biomonitor, yet the cost of growing abalone is high. The results of this study show that the shell of *H. diversicolor supertexta* accumulated Zn and reflected the composition of the sea water in which the organism lived. Although the content of Zn in the shell of *H. diversicolor supertexta* was less than in the soft tissue, the shell is still useful as an indicator. The amount of Zn in the shell was proportional to the concentration in the soft tissue. A similar phenomenon was described by Bertine and Goldberg (1972) and Walsh et al. (1995); they demonstrated that heavy metals were usually higher in the soft parts than that in the solid shell of clams, mussels and shrimps. The presence of contaminants in the shell of *H. diversicolor supertexta* may represent an effective and sensitive means to assess contamination in the soft tissue of this organism and to monitor the aquatic ecosystem.

The shell could act as a toxic waste 'dump' to remove toxic chemicals from the metabolically active tissue and therefore effectively eliminate these chemicals from the food chain (Walsh et al., 1994). The relocation of the contaminants to the shell represents an effective detoxification mechanism.

This research has provided kinetic data for uptake and excretion of Zn by abalone and algae. These data are essential for developing predictive models of Zn accumulation in field aquacultural ecosystems. However, the data from field samples must be interpreted with caution because they were collected at a limited number of sites. Therefore, the comparison with laboratory exposure experiments must be made cautiously.

Further research is required to determine the level of Zn accumulated in key organs such as gill, liver and kidney and to determine if this Zn accumulation is detrimental to abalone in that BCFs for individual tissues may reach levels sufficient to produce toxic effects. Such Zn accumulation may not be acutely toxic, yet it could have deleterious effects on molecular processes essential to long-term survival and reproduction.

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