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Modeling of depuration of paralytic shellfish toxins in *Chlamys nobilis* and *Perna viridis*

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Of the toxic syndromes associated with harmful algal blooms (HABs), paralytic shellfish poisoning (PSP) has attracted much attention due to its public health implications and damaging impact on commercial shellfish industries (millions of US dollars in some incidents) (Chen and Chou, 1998; also see review by Bricelj and Shumway, 1998). Paralytic shellfish toxins (PSTs) can be bioaccumulated in aquatic biota, and biomagnified through the food web (Li et al., 2005). PSTs are fast-acting neurotoxins that inhibit transmission of nerve impulses by blocking sodium channels. These toxins can result in death by respiratory arrest within several minutes to a few hours (Duy et al., 2000). PSTs comprise over 20 molecular forms, and can be grouped into several main categories: the most toxic are the carbamate toxins (e.g. saxitoxin and gonyautoxins), followed by the decarbamoyl toxins and then the *N*-sulfocarbamoyl toxins (e.g. C-toxins).

The PSP causative organisms are dinoflagellates such as *Alexandrium* spp. and *Gymnodinium* spp. Shellfish acquire PSTs mainly through filter feeding of toxic dinoflagellate cells (Bricelj et al., 1990). The biochemical, physiological and behavioral responses of shellfish to PSP-producing dinoflagellates are highly species specific (e.g. Shumway and Cucci, 1987; Smolowitz and Shumway, 1997). Different shellfish species have vastly different (up to 100-fold) abilities to accumulate and depurate toxins (Bricelj and Shumway, 1998 and references therein).

Once absorbed by a shellfish, PSTs will be transported differentially to various tissues, from which they are eliminated at varying rates. The compartmentalization patterns vary with species and with time (e.g. Cembella et al., 1993; Shumway et al., 1994). For example, PSP toxicities in the sea scallop (*Placopectan magellanicus*) follow the order of digestive gland > mantle ≫ gill > gonad ≫ adductor muscle (Cembella et al., 1994). In general, PSP toxins accumulate at highest levels in the viscera of marine shellfish, but the ranking of toxicity among tissues often shifts during depuration (Bricelj et al., 1990; Bricelj and Laby, 1996).

Several studies on temperate bivalves have shown that depuration occurs most rapidly in the viscera (e.g. Waiwood et al., 1995). Based on the rate of toxin elimination, bivalves can be classified as rapid (e.g. the blue mussel, *Mytilus edulis*) or slow detoxifiers (e.g. the sea scallop, *Placopecten magellanicus*) (Bricelj and Shumway, 1998). The former takes weeks to detoxify to below regulatory levels, while the latter could take months to years. Although there is a considerable volume of information on the uptake and depuration kinetics of PSTs in temperate shellfish, similar information is scarce for tropical species.

Exponential equations have previously been used to calculate uptake and depuration rates of PSTs in various bivalve species [Bricelj and Shumway, 1998; and references therein]. In contrast, complete dynamic models, derived from first principles, have rarely been constructed for the biokinetics of PSTs. It is instructive to

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explore the use of such dynamic models to quantitatively describe the transfer of toxins into and out of the shellfish, as well as the contribution of various tissue types in these processes. These types of models can provide quantitative predictions of PST levels in the whole animal and in individual tissue compartments when the shellfish are exposed to PST-laden algal cells, and after the HAB has subsided (Li et al., 2005).

The present study aims to model the depuration of PSTs in two common shellfish in south China, namely the scallop *Chlamys nobilis* and the green-lipped mussel *Perna viridis*. Specifically, this study investigates the anatomical distribution of toxins in two body compartments and depuration of toxins from them. This information is of particular relevance to these shellfish since only a specific body part (e.g. adductor muscle) is generally consumed for the scallop, while all the soft tissues are eaten for the green-lipped mussels.

The data used in this study have already been published, and the details of experimental designs, exposure regimes to toxigenic algae, analytical methods for toxin quantification, and quality assurance/quality control procedures are all given in Choi et al. (2003) and Li et al. (2005). The analytical procedures for PSTs followed those described by Oshima (1995). It is worth noting that in one study (Choi et al., 2003), green-lipped mussels were dissected into the digestive gland and other tissues, and the scallops were dissected into the adductor muscle and other tissues (including the digestive gland). In the second study (Li et al., 2005), each mussel was dissected into five body compartments (hepatopancreas, gill, foot, adductor muscle and viscera). The maximum PST concentrations in the green-lipped mussels studied by Li et al. (2005) were about three orders of magnitude higher than those studied by Choi et al. (2003). This provides an opportunity to investigate whether the PST concentrations in the mussels have effects on the biokinetics.

In the first stage, for both *C. nobilis* and *Perna viridis*, a two-compartment model is employed with the digestive gland (D) as the first compartment and the "remaining tissue" (T) as the second compartment. The biokinetics are modeled by first-order linear differential equations. This preliminary compartment scheme for the two compartments is represented in Fig. 1.

The differential equations governing the biokinetics and the time-dependent toxin concentrations in the two compartments are given below. The differential equation governing depuration for the digestive gland (compartment D) is

$$\frac{dq_D}{dt} = -\lambda_D q_D \tag{1}$$

and that governing depuration for the "remaining tissue" (compartment T) is

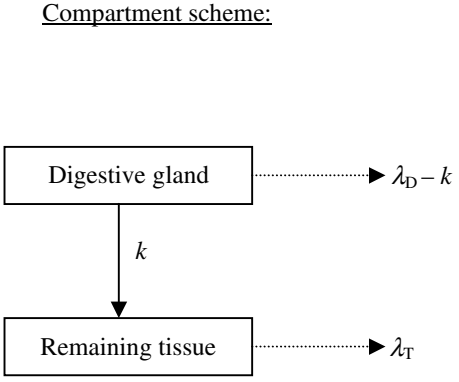

Fig. 1. Preliminary compartment scheme for the two compartments of *Chlamys nobilis* and *Perna viridis*: digestive gland and remaining tissue.

$$\frac{dq_T}{dt} = \frac{m_D}{m_T} k q_D - \lambda_T q_T \tag{2}$$

where q_D and q_T are the concentrations of the toxin in the D and T compartments, respectively, m_D and m_T are the corresponding wet weights, λ_D and λ_T are non-negative removal rates, and k (where $k < \lambda_D$) is a non-negative transfer coefficient from compartment D to compartment T. The parameters k, λ_D and λ_T are to be determined. The time-dependent toxin concentrations in the two compartments during depuration with the initial conditions (on day 0) $q_D(0) = q_{Do}$ and $q_T(0) = q_{To}$:

$$q_D = q_{Do} e^{-\lambda_D t} \tag{3}$$

$$q_T = q_{To} e^{-\lambda_T t} + \frac{m_D}{m_T} k q_{Do} \left(\frac{e^{-\lambda_T t} - e^{-\lambda_D t}}{\lambda_D - \lambda_T} \right) \tag{4}$$

Therefore, if k, λ_D and λ_T are determined, the biokinetics of the toxin in the two compartments, namely, digestive gland and "remaining tissue", can be calculated.

We first studied the biokinetics of C2 toxin in the scallop *C. nobilis*. In the experiments for *C. nobilis*, the "remaining tissue" compartment refers to the adductor muscle. The experimental data for *C. nobilis* were fitted using user-defined expressions in the non-linear curve fit program of the Microcal™ Origin™ (Version 6.0) with the interested parameters as the user-defined parameters. The weights of the digestive gland and the adductor muscle of *Chlamys nobilis* were also measured as 5.561 ± 0.787 g and 1.739 ± 0.365 g, respectively.

From the depuration data shown in Fig. 2, the fitted curve gives the following results:

$$q_{Do} = 3292 \pm 316 \ (\text{pmol g}^{-1})$$

$$\lambda_D = 1.222 \pm 0.295 \ (\text{day}^{-1})$$

From the depuration data in Fig. 3(a), the fitted curve gives the following results:

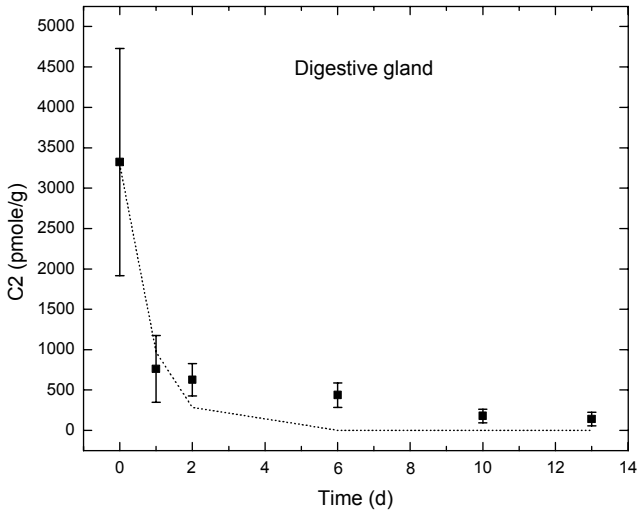

Fig. 2. Concentrations (pmol g^{-1}) of toxin at different depuration times in the digestive gland of *Chlamys nobilis*. Broken line: best fit. Depuration started on day zero. Vertical lines represent ±1 S.D.; $n = 3$.

$$q_{To} = 149.7 \pm 16.7 \ (\text{pmol g}^{-1})$$

$$\lambda_T = 0.1151 \pm 0.1903 \ (\text{day}^{-1})$$

$$k = -0.01305 \ (\text{day}^{-1})$$

However, a negative k violates our compartment scheme, as k should be non-negative as stated above. We attempted to perform the fitting again with the constraint that k should not be negative. The results are shown in Fig. 3(b), and the following parameters were obtained:

$$q_{To} = 150.7 \pm 22.0 \ (\text{pmol g}^{-1})$$

$$\lambda_T = 0.9455 \pm 0.3219 \ (\text{day}^{-1})$$

$$k \approx 0 \ (\text{day}^{-1})$$

In other words, there is essentially no transfer of toxins from the digestive gland to the adductor muscle during

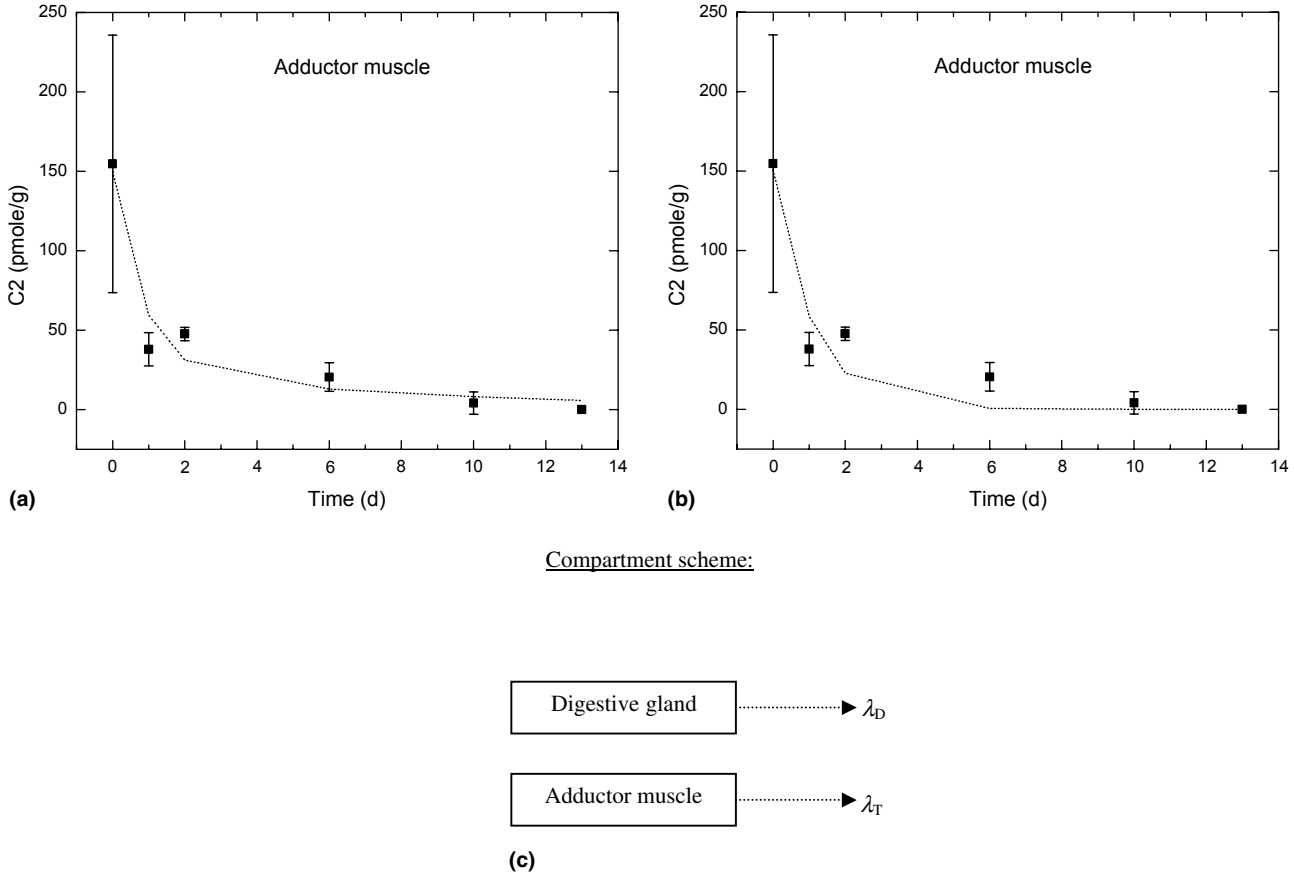

Fig. 3. (a) Concentrations (pmol g^{-1}) of toxin at different depuration times in the adductor muscle of *Chlamys nobilis*. Broken line: best fit without constraints. Depuration started on day zero. Vertical lines represent ±1 S.D.; $n = 3$. (b) Concentrations (pmole g^{-1}) of toxin at different depuration times in the adductor muscle of *Chlamys nobilis*. Broken line: best fit with the constraint that k is non-negative. Depuration started on day zero. Vertical lines represent ±1 S.D.; $n = 3$. (c) Final compartment scheme for the two compartments of *Chlamys nobilis*: digestive gland and adductor muscle.

depuration. The depuration process resembles depuration from two independent compartments when the animal is placed into "toxin-free" seawater as shown by the compartment scheme shown in Fig. 3(c).

The experimental data for the biokinetics of C2 toxin in the green-lipped mussel *Perna viridis* were also fitted using Microcal™ Origin™. The weights of the digestive gland and the remaining tissue of *Perna viridis* were also measured as 0.450 ± 0.102 g and 2.104 ± 0.378 g, respectively.

From the depuration data shown in Fig. 4(a), the fitted curve gives the following results:

$q_{\mathrm{Do}} = 5236 \pm 648$ (pmol g^{-1})

$\lambda_{\mathrm{D}} = 1.125 \pm 0.339$ (day^{-1})

In the present case for *Perna viridis*, fitting the experimental data with the two-compartment model without constraints gives k values larger than λ_{D} which is physically impossible. Therefore, during the fitting procedures, we have to impose an extra condition that k should be at most equal to λ_{D} The results are shown in Fig. 4(b), and the following parameters were obtained:

$q_{\mathrm{To}} = 627.4 \pm 48.7$ (pmol g^{-1})

$\lambda_{\mathrm{T}} = 3.74$ (day^{-1})

$k = 1.1$ (day^{-1})

In other words, essentially all toxins in the digestive gland were transferred to the remaining tissue during depuration. When the animal is placed into "toxin-free" seawater, the depuration proceeds as shown by the compartment scheme shown in Fig. 4(c).

It would be interesting to compare the current results for *Perna viridis* with those of Li et al. (2005), since both have been obtained for paralytic shellfish toxins in *Perna viridis* with compartment modeling involving first-order linear differential equations. The compartment scheme used by Li et al. (2005) is depicted in Fig. 5. Direct comparisons are not possible because five compartments were used by Li et al. (2005), namely, viscera (V), hepatopancreas (H), gill (G), adductor muscle (A) and foot (F) while only two compartments were used in the current work, namely, digestive gland (D) and remaining

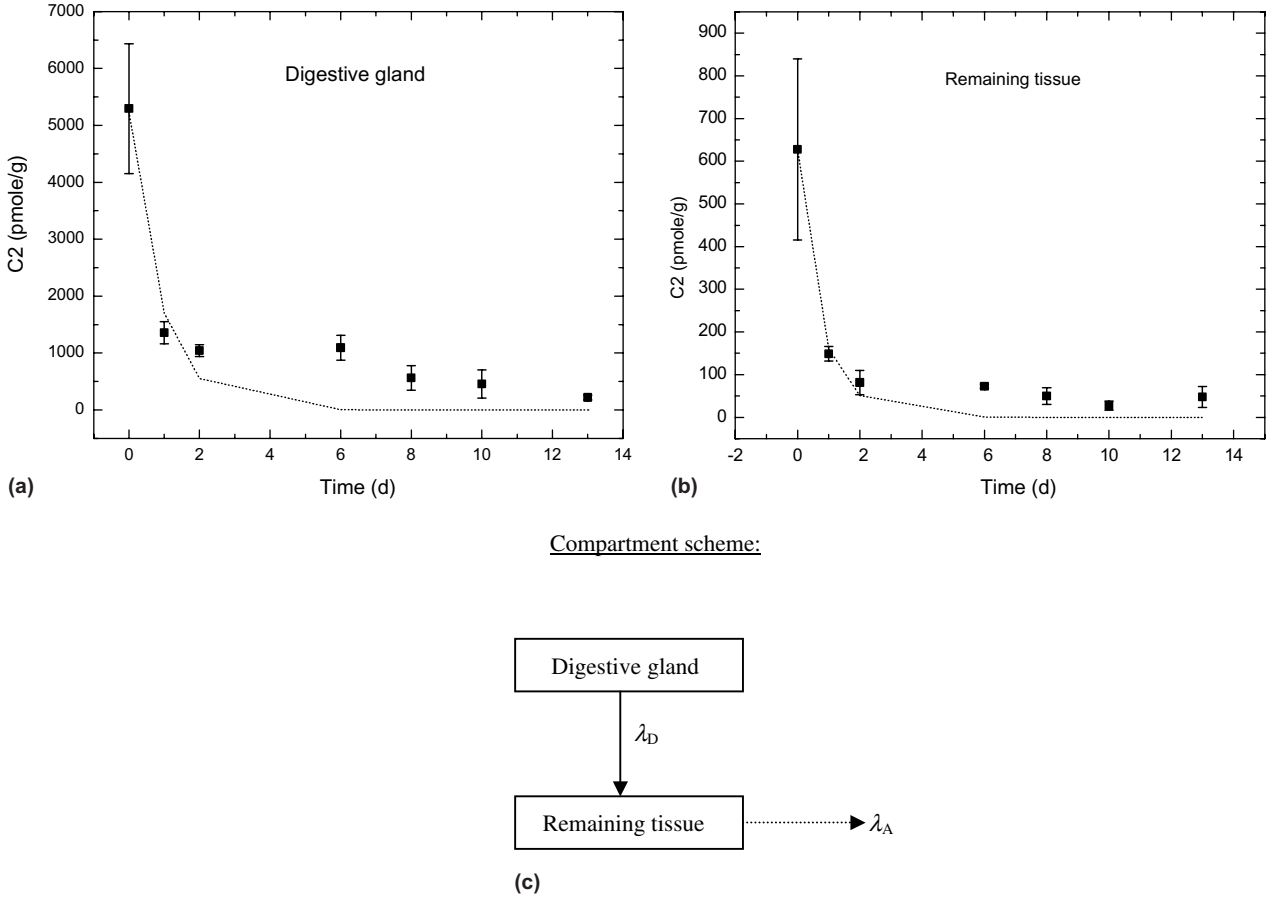

Fig. 4. (a) Concentrations (pmol g^{-1}) of toxin at different depuration times in the digestive gland of *Perna viridis*. Broken line: best fit. Depuration started on day zero. Vertical lines represent ±1 S.D.; $n = 3$. (b) Concentrations (pmol g^{-1}) of toxin at different depuration times in the remaining tissue of *Perna viridis*. Broken line: best fit with the constraint that k is not larger than λ_{D}. Depuration started on day zero. Vertical lines represent ±1 S.D.; $n = 3$. (c) Final compartment scheme for the two compartments of *Perna viridis*: digestive gland and remaining tissue.

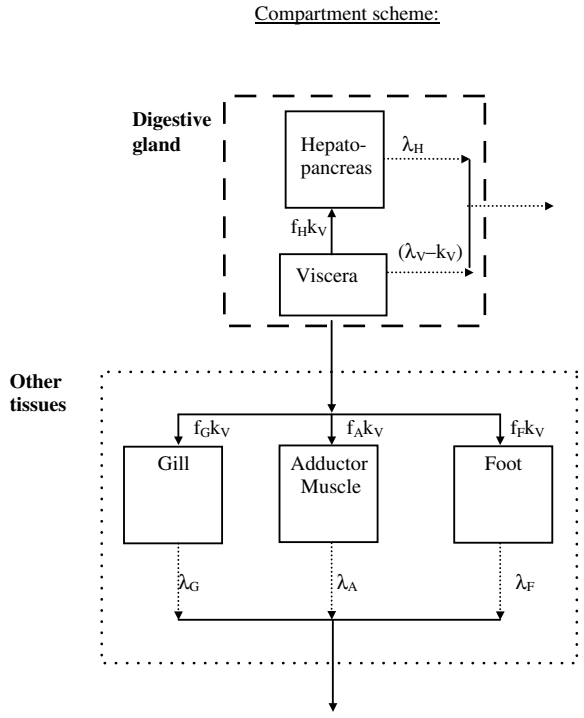

Fig. 5. Compartment scheme for the five compartments of *Perna viridis* used by Li et al. (2005): viscera (V), hepatopancreas (H), gill (G), adductor muscle (A) and foot (F). Combinations of these compartments to obtain the compartment scheme used in the current work are shown, viz., combination of the V and H compartments to form the digestive gland compartment (dashed rectangle) and combination of the G, A and F compartments to form the remaining tissue compartment (dotted rectangle).

tissue (T). However, combinations of compartments used by Li et al. (2005) as follows are possible in order to make comparisons.

First, the hepatopancreas and the viscera compartments were not separated in the present work. However, Li et al. (2005) showed that the transfer coefficient from the viscera to hepatopancreas was about zero during depuration, so these two compartments could be treated as depurating independently. Moreover, the removal rates from these two compartments were close, viz., $\lambda_V = 0.21\ \text{day}^{-1}$ and $\lambda_H = 0.24\ \text{day}^{-1}$, so these two independently depurating compartments can be viewed as a single compartment with a common removal rate. In this way, these two "serial" compartments can be combined as one compartment called "digestive gland", i.e., the compartment represented by the dashed rectangle in Fig. 5. Second, from the work of Li et al. (2005), $\lambda_G = 0.45\ \text{day}^{-1}$, $\lambda_A = 0.28\ \text{day}^{-1}$ and $\lambda_F = 0.38\ \text{day}^{-1}$. Since these three compartments are depurating independently and since λ_G, λ_A and λ_F are not very different, we can approximate these three "parallel" compartments as a single compartment called "remaining tissue", i.e., the compartment represented by the dotted rectangle in Fig. 5. We then effectively get the compartment scheme we used in the present work as shown in Fig. 1.

The first observation is that the removal rates are much larger for the current work, viz. $\lambda_D = 1.125\ \text{day}^{-1}$ and $\lambda_T = 3.74\ \text{day}^{-1}$ (cf. $\lambda_V = 0.21\ \text{day}^{-1}$, $\lambda_H = 0.24\ \text{day}^{-1}$, $\lambda_G = 0.45\ \text{day}^{-1}$, $\lambda_A = 0.28\ \text{day}^{-1}$ and $\lambda_F = 0.38\ \text{day}^{-1}$ obtained by Li et al. (2005)). It is noticed that the toxin levels involved in the work of Li et al. (2005) were orders of magnitude higher than those involved in the current work. It is hypothesized here that exceptionally high toxin levels impair the functionality of the mussel tissues. In this part of discussion, the functionality refers to the capability to eliminate the toxins. If this proposal is correct, the toxin levels involved in the present work are in the low regime so that the toxins can be detoxified or biotransformed at a fast rate, while the toxin levels involved in the work of Li et al. (2005) are in the high regime so that the functionality of the mussel tissues have been impaired and the toxins cannot be eliminated effectively.

The second observation is the different transfer coefficients from the viscera or the digestive glands in the work of Li et al. (2005) and in the current work, respectively. In the work of Li et al. (2005), all toxins were effectively removed from the viscera to the hepatopancreas during uptake, while almost all toxins were eliminated from the viscera out of the mussel during the depuration stage. In contrast, for the current work, essentially all toxins in the digestive gland were transferred to the remaining tissue during depuration. The different behavior can again be explained using the above proposal, with the functionality referring to the assimilation efficiency. The toxin levels involved in the present work are in the low regime so that the toxins are assimilated into the "remaining tissue" efficiently, so there is a high (in fact almost total) transfer from the digestive gland to the "remaining tissue". On the other hand, the toxin levels involved in the work of Li et al. (2005) are in the high regime, so that the toxins are not assimilated efficiently. Instead, the toxins tended to go to the hepatopancreas for storage or for detoxification (during the uptake stage) and directly out of the viscera (during depuration). Future experiments involving different toxin levels in the mussel tissues can validate the proposal.

As regards *C. nobilis*, the results obtained in this work show that there is essentially no transfer of toxins from the digestive gland to the adductor muscle during depuration. If the model proposed above for *Perna viridis* is also applicable to *C. nobilis*, the toxin levels in *C. nobilis* may already be in the high regime (although the toxin levels are comparable to those in *Perna viridis*, which are in the low regime for *Perna viridis*), so that assimilation as well as removal of toxins are not effective. This may suggest that the high and low regimes for toxin levels are species dependent.

To summarize, the results from modeling the depuration of paralytic shellfish toxins in *C. nobilis* and *Perna viridis* in the present work, and comparison with the

results for *Perna viridis* from previous work involving much higher concentrations, have led to the proposal that depuration in shellfish could vary with the concentrations present in the tissues. Future experiments involving different toxin levels in different species of shellfish can help validate the current proposal. Moreover, high and low regimes for the concentrations may also vary with species. The present work shows that a similar toxin level could be in the high regime for *C. nobilis* but at the same time in the low regime for *Perna viridis*.

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Temporal trends in soft tissue metal levels in the periwinkle *Littorina littorea* along the Scheldt estuary, The Netherlands

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Because estuaries are usually important areas for industrial and urban development, they are often used as waste fields for domestic and industrial pollutants, including heavy metals which accumulate in estuarine sediments (e.g. Lam et al., 1997; Verslycke et al., 2004). However, metal bioavailability and the extent to which metals accumulate in, and are released from estuarine sediments, does not depend solely on metal input but is also determined by the spatio-temporal variability of the physico-chemical properties of the estuaries (e.g. Paucot and Wollast, 1997; Nolting et al., 1999; Monbet, 2004). Indeed, environmental factors such as salinity, pH, suspended and particulate matter and oxygen concentration are known to affect metal release and metal speciation (i.e. bioavailability) in a complex manner (e.g. Paucot and Wollast, 1997; Nolting et al., 1999;

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