Acta Oceanol. Sin., 2015, Vol. 34, No. 12, P. 170–174
DOI: 10.1007/s13131-015-0764-y
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Depuration of paralytic shellfish toxins in Japanese scallop (*Patinopecten yessoensis*) in natural environment

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Received 29 May 2014; accepted 5 July 2015

Abstract

To study the paralytic shellfish toxins (PSTs) depuration in Japanese scallop *Patinopecten yessoensis* in natural environment, Japanese scallops naturally contaminated with paralytic shellfish poisoning (PSP) toxins in the Dayao Bay in the northern Huanghai Sea are transited to Qipanmo waters in the Bohai Sea of no reported PSTs incidents. The levels and profile of PSTs during 30-day depuration are detected by the high performance liquid chromatography with fluorescence detection (HPLC-FLD). The results show that the toxicity of the PSTs in soft tissues decreases to a relatively low level at Day 9. Moreover, the depurated ratio at the early stage of the PSTs depuration is higher than that at the later stage. The toxicity analysis of dissected organs reveals that the digestive gland is the most contaminated PSTs part, which is of important implication for the human health and scallop aquiculture. The mortality of Japanese scallops during PSTs depuration experiment is relevant to PSTs level in the soft tissue.

Key words: Paralytic shellfish toxins, toxicity, depuration, toxin transformation, Japanese scallop

Citation: Song Tao, Liu Lei, Song Xiaoping, Liang Yubo, Zhuang Guohong. 2015. Depuration of paralytic shellfish toxins in Japanese scallop (*Patinopecten yessoensis*) in natural environment. Acta Oceanologica Sinica, 34(12): 170–174, doi: 10.1007/s13131-015-0764-y

1 Introduction

Paralytic shellfish toxins (PSTs) are potent marine neurotoxins which block sodium channels of the neuronal cell membrane (Kao, 1993; Chou et al., 2005). The PSTs can be caused by a combination of any of 18 toxins, depending on the species of dinoflagellate, geographic area and type of shellfish involved (Samsur et al., 2006). The primary toxins include the carbamate toxins (saxitoxin, neosaxitoxin and gonyautoxin 1, 2, 3, and 4) and the sulfocarbomoyl toxins (B1, B2, C1, C2, C3, and C4). Filter-feeding molluscs can become poisonous to humans by consuming toxic dinoflagellates and cause human poisoning incidents. The PSTs have become a serious seafood safety issue at the global scale since the early 1970s (La Barbera-Sánchez and Estrella, 1996; Siung-Chang and Lum-Kong, 2001; La Barbera-Sánchez and Gamboa-Maruez, 2001) and there are increasing concerns among scientific community and aquaculture stakeholders (Chen and Chou, 1998; Bricelj and Shumway, 1998).

PSTs caused human poisoning incidents have been reported and confirmed both epidemically and scientifically in the coast of China (Lin et al., 1994; Anderson et al., 1996; Lin et al., 1999; Kong et al., 2007). Japanese scallops *Patinopecten yessoensis*, one of the most popular bivalve species cultured in north China, are more prone to the accumulation of PSTs than other bivalve species under the same environmental conditions. Once contaminated, it would take several months for PSTs in Japanese scallop to be depurated to the safety level (Samsur et al., 2006). Therefore, extra caution should be taken for bivalve aquiculture and seafood safety in study of PSTs depuration in Japanese scallop. The PSP toxin depuration in bivalves has been extensively studied under laboratory conditions using artificially infected bivalve samples (Ichimi et al., 2001; Li et al., 2005; Chen and Chou, 1998; Oikawa et al., 2005). However, little is known about PSTs depuration with bivalves naturally infected with PSTs, especially in the natural environment. In fact, such information is of more practical significance to the bivalve aquiculture management.

Japanese scallop naturally infected with PSTs was used to study the PSTs depuration in bivalve in the outdoor environment in this paper so as to provide detailed information of the PSTs depuration in Japanese scallops in natural environment, such as which organs are more prone to PSTs accumulation and when PSTs can be depurated to a seafood safety level.

2 Materials and methods

2.1 *Materials for depuration of the PSTs in toxic Japanese scallops*

Live Japanese scallops (shell length: 7.1 cm±1.1 cm) were collected from the Dayao Bay (39°09.98'N, 122°04.43'E) in the Huanghai Sea where Japanese scallops were cultured on a large scale with reported PSTs incidents, eutrophic water and frequent algal blooms occurrence. The scallops were maintained for de-

Foundation item: The National Natural Science Foundation of China under contract No. 30470275; the National Special Grant of China under contract Nos 908-01-ZH3 and 908-ZC-I-15; the National Basic Research Grant of China under contract No. 2010CB428706.
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puration of PSTs in Qipanmo waters (39°02.21'N, 122°17.82'E) (temperature (10.6±0.3)°C, salinity 30.2±0.5) in the Bohai Sea for 30 days from April 13 to May 12, 2009. There was no harmful algae bloom documented historically in Qipanmo waters, which are also verified by our PSTs profile analysis result from bivalve samples collected in Qipanmo waters before the study.

Twenty Japanese scallops were collected randomly every 3 days, and transported to the laboratory immediately for toxicity detection and toxin profiles analysis. After being rinsed with distilled water and drained, soft tissues of five Japanese scallops were collected and 15 Japanese scallops remained were dissected according to organ types: digestive gland, mantle, gonad (female or male), gill, and adductor muscle. The corresponding parts of Japanese scallops were pooled into one batch to reduce the intersample variance. The batches were homogenized and kept at -80°C for the PSTs toxicity and profiles analyzed.

2.2 *HPLC-FLD analysis*

PSTs were extracted according to the Oshima's method (1995a). Toxins were extracted from 1 g homogenates that were boiled with 1 mL 0.1 mol/L hydrochloric acid solutions. After cooling, the homogenate was centrifuged at 5 000 r/min for 10 min. The supernatant was adjusted to pH 3.0 with dilute HCl and was filtrated through a 0.45 μm pore membrane. The filtrate was prepared for analysis by high performance liquid chromatography with fluorescence detection (HPLC-FLD).

PSTs toxicity and profiles were analyzed by HPLC-FLD method (Oshima, 1995b) with minor modification. The HPLC-FLD system was Agilent1200 (Agilent, USA) and the post-column derivatization system was Pinnacle PCX (Pickering, USA). The column was a Luna C8 column (5 μm, 250×4.6 mm). Three mobile phases were used for separation of different PSTs groups: (1) 4.3 mmol/L sodium 1-heptanesulfonate in 10 mmol/L ammonium phosphate buffer (pH 7.1) for the GTX groups, (2) 2 mmol/L 1-heptanesulfonic acid in 10 mmol/L ammonium phosphate buffer (pH 7.1): the volume fraction of acetonitrile (20:1) for the saxitoxin group, and (3) 1 mmol/L tetrabutyl ammonium phosphate solution adjusted to pH 5.8 with acetic acid for C toxins. After being separated by the column, the PSTs were oxidized by 10 mmol/L periodic acid in 50 mmol/L sodium phosphate buffer (pH 9.0). The oxidization reaction was stopped by adding 0.5 mol/L acetic acid solution and then the oxidized toxins were detected by a fluorescence detector with excitation at 330 nm and emission at 390 nm.

Twelve PSTs analogues were analyzed in our study with the PSTs standards of C1, C2, GTX1, GTX2, GTX3, GTX4, GTX5, dcGTX2, dcGTX3, neoSTX, dcSTX and STX, purchased from the National Research Council of Canada. The detection limits for individual toxins were 34, 30, 65, 54, 42, 101, 375, 38, 142, 309, 186 and 216 pg, respectively.

2.3 *Data analysis*

Each PSTs analogue was identified and quantified by comparison of retention times and peak areas with those of standards. The ratio of individual PSTs analogue toxicity to the total PSTs toxicity was used to describe PSTs profiles. The toxicity level of each PSTs analogue was expressed as 1 μg STX equivalent to /100 g, which was determined according to Sullivan and Wekell (1987). The total PSTs levels were determined as the sum of the toxicity of carbamate toxins (GTX1-4), *N*-sulfocarbamoyl toxins (C1-2 and GTX5) and decarbamoyl toxins (dcGTX2-3).

3 Results

3.1 *Toxicity variations of PSTs during the depuration period*

Japanese scallops were collected regularly and were analyzed by HPLC-FLD during the depuration period. Typical HPLC-FLD profiles of toxins were extracted from the samples, which consisted of ten components (Fig. 1).

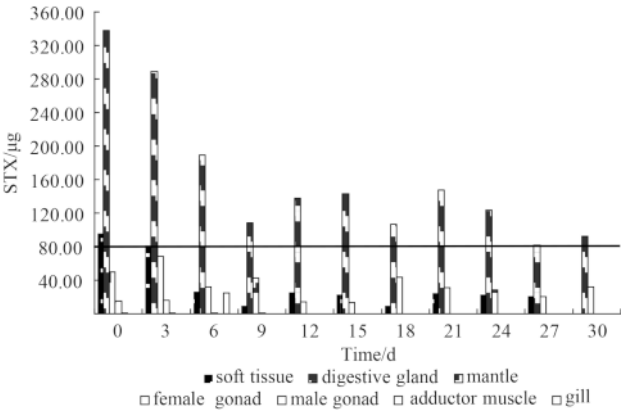

Fig. 1. Changes in total PSTs content obtained from soft tissues of five scallops and digestive glands, mantles, female gonads or male gonads, adductor muscles and gills 15 scallops by HPLC-FLD analysis. The black line represents the regulatory limit of PSTs.

The toxicity levels of PSTs in soft tissues (Fig. 2a), digestive glands (Fig. 2b), male gonads and female gonads (figure not shown) show a continued decline in 9 d and then a fluctuating drift, while, in mantles (Fig. 2c), PSTs level change presented an undulate declining curve during depuration period. However, from Day 0 to Day 30, the toxin concentration declined totally.

The toxicity level of PSTs in soft tissues reached 96.01 μg/(100 g) tissues at the beginning of the depuration period, while, the PSTs almost completely depurated at the end of experiment. The toxicity levels rapidly reduced to 19.96 μg/(100 g) tissues at day 9 and the depuration rate of that in Japanese scallops slowed down continually. The results also show that the PSTs toxicity levels increased slowly to the relative peak at Day 15 and 21 (Fig. 2a), then declined after 21 d.

In digestive glands, the toxicity levels of the PSTs decreased with fluctuation, from 338.19 μg/(100 g) tissues at Day 0 to 92.37 μg/(100 g) tissues at Day 30, which was still higher than the regulatory level. In mantles, the initial PSP toxins concentration in Japanese scallops was 49.91 μg/(100 g) tissues. The toxicity levels increased slightly to 68.65 μg/(100 g) tissues at Day 3, and then decreased rapidly to 32.26 μg/(100 g) tissues at Day 6, with three fluctuations of detoxification during subsequent time periods, dropping to 32.10 μg/(100 g) tissues at the end of the toxicity depuration period. After depurating for 12 d, male and female gonads had lost 100% of PSTs that they contaminated. The results also show that adductor muscles and gills were intermittent toxic during the depuration period with the trace levels of PSTs being detected only in adductor muscles at Day 6 and in gills at Day 0, 3 and 9.

3.2 *PSTs profiles changes during the depuration period*

PSTs profiles of soft tissues and other organs of the scallops

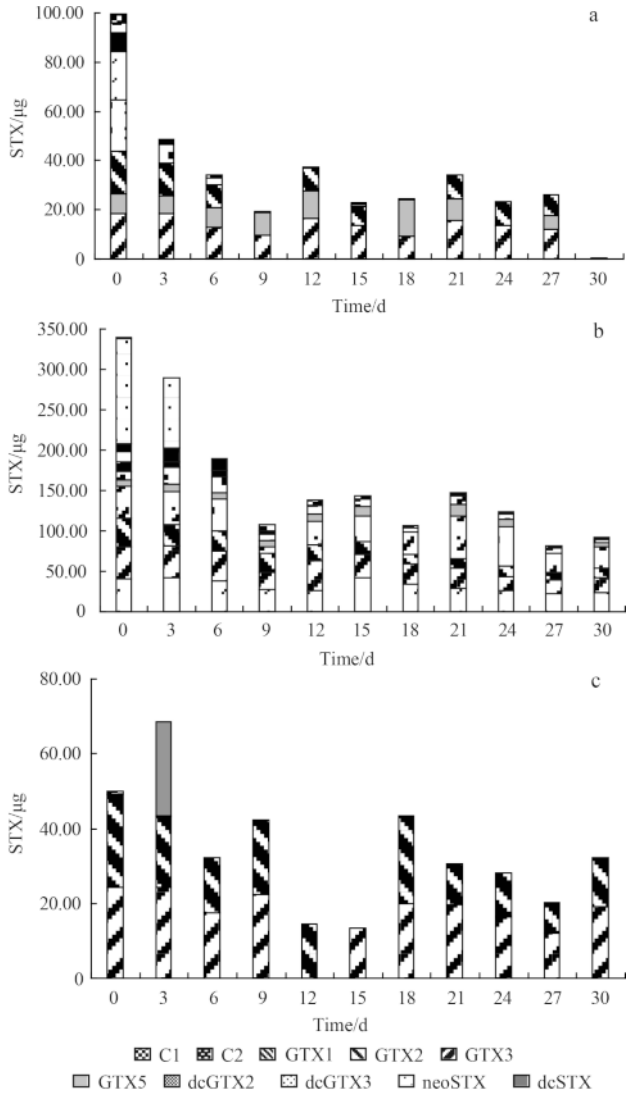

Fig. 2. Changes of PSP toxins profiles contents of soft tissues (a), digestive glands (b) and mantles(c) from 15 scallops, during the excreting experiment by HPLC-FLD.

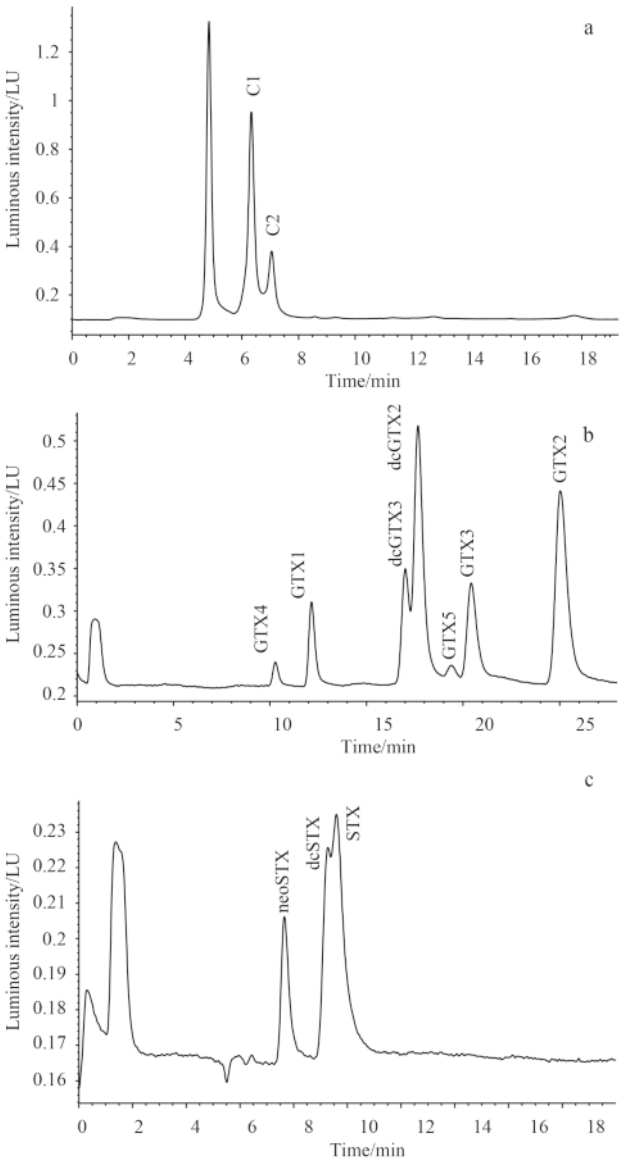

Fig. 3. Typical HPLC-FLD chromatograms of paralytic shellfish toxins in the digestive gland of Japanese scallop showing three main toxins: C-toxins at Day 3 (a), GTXs and STXs at Day 0 (b and c).

have been detected and three main toxins in the digestive gland of Japanese scallops are shown in Fig. 3.

The epimers were combined to simplify the comparison of the toxin profiles. During the depuration period, the predominant toxin profiles detected in samples were C1-2, dcGTX2-3, GTX1-3, and GTX5, whereas toxin profiles STX and GTX4 were not detected in any samples. The toxin profiles of the soft tissues were similar to those of digestive glands and mantles. Furthermore, neoSTX was only detected in digestive glands at Day 0 and 3, while dcSTX was detected in mantles at Day 3 and in adductor muscles at Day 6. Surprisingly, no significant STX toxin profiles were observed at any time and in any samples.

Except GTX4, neoSTX, dcSTX and STX, the other eight toxin analogues were all detected in the soft tissues during the depuration period (Fig. 3), with C1-2 and GTX2-3 being the major toxin and GTX1 and dcGTX2-3 only detected at Day 0 (Fig. 3a). Digestive glands shared the same PSTs components with soft tissues except neoSTX, but they showed different proportions of the components. In digestive glands, GTX1-3 and dcGTX2 were dominant PSTs components whereas dcGTX3 and dcSTX were only in trace amount. It is worth pointing out that GTX4 and STX were not detected at all in these samples (Fig. 3b). In contrast with the soft tissues and the digestive glands, only three toxin components, namely C1, GTX2 and GTX3, were found in the mantles (Fig. 3c). As for gills and gonads, C1 and C2 were only detected with trace amount (not shown in figure).

3.3 *Relationship between cumulative mortality of Japanese scallops and toxicity level of PSTs in soft tissues*

The mortalities of Japanese scallops had occurred during PSTs depuration experiment under outdoor condition and the final cumulative mortality reached 67.36% at the end of the experiment (Fig. 4).

The changes of the cumulative mortalities of Japanese scallops can be divided into two stages: (1) from Day 0 to 9, the cumulative mortality increased abruptly from 17.40% to 61.39%;

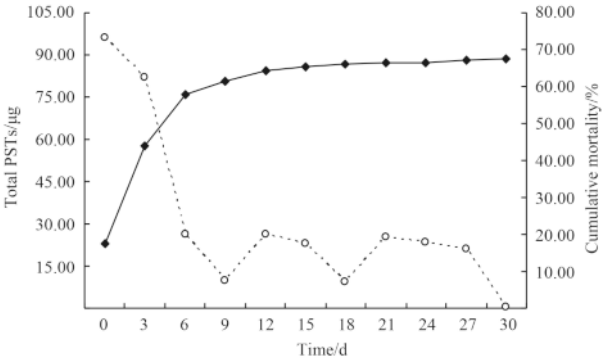

Fig. 4. Changes in cumulative mortalities of *Patinopecten yessoensis* and PSTs toxicity level in soft parts during PSTs depuration experiment. The white circles and the black diamonds represent PSTs toxicity level and the cumulative mortality, respectively.

and (2) from Day 9 to 30, the cumulative mortality increased tardily from 61.39% to 67.36%. The changes in cumulative mortalities are clearly related to these of PSTs levels in the soft tissues. Following the PSTs toxicity level dropped from 96.01 to 19.96 μg /(100 g) tissues,, the number of dead Japanese scallops decreases abruptly from 223 at the earlier stage to 31 at the latter stage. With the PSTs toxicity level drops to a relatively low level, there are less dead Japanese scallops at the latter stage. The death count of Japanese scallops is 10.3 times higher at the earlier stage than that at the latter stage.

4 Discussion

The bivalves can be classified into rapid and slow detoxifiers. The former takes weeks to detoxify toxin to food safety levels while the latter could take months to years to reach the similar level. Our results suggest that Japanese scallop belongs to rapid detoxifier. The digestive gland is the most contaminated PSTs part, and the toxicity in organs of Japanese scallops followed the order of the digestive gland greater than mantle greater than remaining tissues, which is consistent with the study by Cembella et al. (1994). The result shows that the toxicity level in the gonad is higher than that in the gill and that the toxicity level in female gonad was slightly higher than that in male gonad. The toxicity of Japanese scallops had decreased to relatively low level at Day 9 and the depurated ratio at the early stage of the PSTs depuration period is higher than that at the later stage. It is noteworthy that the toxicity level in the digestive gland of Japanese scallop during depuration is above the maximum permissible food safety level, and the adductor muscles are far below the safety level. It indicates that digestive gland in Japanese scallop from the Dayao Bay could be harmful to seafood consumers while the adductor muscles are still safe without depuration. In the present study, although the toxicity had been eliminated mostly in some organs, there is a rising trend at the later stage of the depuration. Important information is provided on when PSTs in scallops can be depurated to seafood safety level and which organs are safer for consumers.

There were significant differences in the toxin profiles in the dissected tissues, which is consistent with previous studies (Jiang et al., 2006; Kong et al., 2007) and it may be attributed to the possible PSTs transformation in Japanese scallops (Shimizu and Yoshioka, 1981, Sullivan et al., 1983, Oshima et al., 1990, Oshima, 1995b). Not agreeing with what Jiang and Kong were found (Jiang et al., 2006; Kong et al., 2007), the results of the present study indicates that the toxicity level of GTXs is higher than those of C toxins when they are both existent. Our explanation for this result is that the lower toxicity N-sulfocarbamoyl toxin C1 and C2 could be transformed into the higher toxicity carbamate toxin GTX2, 3 in Japanese scallop (Jiang et al., 2006). In our study, it is also showed that elimination of neoSTX and dcGTX3 from Japanese scallops is fast with their appearance only from Day 0 to 3 and Day 0 to 9 during the depuration period, respectively. However, GTX1, 2, 3, 5 and dcGTX2 in all detected PSPs profiles degraded very slowly, in that the hydroxyl group as GTX1-5 had a longer half-life, meanwhile, GTX4 was transformed to GTX1 quickly after feeding with toxic dinoflagellates (Chen and Chou, 2002), which might be the reason that the GTX4 was not detected in the study. Oshima (1995b) considered that it existed a chemical transformation that formation of decarbamoyl derivatives (dcGTXs and dcSTXs) might occur via the hydrolysis of N-sulfocarbamoyl toxins at neutral pH in live shellfish, as was well-known that pH in algae blooming sea was above that in normal sea water, which prove the longer degradation time of GTXs in Japanese scallops in the experiment. Additionally, the ratio of total α PSTs (GTX2, dcGTX2 and C1) to total β PSTs (GTX3, dcGTX3 and C2) in the digestive glands is 1.84, higher than that reported by Kong et al. (2007), which indicates the longer time of accumulating toxin. The capacity of toxin decomposition is probably both species-specific and toxin-specific. Therefore, further studies using other bivalve species with different toxin profiles are necessary for comprehensive understanding of decontamination mechanisms of PSTs in bivalves.

PSP toxins affect the action of nerves in bivalves by blocking the sodium channel (Bricelj et al, 2005; Llewellyn, 2006), and the high cumulative mortalities of some bivalve species have been recorded coinciding with outbreaks of PSTs producing microalgae, such as *Crassostrea virginica*, *Ostrea edulis* (Lesser and Shumway, 1993), *Mytilus edulis* (Shumway and Cucci, 1987), *Crassostrea gigas* larvae (Matsuyama et al., 2001), *Mytilus edulis* (Blanco and Fuentes, 2002) and *Lyropecten nodosus* (Lodeiros et al., 1998). Our study also shows that the massive mortalities of *Patinopecten yessoensis* occur at the beginning of depuration. In addition to the higher PSTs toxicity level in Japanese scallops, poor adaptability to a new environment may be one of the reasons for the higher cumulative mortalities.

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