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Trophic transfer of paralytic shellfish toxins from clams (*Ruditapes philippinarum*) to gastropods (*Nassarius festivus*)

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Abstract

A local strain of the dinoflagellate *Alexandrium tamarense* (ATCI01), which predominantly produces C2 toxin, was fed to the clams (*Ruditapes philippinarum*) under laboratory conditions. Concentrations of paralytic shellfish toxins (PSTs) in the dosed clams were determined by High Performance Liquid Chromatographic (HPLC) analyses, and the clams were homogenized and then fed to the gastropods (*Nassarius festivus*). In the toxin accumulation phase, which lasted for 42 days, concentrations of PSTs increased in the snails gradually, reaching a maximum of 1.10 nmole g^{-1} at the end of the exposure period. The toxin content of the homogenized clams (food) was 13.18 nmole g^{-1}, which was about 12-fold higher than the PST content in the snails. Between day 43 and day 82, the snails were fed with non-toxic clams, and this period represented the depuration phase. Accumulation and depuration rates of PSTs in the snails, *N. festivus*, were determined by fitting the experimental data to user-defined parameters program using a one-compartment model. Two different modeling approaches were used to derive the accumulation and depuration rates. The first approach is to derive both values from the data for the toxin uptake. The second approach is to derive depuration rate from the depuration data and then to derive uptake rate, allowing for toxin depuration, from the data for toxin uptake. The first approach yielded more consistent results for the toxin concentration at the end of the uptake period, when compared with the experimental data. The toxin uptake and depuration rates were 1.64 (pmole of toxin into snail per day) per (nmole g^{-1} of toxin in food) and 0.06 ± 0.02 day^{-1} (mean $\pm$ SE), respectively. The toxin profiles of snails were similar to the clams, but different from the algae. Besides C toxins (C1 and C2), dcGTX2 and dcGTX3 were also detected in both clams and snails. The β:α epimer ratio gradually decreased during trophic transfer and approached a ratio of 1:3 (26.4 mol%:73.6 mol% at day 42) in the snails, near the end of the accumulation period.

Keywords: Paralytic shellfish toxins; Trophic transfer; Clams; Gastropods

1. Introduction

Although filter-feeding bivalve molluscs are major vectors of phycotoxins, marine scavengers such as gastropods and crabs are also important for transferring phycotoxins to humans via food consumption (Shumway, 1995). Paralytic shellfish toxins (PSTs) are one class of phycotoxins that have attracted public attention due to their lethal effects and wide geographic distribution. In contrast to North America, the biokinetics of PSTs in commercially important species of the subtropics are less well studied (Li et al., 2005). Because of potential differences in physiology, biochemistry and behaviour amongst species, as well as variations in environmental conditions between sites, information derived from northern latitude locations may not be directly applicable to subtropical environments.

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In China, there have been several reports of paralytic shellfish poisoning (PSP) associated with the consumption of marine gastropods, indicating the potential of these species to cause PSP intoxication, sickness, and fatalities. Between March and August of 1979, there were 68 reported cases of shellfish poisoning in Zhejiang Province due to the consumption of the snails, *Nassarius succinctus*. In August 1979, one person died, and seven people were hospitalized following the consumption of the snails, *N. succinctus*, and all of the victims reported symptoms typical of PSP (Li and Chen, 1981). Between April and May 2002, 50 people were intoxicated with three fatalities in Fujian Province due to the consumption of PST-laden snails, *Nassarius* spp. (Ming Pao News, 2002). A more recent incident happened during summer 2004, where 55 food poisoning cases with one fatality were recorded in Yin Chuan city, and another 15 reported cases in Fujian Province due to the consumption of *Nassarius* spp. (Gao, 2004). A survey of the PSTs in snails, *Nassarius* spp., collected from different areas in Zhoushan Islands revealed PSTs up to 107413 MU 100 g^{-1}, which is more than 200 times above the WHO health limit for human consumption (Fu et al., 1982). Another survey on PSTs in shellfish conducted along the coast of China found that *N.* (*Zeuxis*) *siguijorensis* collected in Daya Bay, Guangdong Province, contained PSTs with an average level of 370 MU 100 g^{-1}, while bivalves collected from the same area exhibited higher toxin concentrations ranging from 175 to 3510 MU 100 g^{-1} (Li et al., 1999).

PSTs are normally produced by phytoplankton, and accumulated by filter-feeding bivalve molluscs. Shumway (1995) reported that gastropods could concentrate phycotoxins by feeding on toxin-contaminated, filter-feeding bivalves. In general, the mechanisms of PST accumulation and depuration in filter-feeding bivalves have been studied in greater detail than those of marine gastropods. In a recent study, Chen and Chou (1998) studied the trophic transfer of PSTs from the toxigenic dinoflagellate *Alexandrium minutum* to a carnivorous gastropod, *Babylonia areolata*, in a laboratory-based feeding experiment. In this study, purple clams, *Hiatula diphos*, were dosed with the toxigenic dinoflagellate. The dosed purple clams were, in turn, fed to the gastropods. The results provided direct evidence for the transmission of dinoflagellate PSTs to carnivorous gastropods. This study, however, did not investigate the kinetics of PST uptake and depuration, and this information is necessary to understand the fate of PSTs in marine food chains.

The major PST found in the waters of Hong Kong and southern China (Wang and Hsieh, 2001) is the C2 toxin, which has been the subject of a number of recent studies (Choi et al., 2003; Mak et al., 2003; Li et al., 2005). The present study investigates the uptake and depuration dynamics of PSTs in a subtropical predatory marine gastropod, exposed to toxins that have been transferred through an intermediate food source, the Manila clam, *Ruditapes philippinarum*. A secondary and complimentary aim is to develop a biokinetic model for PSTs that will allow an estimation of PST levels in marine gastropod predators via the consumption of toxin-laden bivalve prey. The modeling approach is to develop a one-compartment model utilizing first-order linear differential equations. The solutions to the equations will then be used to fit the experimental data. The most significant parameters governing the biokinetic model, primarily dependent upon the uptake and depuration rates, will be estimated.

2. Materials and methods

In the present study, a local strain of *Alexandrium tamarense* (ATCI01) (*A. tamarense*), which predominately produces low potency C2 toxin, was fed to clams (*R. philippinarum*) for 15 days, and "toxin-dosed" clam tissues were, in turn, fed to gastropods (*N. festivus*) intermittently for 42 days, followed by a 42-day depuration period.

2.1. Snail exposure and depuration experiments

The *A. tamarense* (ATCI01) was obtained from the Institute of Hydrobiology, Jinan University, China. It was cultured in 0.22 μm-filtered natural seawater containing K-medium (Keller et al., 1987) at 21–23 °C, on a 16:8 h light:dark cycle. A non-toxin producing alga (*Dunaliella tertiolecta*), used as food for the non-toxic clams, was also maintained under the same culture conditions. Algal cells of both toxic and non-toxic species were harvested for feeding experiments at their exponential growth phase.

Clams, *R. philippinarum* (length 3.94 ± 0.23 cm, tissue wet weight 1.88 ± 0.41 g), were collected by a local fisherman in Sha Tau Kok, Hong Kong in December, 2002. Six hundred and thirty animals were divided into two groups: the exposure group and the control group. Each group was then divided into seven subgroups and placed into individual 30 l glass tanks containing 20 l Glass Fiber Filtered (GFF) seawater. The seawater was obtained from Ocean Park, Hong Kong, China (Li et al., 2005). Animals from the two groups were kept in an environmental chamber with temperature controlled at 21–23 °C. Salinity and dissolved oxygen, ranged between 30‰ and 35‰ and >8.2 mg l^{-1} respectively, were monitored daily. For the exposure group, clams were fed with toxigenic dinoflagellate *A. tamarense* at 2.57×10^7 cells per tank each day, while the clams in the control group were fed with *D. tertiolecta* at 3.15×10^8 cells per tank each day. This part of the experiment lasted for 15 days. Seawater in each tank was changed every two days. The total numbers of toxic dinoflagellate cells ingested by the filter-feeding clams were estimated following Li et al. (2005). In brief, algal samples were taken everyday for toxin analysis and cell counting. Algal cells were preserved in Lugol's iodine for cell counting under a light microscope. Algal samples used for toxin analysis were concentrated by centrifugation and the cell pellets were extracted in 0.05 M acetic acid aided by sonication in an ice bath for 1 min. The crude

extract was centrifuged again at 16 600 × *g* for 15 min, and the supernatant was passed through an ultrafiltration unit (Millipore, 10 000 MW) at 4 °C for subsequent toxin analysis. On day 15, clams were harvested and tissues were homogenized. The homogenized clams were divided into separate portions and stored at −20 °C for future feeding experiment.

About 1900 carnivorous marine snails, *N. festivus* (shell length 1.24 ± 0.09 cm, tissue wet weight 0.06 ± 0.10 g), were collected from Lok Wor Sha, Hong Kong in February, 2003. They were acclimated for 3 weeks in the laboratory under the same conditions described previously for clams. Prior to the uptake experiment, five groups of snails (five individuals each) were taken randomly, and tested for PSTs by HPLC analysis. The remaining stock of snails was then separated into exposure and control groups. Each group was subdivided into 42 holding chambers, each containing 20 individuals, and placed into three different holding aquaria [520 mm (width) × 420 mm (length) × 220 mm (height)]. Natural GFF seawater was used for the experiment. Water depth was maintained at 150 mm in the test aquaria. Seawater in the aquaria was changed at each feeding occasion (at 3 day intervals). A 12:12 h light/dark photoperiod was maintained throughout the experimental period. Each holding chamber was 70 mm (diameter) × 120 mm (height), covered with a 100 μm nylon mesh on the top of the chamber to contain the snails. Holding chambers were numbered to allow random sampling and identification of exposure histories.

During the initial accumulation phase, snails were fed with PST-exposed clam tissues once every 3 days for 42 days. Each chamber contained 20 snails and received 0.2 g (wet weight) clam tissue per feeding. Average PST content in clam tissue was 13.18 nmole g^{-1}. All experimental conditions for the depuration phase were identical to the accumulation phase, except for the type of food introduced. Salinity, pH, and dissolved oxygen (ranged between 30‰ and 34‰, 7.8–8.7, and >8 mg l^{-1}, respectively) were monitored daily throughout the 84 days. Mortality of gastropods in the feeding chambers was recorded daily. Mortality rates for both the control and exposure groups were <2%.

PST analyses were conducted on snails every 6 days during the accumulation phase and on days 43, 45, 48, 54, 60, 72 and 80 during depuration. On each sampling occasion, three chambers were randomly selected, and five individuals from each chamber were removed and pooled to form one composite sample for toxin analysis.

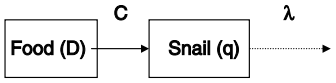
2.2. Toxin extraction and analysis

Snail soft tissues were dissected from the shell and homogenized with 1:1 volume of 0.2 M acetic acid, followed by sonication with an ultrasonic probe (High Intensity Ultrasonic Processor: An Auto-Tune Series; Sonics, Danbury, CT, USA) for 1 min. The homogenates were centrifuged at 16 600 × *g* for 15 min; the supernatant was decanted, passed through a Sep-Pak C18 cartridge column (Waters) and stored at −20 °C until analyzed.

Samples were analyzed for PSTs by High Performance Liquid Chromatography (HPLC) with post-column derivatization and fluorescence detection (Oshima, 1995a, with modifications of Anderson et al., 1996). A more complete description of the toxin extraction and analysis is given by Li et al. (2005). The HPLC system was from Waters. A stainless-steel column containing reverse-phase packing [Inertsil C8 (film thickness, 3 μm; length, 150 mm; inner diameter, 4.6 mm) with Inertsil C8 All-Guard cartridge; Alltech, Deerfield, IL, USA] was used. Saxitoxin (STX), neosaxitoxin (neoSTX), decarbamoyl saxitoxin (dcSTX), gonyautoxin I, II, III, IV, V (GTX1-5), decarbamoyl gonyautoxin II, III (dcGTX2 and dcGTX3)] standards, were obtained from the National Research Council of Canada (NRC) and were used to quantify PSTs in the samples. A C2 toxin standard was kindly provided by Professor Y. Oshima, Tohoku University, Sendai, Japan and reconfirmed with purified C2 toxin from our own production. The C1 toxin standard was prepared in our laboratory from the dinoflagellate *A. tamarense* (clone ATDP). Total toxicity of PSTs was calculated using the toxicity data reported by Oshima (1995b). The relative toxicities of different PSTs in MU μmol^{-1} are 15 (C1), 239 (C2), 892 (GTX2), 1584 (GTX3), 1617 (dcGTX2) and 1872 (dcGTX3). Mouse units were then converted to STX equivalents by using a conversion factor of 0.23 μg STX eq. MU^{-1}.

2.3. Model development

A one-compartment model (for the whole animal) is employed to model the uptake and depuration of PSTs in snails feeding on clams dosed with PSTs. The compartment scheme and the parameters used are given in Fig. 1. The toxin concentration in the food is denoted as *D* (in nmole g^{-1}). The differential equations governing the biokinetics and the time-dependent toxin concentrations in the snail are given below:



Parameter	Description	Unit
C	(pmole of toxin into snail per day) per(nmole g^{-1} of toxin in food)	mgd^{-1}
q	pmole g^{-1} of toxin in the snail	pmole g^{-1}
m	wet mass of the snail	g
λ	removal rate from the snail	d^{-1}
D	toxin concentration in food	nmole g^{-1}

Fig. 1. *Nassarius festivus*. One-compartment scheme and the parameters used for uptake and depuration of PSTs in snails feeding on clams dosed with PSTs.

Differential equations governing the biokinetics for the snail:

$$\text{Uptake:} \quad \frac{dq}{dt} = \frac{CD}{m} - \lambda q \tag{1}$$

$$\text{Depuration:} \quad \frac{dq}{dt} = -\lambda q \tag{2}$$

Time-dependent toxin concentration in the snail during uptake (days 0–42) with the initial condition $q(0) = 0$:

$$q = \frac{CD}{m\lambda}(1 - e^{-\lambda t}) \tag{3}$$

Time-dependent toxin concentrations in the snail during depuration (days 42–84) with the initial conditions (on day 42) $q(0) = q_o$:

$$q = q_o e^{-\lambda t} \tag{4}$$

Therefore, if λ and C are determined, the biokinetics of the toxin in the snail can be determined.

3. Results

3.1. Toxin content and toxicity

Changes in total PST concentrations in individual clams and the total numbers of toxic dinoflagellate cells ingested by the filter-feeding clams during the accumulation phase are shown in Fig. 2. The toxin content and relative STX-equivalent toxicity of the toxic dinoflagellate, *A. tamarense*, averaged 28.73 fmole cell^{-1} or 1.57 pg STX eq. cell^{-1}, respectively. Clams gradually accumulated PSTs from the toxic dinoflagellate exposures, reaching a maximum toxin concentration of 13.18 nmole g^{-1} (52.66 μg STX eq. 100 g^{-1} tissue wet weight) on the final day of exposure. In the snails, toxicities increased with period of exposure (i.e. time of feeding on the toxic clam homogenates from day 0 to day 42; Fig. 3). The pattern of accumulation speared to be "saturated" from day 18 to day 36. The maximum toxin content and toxicities on day 36 were 1.47 nmole g^{-1} and 6.43 μg STX eq. 100 g^{-1} tissue wet weight, respectively. Changes in total toxin concentrations over the 42-day depuration period showed that toxins decreased gradually over the entire depuration period; toxin concentrations approached initial concentrations by the end of the depuration period (day 84).

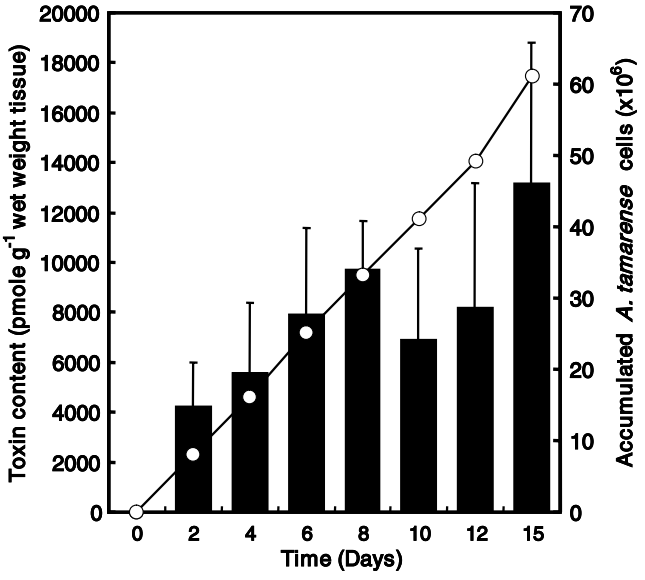


Fig. 2. *Ruditapes philippinarum* accumulation in a 15-day exposure. Total paralytic shellfish toxin concentration (left-side legend) in clams from exposure to *Alexandrium tamarense*, during the course of accumulation period (bar); mean ± SD ($n = 7$), and the number of ingested toxic dinoflagellate cells (line).

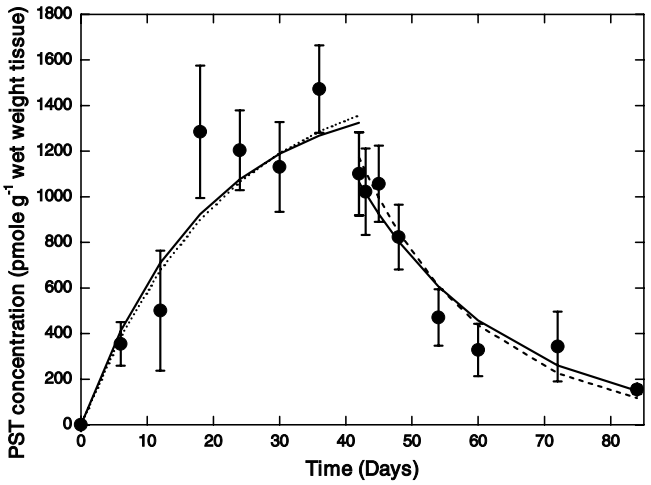


Fig. 3. *Nassarius festivus* exposure to toxic clam homogenates. Concentration (pmole g^{-1}) of toxin at different culture time in the snail, and the best fit results for the uptake phase (dotted line: free fitted; solid line: fitted using k derived from depuration data) and for the depuration phase (dotted line: free fitted; solid line: fitted using k derived from uptake data). Depuration started on the 42nd day. Mean ± SD ($n = 6$).

3.2. Model of toxin kinetics

To model toxin accumulation and depuration rates for snails, the experimental data were fitted using user-defined expressions from the non-linear curve fit program of the Microcal™ Origin™ (Version 6.0) with the interested parameters as the user-defined parameters.

The parameter λ can be derived either using Eq. (3) with the data for the uptake, or using Eq. (4) with the data for the depuration. If a single λ is desired, two different approaches can be used. The first one is to derive both λ and C from the data for the uptake using Eq. (3). The second one is to derive λ from the data for the depuration from Eq. (4), and then to derive C with this λ from the data for the uptake using Eq. (3). Both approaches were utilized here, and the results are shown in Fig. 3, and are summarized as follows:

3.2.1. The first approach

From the uptake data, and by using Eq. (3), the best fit results give

$\lambda = 0.05 \pm 0.02\ \text{day}^{-1}$

$CD/m\lambda = 1500 \pm 260\ \text{pmole g}^{-1}\ \text{of toxin}$

These parameters predict a concentration in the snail of 1300 pmole g^{-1} on the 42nd day. With a mean mass of snail as 0.27 ± 0.04 g wet weight (composite of five snails), and $D = 13.18$ nmole g^{-1}, there is a calculated toxin concentration rate per day or $C = 1.64$ (pmole of toxin into snail per day) per (nmole g^{-1} of toxin in food). The fitted results are shown as the solid line (in the uptake phase, Fig. 3). If we use this value of λ, by using the depuration data and Eq. (4), we obtain

$q_o = 1200 \pm 78\ \text{pmole g}^{-1}\ \text{of toxin}$

which is similar to the predicted concentration in snail on the 42nd day. This value is consistent with the values predicted by using the uptake data (1300 pmole g^{-1}) or the experimental data (1100 ± 180 pmole g^{-1}). It is also observed that the relative error for the value of $CD/m\lambda$ derived here is the smallest of the methods employed, primarily due to smaller variation in the depuration data. The fitted results are shown as the dotted line (in the depuration phase, Fig. 3).

3.2.2. The second approach

From the depuration data, and by using Eq. (4), the best fit results give

$\lambda = 0.05 \pm 0.003\ \text{day}^{-1}$

$q_o = 1100 \pm 73\ \text{pmole g}^{-1}$

The relative errors for the derived λ and q_o terms are small, reflecting small variation in depuration data. The fitted results are shown as the solid line (in the depuration phase, Fig. 3). The predicted concentration in snail tissue on the 42nd day or q_o is consistent with the experimental data (1100 ± 180 pmole g^{-1}). If λ is derived the uptake data and Eq. (3), we obtain

$CD/m\lambda = 1600 \pm 100\ \text{pmole g}^{-1}$

From this, we calculate C to be 1.75 (pmole of toxin into snail per day) per (nmole g^{-1} of toxin in food). These parameters predict a concentration in snail tissue of 1400 pmole g^{-1} on the 42nd day. The fitted results are shown as the dotted line (in the uptake phase, Fig. 3).

Both approaches give similar results, with the first approach giving results closer to those obtained in the experiment at the end of the uptake period (42-day exposure).

3.3. Toxin profiles and specific toxin compositions

The toxin profiles of clams and snails are shown in Fig. 4a and b, respectively. In the toxic dinoflagellates, C2 toxins contributed nearly 99% of the total PSTs, with only trace amounts of C1 and GTX3 detected. The proportion of C2 toxin decreased to 71% (day 2, Fig. 4a) when PSTs were transferred from algae to the clams.

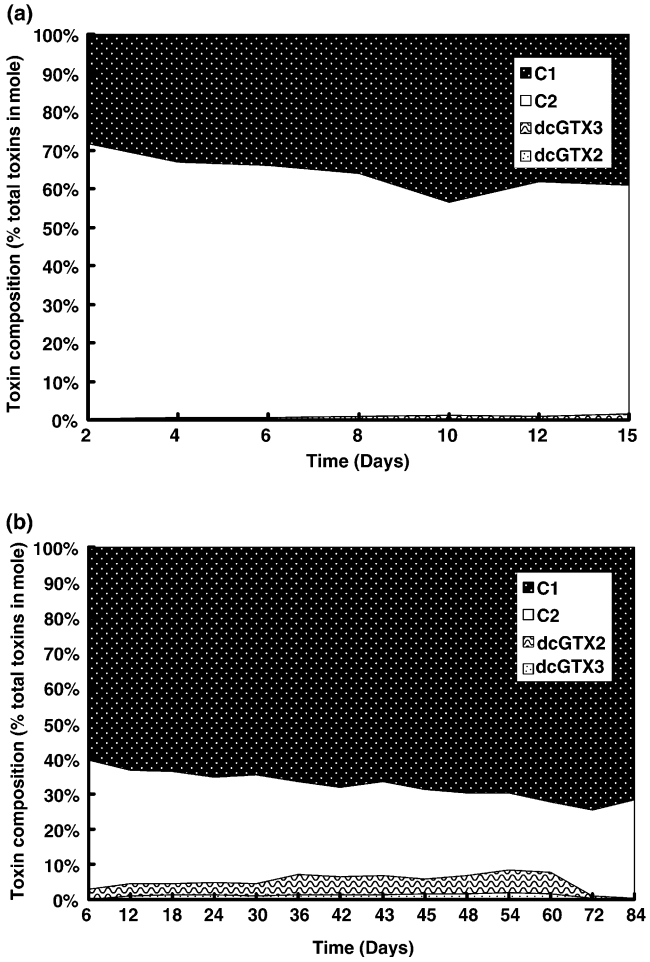


Fig. 4. *Ruditapes philippinarum* uptake (a) and *Nassarius festivus* uptake and depuration (b). Relative distribution of different derivatives of paralytic shellfish toxins in the clams; Mean ± SD ($n = 7$) and snails; Mean ± SD ($n = 6$).

In the clam homogenates, C1 and C2 toxins were the predominant toxin species, accounting for up to 40% and 60% of the total PSTs (10-day exposure; Fig. 4a) respectively, with <1% dcGTX2 and dcGTX3. Trace amounts, in equal proportions, of dcGTX2 and dcGTX3 were detected in the clams throughout the whole 15-day feeding period.

The PST profile of the snail was similar to that of the clam with respect to C1, C2, dcGTX2 and dcGTX3 concentrations, but different from that of the algae (containing 99% C2 toxins). With a decrease in the proportion of C2 toxin, C1 became the major toxin in the snails and the relative proportion of dcGTX2 and dcGTX3 also increased with period of accumulation (Fig. 4b). During the depuration phase, most of the dcGTXs disappeared on day 72. Even though the relative proportion of C1 and dcGTX2 increased with increasing duration of depuration, the absolute amount of these toxins decreased over the depuration period (Figs. 4b and 5). The amount and proportion of dcGTX2 were greater than those of dcGTX3 in the snails, but greater amount and proportion of dcGTX3 were detected in the clams.

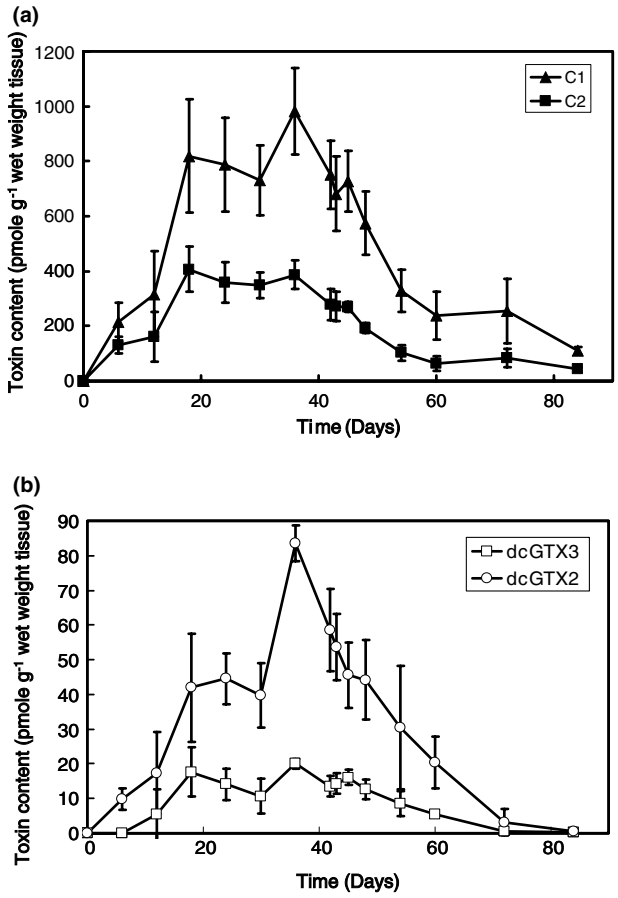


Fig. 5. *Nassarius festivus*. Changes in different derivatives of paralytic shellfish toxins in snails (a) C1 and C2; (b) dcGTX2 and dcGTX3. Mean ± SD ($n = 6$).

4. Discussion

4.1. Toxin uptake kinetics

The maximum PST concentrations and weight-specific toxicities of clams are larger than those of the snails. A similar finding has also been reported for the short-neck clam, *Tapes* (*Amygdara*) *japonica*, and a carnivorous predator, the rapa whelk, *Rapana venosa* (Ito et al., 2004). In this field-based study, the authors reported that the PSTs found in the gastropods were likely to have originated from predation on short-neck clams based on the similarities of the toxin profiles. They found that the maximum toxicity in the viscera of *R. venosa* was only 11.4 MU g^{-1}, while the maximum toxicity of the short-necked clams from the same area was 49.6 MU g^{-1} (Ito et al., 2004). In contrast, Oikawa et al. (2002) reported that other carnivores that fed on bivalves, such as crab, could attain a maximum toxicity of 30–80 MU g^{-1} in the viscera, while the toxicity of its prey, *Mytilus galloprovincialis*, was only 9.6 MU g^{-1}. It is worth noting that the greater PST concentrations in the clams as compared to the snails observed in the present study could be a function of the feeding levels and times. Bricelj and Shumway (1998) suggested that the differences in toxin accumulation abilities in different bivalve species may be related to the species-specific sensitivities to PSTs. In species insensitive to PSTs, special mechanisms may have developed which may reduce the risk of exposure to PSTs through complexation with STX-binding proteins (Daigo et al., 1988; Mahar et al., 1991; Llewellyn, 1997). Because of such adaptations, these animals can readily feed on toxic dinoflagellates and accumulate high concentrations of toxins without incurring injury or death (Bricelj et al., 1990). For sensitive species, physiological and behavioural mechanisms may have developed which limit exposure to toxic dinoflagellates, and as a consequence relatively low levels of toxins are found in their tissues (Bricelj and Shumway, 1998). Such avoidance behaviours may include shell valve closure (Gainey and Shumway, 1988), increased burrowing activity (Bricelj et al., 1996), and reduced clearance rates in the presence of toxic dinoflagellates (Bricelj and Shumway, 1998). In the present study, no inhibition of ingestion of toxic clam homogenate by the snails was observed when compared to the control group. Therefore, the relatively low maximum toxin content detected in the snails was likely due to the low assimilation rate of toxins into the snails [C was only 1.64 (pmole of toxin into snail per day) per (nmole g^{-1} of toxin in food)]. It is also conceivable that a lower binding affinity of certain PSTs to proteins such as saxiphilin may account for the low assimilation rate into snails (Llewellyn, 1997).

In this study, an exponential decay curve was adequate to describe the depuration of PSTs in the snail. In contrast, Choi et al. (2003) found that two distinct phases were required to simulate the detoxification patterns of PSTs in scallops and mussels. In this earlier study, scallops and mussels depurated most of the PSTs (>50%) in the first day, followed by a reduced rate of depuration thereafter. Such an observation may suggest a rapid elimination of unassimilated toxins followed by a loss of assimilated toxins from the tissues (Lee, 1993; Silvert and Cembella, 1995). As there is little information on the depuration patterns of PSTs in gastropods, the depuration rate constant of PSTs in *N. festivus* was compared with those reported for bivalves in the previous study as the toxin compositions were similar in the two studies. Although the rate constants for the slow depuration phase for the scallops and mussels (0.063 day^{-1} and 0.040 day^{-1}, respectively) were comparable to the snails (0.055 day^{-1}), the overall depuration rates (i.e. including the fast depuration phase) were faster in the two bivalves than that of the snails. This may be due to the fact that the toxins being depurated have likely been assimilated into the snail tissues. Other gastropods, such as abalone (*Haliotis tuberculata*), have exhibited slow depuration of PSTs (Bravo et al., 1996). In that study, abalone with high toxin contents in tissues were collected from the northern and southern coasts of Spain, held in the laboratory and fed with non-toxic macroalgae. A reduction in toxin content did not occur until after 3 months.

4.2. Toxin composition

The appearance of toxins such as dcGTX2 and dcGTX3 in the clams (*R. philippinarum*) and gastropods (*N. festivus*) was not observed in the toxic dinoflagellates, suggesting that molluscs could biotransform toxins (Bricelj and Shumway, 1998). In contrast, Chen and Chou (1998) found no evidence of toxin transformation in the purple clam, *H. diphos*, and the gastropod, *B. areolata*, where the exposed animals possessed similar toxin compositions (or profiles) to those of the algae, *A. minutum*. One possible explanation for these differences in results could be that the *A. tamarense* culture used in this study produces predominately low potency *N*-sulfocarbamoyl C2 toxin, which may be more labile and susceptible to conversion when compared with the gonyautoxins (GTXs) produced by *A. minutum*. The results of Choi et al. (2003) also suggest that different mollusc species have varying abilities to transform toxins.

In the present study, only a few toxin derivatives (e.g. C1, dcGTX3 and dcGTX2) were found in the clams and snails, whereas Choi et al. (2003) found derivative toxins, such as GTX2, GTX3 and GTX5, in mussels and scallops after toxin transformation from C2. These observations suggest that the toxin transformation abilities of scallops and mussels may be higher than those of clams and snails. In addition, the conversion of C2 to dcGTX2, rather than dcGTX3, is more easily accomplished in snails than clams. However, previous studies have shown that decarbamoylation is highly substrate specific and can occur faster for β- (e.g. C2 with dcGTX3 as its corresponding decarbamoyl derivatives) than α-epimer (Buzy et al., 1994; Oshima, 1995b). The faster toxin conversion to dcGTX2 might be due to the increase in the amount of C1 toxin in the snails during trophic transfer, allowing the conversion of more C1 to its corresponding decarbamoyl derivative dcGTX2 in the snails. Moreover, there was a gradual decrease in β:α epimer ratio as the C2 toxin was converted to a more stable α-epimer C1 toxin through epimerization at the C-11 hydroxysulfate moiety (Oshima, 1995b). The ratio of β:α was 99:1 (98.7 mol%:1.3 mol%) in the toxic dinoflagellates, 3:2 (60.7 mol%:39.3 mol%) in the clams, and finally about 1:3 (26.4 mol%:73.6 mol% on day 42) in the snails. These findings are consistent with the previous study that β:α would decrease when toxins are transferred from toxic dinoflagellates to shellfish through the food chain, leading to a β:α ratio of 1:3. Bricelj and Shumway (1998) suggested that the epimer ratio might be a useful parameter to predict the retention time of toxins in bivalves after the initial toxin accumulation from toxic dinoflagellates.

In China, the consumption of *Nassarius* spp. has resulted in many cases of paralytic shellfish poisoning. Studies have shown that paralytic shellfish toxins can be detected in different species of gastropods such as basket shell, *Niotha clathrata* (Hwang et al., 1994), turban shells, *Turbo marmorata* and *T. argyrostoma* and top shells, *Tectus pyramis* and *T. nilotica maxima* (Oshima et al., 1984) and cultured abalone (*H. midae* and *H. turberculata*) (Bravo et al., 1996; Pitcher and Franco, 2000). This study illustrated how a model can be constructed for the trophic transfer of paralytic shellfish toxins from clams (*R. philippinarum*) to gastropods (*N. festivus*). This may help to predict toxin concentrations in gastropods via the consumption of toxin-laden bivalves, and may provide useful information for the assessment of the public health risks of PSTs.

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