Dietary exposure assessment of paralytic shellfish toxins through shellfish consumption in Shenzhen population, China

Yan Zhou 1 · Shenpan Li 1 · Jianying Zhang 2 · Jinzhou Zhang 1 · Zhou Wang 1 · Liubo Pan 1 · Baiqiang Huang 1,3 · Ke Huang 2 · Xiao Chen 1 · Qionghui Zhao 2 · Tianjui Jiang 3 · Jianjun Liu 1

Received: 18 June 2021 / Accepted: 26 August 2021 / Published online: 13 September 2021
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Abstract
Paralytic shellfish toxins (PSTs) produced by certain marine dinoflagellates accumulate in filter-feeding marine bivalves. We used LC-MS/MS to detect and quantify 13 PSTs in 188 shellfish samples of 14 species collected from Shenzhen city’s Buji seafood wholesale market from March 2019 to February 2020. Twenty-six of 188 shellfish samples (13.8%) were PSTs detectable. Within 14 species, 10 out of 34 noble clam Chlamys nobilis samples contain detectable PSTs with the highest detection rate 29.4%. Seven out of 17 samples from Nan’ao island contained detectable PSTs with the highest detection rate 41.2% among 11 origins. Samples containing PSTs were concentrated in spring and winter, with the highest levels in March>December>January. Among PSTs detected, C1 was dominant. Acute dietary exposure assessment for Shenzhen residents were based on mean adult body weight, 99th percentile daily shellfish consumption of Shenzhen food consumption survey 2008 and maximum PSTs concentration for each shellfish species. The outcome for Chlamys nobilis was 2.4–3.7-fold higher than recommended ARfDs. Mean PSTs concentration, P99, and mean shellfish consumption were used to assess chronic dietary exposure. The results were lower than recommended ARfDs. In conclusion, residents in Shenzhen are at risk for acute PSTs poisoning, while relatively safe from chronic PSTs exposure.

Keywords Paralytic Shellfish toxins · Saxitoxin · Dietary exposure assessment

Abbreviations
ARfD Acute reference dose
dcNEO Neo-dc saxitoxin
dcSTX Dc saxitoxin
bw Body weight
EFSA European Food Safety Authority
FAO Food and Agriculture Organization
GTX5 Pgonyautoxin 5
IOC Intergovernmental Oceanographic Commission of UNESCO
LB Lower bound
LOD(s) Limits of detection
P99 Low observed adverse effect level
ND Non-detected
NEO Neosaxitoxin
PSP Paralytical shellfish poisoning
PST(s) Paralytic shellfish toxin(s)
STX Saxitoxin
STX eq. Saxitoxin equivalent(s)
SZFCS Shenzhen Food Consumption Survey 2008
STX eq. Saxitoxin equivalent(s)
TEF(s) Toxicity-equivalence factors
TDI Tolerable daily intake
UB Upper bound
Introduction

The escalating incidence of paralytic shellfish poisoning (PSP) is associated with increasing harmful algal blooms linked to global ocean warming and anthropogenic activity that promotes eutrophication (Visciano et al. 2016). Paralytic shellfish toxins (PSTs) are mainly produced by marine dinoflagellates, such as *Alexandrium*, *Gymnodinium*, and *Gonyaulax* spp., and accumulated in filter-feeding bivalves (Liu et al. 2004; Asakawa et al. 2006; Shin et al. 2018). Bivalves commonly contaminated with PSTs include saltwater clams (*Meretrix lyrata*, *Siliqua patula*, *Paphi subtriangulata*, *Saxidomus giganteus*), mussels (*Perna viridis*, *Mytilus edulis*), scallops (*Amusium pleuonectes*, *Mimachlamys nobilis*, *Argopecten irradians*), oysters (*Crassostrea rivularis*), sea snail (*Haliotis tuberculata*), and crab (*Zosimus aeneus*), among other edible marine organisms (Bricelj and Shumway 1998; Tan and Ransangan 2015).

PST intoxication results from ingestion of seafood containing potent algal toxins, the parent molecule of which is saxitoxin (STX), which acts by blocking sodium channels in excitable membranes of cells, notably neurons (Pellegrino et al. 1984). Clinical symptoms of PST intoxication in mild cases begin with circumoral paresthesia that slowly spreads to the face, neck, fingers, and other parts, and dizziness, nausea. Symptoms usually begin 2–12 h after ingestion of the contaminated products, with gradual resolution over 24 h (FAO/IOC/WHO 2004). Severe poisoning results in muscle paralysis, dyspnea, and death from respiratory paralysis (FAO/IOC/WHO 2004), with symptom onset 2–12 h after consumption of contaminated shellfish. An antidote for PST intoxication has yet to be found.

STX is a trialkyl tetrahydropurine derivative (Fig. 1) that is soluble in water and has thermal and acid stability (Wang 2008). Wiese et al. (2010) collected 57 PST derivatives, ~20 of which can be extracted from shellfish. The toxic potencies of individual PSTs are expressed in relation to STX, and the relevant toxicity-equivalence factors (TEFs) are shown in Table 1 (European Food Safety Authority (EFSA) 2009; Van de Riet et al. 2009). International regulation limit for PSTs in shellfish is set at 800 μg STX eq. kg⁻¹ shellfish meat (Vale and Taleb 2005; European Food Safety Authority (EFSA) 2009).

In recent years, outbreaks of human PSP have occurred in coastal areas around the world, particularly in countries with Atlantic and Pacific coastlines. More than 2100 cases of PSP with 120 deaths were reported in the Philippines between 1983 and 2002 (Visciano et al. 2016; Ching et al. 2015). Hundreds of other PSP cases have been reported in Asia over the past decade (Nicolas et al. 2017; Ching et al. 2015). China has experienced many PSP cases and fatalities in coastal cities. Between 1991 and 2003, the coastal city of Lianyungang City in Chinese Jiangsu Province reported 11 PSP events, which together sickened 40 individuals and resulted in 8 fatalities, and coastal Fujian Province in S.E. China reported a PSP incident involving 164 cases in 2017 (Lin et al. 2005; Zhou et al. 2013; Chen et al. 2018). Additionally, the southern coast of China has experienced sporadic outbreaks of PSP (Anderson et al. 1996) and, at the turn of the twenty-first century, up to 30% of shellfish samples contained algal toxins in the Shenzhen area of the South China Sea (Jiang et al. 2003), where the predominant PSP source was the dinoflagellate *Alexandrium pacificum* (Liu et al. 2021). In March 2005, Shenzhen’s neighbor Hong Kong experienced 36 clusters of shellfish poisoning related to consumption of fresh scallops (*Atrina vexillum*). Since Shenzhen and Hong Kong have a

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**Table 1** The toxic factor (TEF) and standard limit of PSTs

| PST  | TEF  | Limit*         |
|------|------|----------------|
| dcNEO| 0.4  | 800 μg STX eq. kg⁻¹ |
| GTX5 | 0.1  |                 |
| NEO  | 1    |                 |
| dcSTX| 1    |                 |
| STX  | 1    |                 |
| C1   | 0.01 |                 |
| C2   | 0.1  |                 |
| dcGTX2 | 0.2|                 |
| GTX1 | 1    |                 |
| GTX2 | 0.4  |                 |
| GTX4 | 0.7  |                 |
| GTX3 | 0.6  |                 |
| dcGTX3| 0.4|                 |

* International regulation limit for PSTs in shellfish are 800 μg STX eq. kg⁻¹ shellfish meat (Vale and Taleb 2005; European Food Safety Authority (EFSA) 2009)
combined population of 20 million residents, all of whom are at risk for exposure to PSP, the present study assesses the current level of PST contamination of edible shellfish as well as the extent of local resident dietary exposure to these dangerous toxins.

**Material and methods**

**Sampling**

Every month from March 2019 to February 2020, shellfish samples were collected from the Buji seafood wholesale market in Luohu, which supplies 90% of shellfish consumed in Shenzhen. In total, 188 shellfish samples of 14 species were collected, including *Chlamys nobilis* (scallop), *Crassostrea rivularis* (oyster), *Perna viridis* (mussel), *Paphia undulata* (clam), *Atrina pectinata* (bivalve), *Babylonia areolate* (sea snail), *Scapharca broughtoni* (clam), *Scapharca subcrenata* (bivalve), *Meretrix meretrix* (razor clam), *Ruditapes philippinara* (bivalve), *Sinonovacula constricta* (sea scallop), *Solen gouldi* (bivalve), and *Mytilus edulis* (mussel). Sampling methods were made based on shellfish species monthly availability on the market, and 14–17 samples were collected each month.

**Reagents**

Methanol, acetonitrile, and formic acid were purchased from Merck (Darmstadt, Germany). Methylene chloride was obtained from Damao Chemical Reagent Factory (Tianjin, China). Water was distilled and passed through a MilliQ water purification system (Millipore, Burlington, Mass. USA). Ammonium formate was purchased from Sigma (Bangalore, India). PST standard reagents (C1, C2, dcGTX2, dcGTX3, dcNEO, dcSTX, GTX1, GTX2, GTX3, GTX4, GSTX, STX, NEO) were purchased from Bedford Institute of Oceanography (Dartmouth, Nova Scotia, Canada).

**Toxin extraction**

Shellfish samples were cleaned with fresh water, removed from their shells, and rinsed again with MilliQ water to remove silt from the flesh. Shellfish meat and viscera (200 g of each batch) was removed and homogenized. A 5-g aliquot of homogenized sample was transferred to a centrifuge tube, 8 mL of 0.5% formic acid solution added, for vortex-mixed for 5 min, ultrasonically (200 W, 40 kHz, Kunshanshumei, China) extracted for 10 min, followed by centrifugation at 9500 rpm/min for 10 min (Mattarozzi et al. 2016; Yang 2020). The suspension was immersed in 0.5% formic acid solution to 10 mL. Two milliliters of this mixture were added to 5 mL dichloromethane, vortex-mixed for 3 min, and then centrifuged for 5 min at 9500 rpm. Then, 1 mL suspension was passed through a C-18 solid-phase extraction cartridge (Waters, Millford, Mass, USA) that had been activated by adding 3 mL acetonitrile and 3 mL 0.5% formic acid solution in that order, and then immersed in 1.5 mL 0.5% formic acid solution. The eluate was collected, adjusted to 6 mL acetonitrile solution, and vortexed. The resulting solution was allowed to rest for 30 min in a −4 °C refrigerator, then centrifuged at 9500 t/min for 5 min, passed through 0.22 μm nylon syringe filter and collected in a brown test tube.

**Detection and quantification of individual PSTs**

Firstly, a chromatographic analysis was performed with a Nexera X2 HPLC system (Shimadzu, Kyoto, Japan) comprising a degassing unit, an auto-sampler, and a micro binary pump and equipped with a Waters Amide column (2.1*100 mm, 3.5 μm, Waters, USA). The temperature of the column was set at 40 °C. The injection volume was 5 μL, and the flow rate was set to 0.5 mL min⁻¹ in all time. The mobile phase consisted of two eluates: A (1 mmol L⁻¹ ammonium formate solution) and B (acetonitrile), both of which contained 1.3 mmol L⁻¹ formic acid. The elution was performed starting with 5% A for 2 min, increasing to 15–30% for 16 min, and finally 5% A for 2.5 min for column re-equilibration. PSTs were detected by mass spectrometry analysis using a Shimadzu 8050 series mass spectrometer equipped with an electrospray interface set in the positive ionization mode (ESI+). PSTs (and their corresponding PubChem CID) included neo-de saxitoxin (dcNEO, 100962170), gonyautoxin 5 (GTX5, 49789073), neosaxitoxin (NEO, 135562690), dc saxitoxin (dcSTX, 101936522), and saxitoxin (STX, 56947150). A negative ionization mode (ESI−) was used to detect: N-sulfocarbamoyl-gonyautoxin-2 and -3(C1, 2, 49789085), and other gonyautoxins, including dcGTX2 (101034662), GTX1 (135061918), GTX2 (101650338), GTX4 (440699577), GTX3 (46217347), and dcGTX3 (101034664). The multiple reaction monitoring (MRM) mode was used, with specific transition parameters as shown in Table 2. The capillary voltage was 4000 V, the nebulizer gas flow rate was 3 L min⁻¹, the drying gas flow rate was 10 L min⁻¹, and the temperature of the ion source was 300 °C. The limits of detection (LOD, μg kg⁻¹) were dcNEO (24), GTX5 (60), NEO (30), dcSTX (30), STX (30), C1(40.8), C2 (24), dcGTX2 (42), GTX1 (38.4), GTX2 (28.8), GTX4 (48), GTX3 (48), and dcGTX3 (48).

**Statistical analysis**

Statistical analysis was performed using SPSS (version 22, IBM SPSS Statistics). The number of PSTs detected and non-detected sample in different subgroups of species/
Fisher’s exact test was used to analyze whether detection rates of shellfish depend on species, origins, as well as time of sampling. Fisher’s exact test \( \leq 0.05 \) was regarded as statistically significant.

Dietary exposure assessment

For both acute and chronic dietary exposure assessment, the general equation is (World Health Organization and Food and Agriculture Organization of the United Nations 2009; Wong et al. 2013):

\[
PSTs \text{ Dietary exposure (} \mu g kg^{-1} \text{ bw day}^{-1}) = \frac{\text{Concentration of } PSTs \text{ in shellfish (} \mu g \text{ STX eq. kg}^{-1}) \times \text{Shellfish consumption (g day}^{-1})}{\text{Body weight (kg) \times 10^3}}
\]

Shellfish consumption and body weight data

The shellfish consumption data (\( \mu g \text{ STX eq. kg}^{-1} \)) and adult mean body weight (60.2 kg) of the target population were obtained from the individual-based Shenzhen Food Consumption Survey 2008 (Huang et al. 2015), which was conducted by the Shenzhen Center for Disease Control and Prevention. A three-stage cluster sampling was performed taking account of local population flow and density, together with geographic and economic characteristics (Yang et al. 2014). Referring to the sampling principles of China National Health and Nutrition Survey 2002 (Huang et al. 2015), 244 households were selected throughout 4 urban regions and 2 rural regions, a total of 853 individuals was included. Food consumption data were assessed by a continuous 3-day door-to-door interview, which included weighing that was consumed at home and using a 24-h dietary-recall questionnaire to record food intake outside of the home. Different values of shellfish consumption used in acute/chronic dietary exposure assessment were detailed in the “Results” section.

PSTs concentration data

PSTs concentration in each shellfish sample was calculated by summing up 13 individual PSTs STX equivalents, and the unit was \( \mu g \text{ STX eq. kg}^{-1} \). Thirteen individual PST (dcNEO, GTX5, NEO, dcSTX, STX, C1, C2, dcGTX2, GTX1, GTX2, GTX4, GTX3, and dcGTX3) concentrations were quantified respectively using liquid chromatograph-tandem mass spectrometry (LC-MS/MS). Since the data were left-censored, the protocol referring to FAO/IOC/WHO (2004) for assigning concentration values to non-detected (ND) results was applied in this study. In a lower bound (LB) scenario, 0 was used to substitute for ND results (World Health Organization and Food and Agriculture Organization of the United Nations 2009; European Food Safety Authority (EFSA) 2010), while in an upper bound (UB) scenario, 1/2 LOD was used to substitute for ND results.

The concentration of individual PST concentration was converted to the STX-equivalent (STX eq.) by multiplying the appropriate toxic equivalent factors (TEFs) (Shin et al. 2014). Referring to the sampling principles of China National Health and Nutrition Survey 2002 (Huang et al. 2015), 244 households were selected throughout 4 urban regions and 2 rural regions, a total of 853 individuals was included. Food consumption data were assessed by a continuous 3-day door-to-door interview, which included weighing that was consumed at home and using a 24-h dietary-recall questionnaire to record food intake outside of the home. Different values of shellfish consumption used in acute/chronic dietary exposure assessment were detailed in the “Results” section.

### Table 2 Mass spectrum parameters, limits of detection, and limits of quantification for PSTs

| PST Precursor-product ions | Positive/Negative | Linear range (ng mL\(^{-1}\)) | Limit of detection (\( \mu g \text{ kg}^{-1} \)) | Limit of quantification (\( \mu g \text{ kg}^{-1} \)) |
|---------------------------|------------------|----------------|-----------------|-----------------|
| dcNEO 273.1–126.0 ESI+    | 4–80             | 24             | 48              |
| GTX5 380.1–300.1 ESI+     | 10–500           | 60             | 120             |
| NEO 316.0–220.0 ESI+      | 10–500           | 30             | 60              |
| dcSTX 257.1–126.0 ESI+    | 5–200            | 30             | 60              |
| STX 300.0–204.0 ESI+      | 5–200            | 30             | 60              |
| C1 474.0–357.0 ESI-       | 6.8–270          | 40.8           | 81.6            |
| C2 474.0–121.9 ESI-       | 4–80             | 24             | 48              |
| dcGTX2 351.1–333.1 ESI-   | 7–275            | 42             | 84              |
| CTX1 410.1–367.1 ESI-     | 6.4–254          | 38.4           | 76.8            |
| GTX2 394.0–351.1 ESI-     | 4.7–187          | 28.8           | 56.4            |
| GTX4 410.1–367.1 ESI-     | 8–160            | 48             | 96              |
| GTX3 394.0–351.1 ESI-     | 8–160            | 48             | 96              |
| dcGTX3 351.1–333.1 ESI-   | 8–160            | 48             | 96              |
13 individual STX-equivalents to obtain the total PST concentration of each sample. Different values of PSTs concentration used in acute/chronic dietary exposure assessment were detailed in the “Results” section.

### Table 3 Detection of PST contamination in annual shellfish samples (n = 188)

| Species                      | Annual sample size (n=188,100%) | Detected (n=26, 13.8%) | Non-detected (n=162, 86.2%) | *       |
|------------------------------|----------------------------------|-------------------------|-----------------------------|---------|
|                              | n %a                             | n %b                    | n %c                        |         |
| **Species**                  |                                  |                         |                             |         |
| *Chlamys nobilis*            | 34 18.1                          | 10 29.4                 | 24 70.6                     | 0.0533  |
| *Crassostrea rivularis*      | 23 12.2                          | 5 21.7                  | 18 78.3                     |         |
| *Perna viridis*              | 20 10.6                          | 4 20.0                  | 16 80.0                     |         |
| *Paphia undulata*            | 40 21.3                          | 3 7.5                   | 37 92.5                     |         |
| *Atrina pectinate*           | 12 6.4                           | 1 8.3                   | 11 91.7                     |         |
| *Babylonia areolata*         | 10 5.3                           | 1 10.0                  | 9 90.0                      |         |
| *Scapharca broughtoni*       | 3 1.6                            | 1 33.3                  | 2 66.7                      |         |
| *Scapharca subcrenata*       | 2 1.1                            | 1 50.0                  | 1 50.0                      |         |
| *Meretrix meretrix*          | 18 9.6                           | 0 0.0                   | 18 100                      |         |
| *Sinonovacula constricta*    | 11 5.9                           | 0 0.0                   | 11 100                      |         |
| *Ruditapes philippinarum*    | 6 3.2                            | 0 0.0                   | 6 100                       |         |
| *Patinopcten yessoensis*      | 5 2.7                            | 0 0.0                   | 5 100                       |         |
| *Solen gouldii*              | 2 1.1                            | 0 0.0                   | 2 100                       |         |
| *Mytilus edulis*             | 2 1.1                            | 0 0.0                   | 2 100                       |         |
| **Origins**                  |                                  |                         |                             |         |
| Nan‘ao                       | 17 9.0                           | 7 41.2                  | 10 58.8                     | 0.074   |
| Huidong                      | 31 16.5                          | 6 19.4                  | 25 80.7                     |         |
| Zhanjiang                    | 31 16.5                          | 6 19.4                  | 25 80.7                     |         |
| Aotou                        | 21 11.2                          | 2 9.5                   | 19 90.5                     |         |
| Guangxi                      | 16 8.5                           | 2 12.5                  | 14 87.5                     |         |
| Fukien                       | 17 9.0                           | 1 5.9                   | 16 94.1                     |         |
| Hainan                       | 10 5.3                           | 1 10.0                  | 9 90.0                      |         |
| Zhubai                       | 12 6.4                           | 1 8.3                   | 11 91.7                     |         |
| Dianbai                      | 6 3.2                            | 0 0.0                   | 6 100                       |         |
| Shantung                     | 9 4.8                            | 0 0.0                   | 9 100                       |         |
| Yangjiang                    | 18 9.6                           | 0 0.0                   | 18 100                      |         |
| **Time of sampling**         |                                  |                         |                             | <0.01   |
| 2019 March                   | 15 8.0                           | 5 33.3                  | 10 66.7                     |         |
| April                        | 15 8.0                           | 2 13.3                  | 13 86.7                     |         |
| May                          | 14 7.4                           | 4 28.6                  | 10 71.4                     |         |
| June                         | 14 7.4                           | 0 0.0                   | 14 100                      |         |
| July                         | 17 9.0                           | 0 0.0                   | 17 100                      |         |
| August                       | 15 8.0                           | 0 0.0                   | 15 100                      |         |
| September                    | 16 8.5                           | 0 0.0                   | 16 100                      |         |
| October                      | 16 8.5                           | 0 0.0                   | 16 100                      |         |
| November                     | 17 9.0                           | 1 0.0                   | 16 94.1                     |         |
| December                     | 16 8.5                           | 5 31.3                  | 11 68.8                     |         |
| 2020 January                 | 16 8.5                           | 5 31.3                  | 11 68.8                     |         |
| February                     | 17 9.0                           | 4 23.5                  | 13 76.5                     |         |

*a* calculated by annual number of samples in the sub-group/ total number of shellfish sample (n=188)  
*b* calculated by detected sample number of the sub-group/ annual sample number of the sub-group  
*c* calculated by non-detected sample number of the sub-group/ annual sample number of the sub-group  
*Fisher’s exact test p value of comparing annual detected and non-detected groups*
Results

Detection rate of PSTs in shellfish

Among 188 shellfish samples, 26 samples (13.8%) were PSTs detectable. Samples were PSTs detectable meaning they were detected at least 1 individual PST. Annual number of samples, number of detected/non-detected samples, as well as detected rate (number of detected samples/annual number of samples)/non-detected rate (number of non-detected samples/annual number of samples) in sub-groups of species/origins/time of sampling were showed in Table 3. Among 14 species, year-round samples of the noble scallop *Chamys nobilis* had the highest PST detected rate: 10 of 34 samples (29.4%) contain detectable PSTs. Annual high-detected rates were also record-ed for the oyster *Crassostrea rivularis*, 5 out of 18 annual samples (21.7%) were detected containing PSTs, and the Asian green mussel *Perna viridis*, 4 out of 20 annual samples (20.0%) were PSTs-detected. PSTs were not detected in samples of 7 shellfish species (*Meretrix meretrix*, *Sinonovacula constricta*, *Ruditapes philippinarum*, *Patinopecten yessoensis*, *Solen gouldi*, and *Mytilus edulis*). Shellfish PST contamination varied by the origin location in China (Fig. 2). Nan‘ao or Nanao, formerly romanized Namoa, is an island and county of the prefecture-level city of Shantou in Guangdong Province, China. Most contamination was detected in samples taken from waters around Nan’ao island, (7/17, 41.2%), followed by Huidong (6/31, 19.4%) and Zhanjiang (6/31, 19.4%). Shellfish samples from coastal Dianbai, Shantung, and Yangjiang were PST undetectable (Table 3). Only samples distributed in spring and winter were PSTs detectable: the highest detected rate was in March (5/15, 33.3%), followed by December (5/16, 34.3%) and January (5/16, 31.3%). The contamination of PSTs of samples collected from June to October 2019 was not detectable (Table 3). Results of Fisher’s exact test showed that detected rates among the time-sampled groups differed significantly ($p \leq 0.05$).

Concentration of PSTs in shellfish

Annual shellfish samples ($n = 188$) have been quantified for 13 individual PSTs (dcNEO, GTX5, NEO, dcSTX, STX, C1, C2, dcGTX2, GTX1, GTX2, GTX4, GTX3, dcGTX3) and the distribution of the detected/non-detected number of samples were shown in Fig. 3. Most samples were C1 detectable (19/188, 10.1%), with the widest concentration range of 0–1596.5 μg kg$^{-1}$, followed by C2 (14/188, 7.5%), GTX5 (8/188, 4.3%), and others. NEO, STX, GTX3, and GTX4 were not detected in any sample. The concentration ranges for individual PSTs are summarized in Fig. 4.

After converting individual PST concentrations to STX-equivalents by multiplying the appropriate TEFs (Shin et al. 2018), sample PSTs concentration was obtained by summing up 13 individual STX-equivalents. Among all samples, mean PST concentration ranged from 10.85 (LB)–134.06 (UB) μg STX eq. kg$^{-1}$, with maximum values ranging from 715.60 (LB) to 796.00 (UB) μg STX eq. kg$^{-1}$. Both mean and maximum PST concentrations were highest in *Chlamys nobilis* and samples obtained from Nan‘ao. Samples collected in March and April contained similar mean and maximum

![Fig. 2 Locations of sample origins in China](image-url)
PSTs concentrations, and these values were higher than in samples collected in other months. Detailed PST concentration sets for all samples, by shellfish species, origins, and time of sampling, are integrated in Table 4.

**Acute dietary exposure assessment**

International guidance dictates that large portions of shellfish should be used to conduct an acute dietary exposure assessment (WHO 2009). A large portion consumption was estimated as 139.2 g day$^{-1}$ in the present study, which corresponded to 99th percentile daily shellfish consumption ($P_{99}$ Shellfish consumption) of individuals included in SZFCS 2008, from which adult mean body weight (60.2 kg) were also acquired. Both upper and lower bounds of maximum PST concentrations were used to calculate the acute dietary exposure level using the equation proposed by WHO (2009) which was demonstrated in “Dietary exposure assessment” section. Results of acute dietary exposure level with respect to individual seafood species are summarized in Fig. 5.

![Fig. 3 Occurrence of individual PSTs in shellfish samples. > LOD means number of samples with detectable individual PST; ND means number of samples with undetectable individual PST](image1)

![Fig. 4 Raw detected concentration ranges of individual PST in shellfish samples](image2)
In studies conducted by the IOC/WHO Expert Consultation on Biotoxins in Molluscan Bivalves, the PST toxicological reference value of provisional low observed adverse effect level (LOAEL) was established as 2.0 μg kg\(^{-1}\) bw day\(^{-1}\). A safety factor of 3 was used to drive an Acute Reference Dose (ARfD) of 0.7 μg STX eq. kg\(^{-1}\) bw.

Table 4  Concentration data set of PSTs of all samples and by shellfish species, origins, time of sampling

| Species                  | Annual sample size | Mean (μg kg\(^{-1}\)) | P\(_{95}\) (μg kg\(^{-1}\)) | Max (μg kg\(^{-1}\)) |
|--------------------------|--------------------|------------------------|----------------------------|----------------------|
|                          | n | n% | LB | UB | LB | UB | LB | UB | LB | UB |
| Total species            | 188 | 100 | 10.85 | 134.06 | 91.09 | 195.09 | 715.60 | 796.00 |
| **Species**              |    |    |    |    |    |    |    |    |    |    |
| Paphia undulata          | 40 | 21.28 | 1.29 | 125.42 | 0.99 | 124.95 | 49.81 | 172.78 |
| Chlamys nobilis          | 34 | 18.09 | 50.84 | 170.90 | 710.26 | 790.66 | 715.60 | 796.00 |
| Crassostrea rivularis    | 23 | 12.23 | 5.88 | 129.09 | 54.42 | 168.42 | 60.79 | 174.79 |
| Perna viridis            | 20 | 10.64 | 1.64 | 125.41 | 12.37 | 134.23 | 13.68 | 124.65 |
| Meretrix meretrix        | 18 | 9.57 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| Atrina pectinate         | 12 | 6.38 | 0.06 | 124.20 | 0.69 | 124.65 | 0.69 | 124.65 |
| Sinonovacula constricta. constricta | 11 | 5.85 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| Balbina areolate         | 10 | 5.32 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| Ruditapes philippinara   | 6  | 3.19 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| Patinopecten yessoensis   | 5  | 2.66 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| Scapharca broughtoni     | 3  | 1.60 | 0.27 | 124.36 | 0.80 | 124.74 | 0.80 | 124.76 |
| Solen gouldi             | 2  | 1.06 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| Mytilus edulis           | 2  | 1.06 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| Scapharca subcrenata     | 2  | 1.06 | 14.61 | 137.27 | 29.22 | 150.38 | 29.22 | 150.38 |
| **Origins**              |    |    |    |    |    |    |    |    |    |    |
| Huidong                  | 31 | 16.49 | 2.49 | 126.20 | 13.68 | 134.64 | 60.79 | 174.79 |
| Zhanjiang                | 31 | 16.49 | 6.24 | 129.19 | 54.42 | 168.42 | 78.02 | 181.58 |
| Aotou                    | 21 | 11.17 | 0.24 | 124.33 | 0.55 | 124.51 | 4.54 | 127.30 |
| Yangjiang                | 18 | 9.57 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| Fukien                   | 17 | 9.04 | 0.03 | 124.18 | 0.52 | 124.48 | 0.52 | 124.48 |
| Nan’ao                   | 17 | 9.04 | 97.12 | 214.29 | 715.60 | 796.00 | 715.60 | 796.00 |
| Guangxi                  | 16 | 8.51 | 3.16 | 127.23 | 49.81 | 172.78 | 49.81 | 172.78 |
| Zhuhai                   | 12 | 6.38 | 0.10 | 124.25 | 1.18 | 125.14 | 1.18 | 125.14 |
| Hainan                   | 10 | 5.32 | 0.69 | 129.77 | 60.86 | 180.23 | 60.86 | 180.23 |
| Shantung                 | 9  | 4.79 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| Dianbai                  | 6  | 3.19 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| **Time of sampling**     |    |    |    |    |    |    |    |    |    |    |
| 2019                     |    |    |    |    |    |    |    |    |    |    |
| March                    | 15 | 7.98 | 49.45 | 170.46 | 710.26 | 790.66 | 710.26 | 790.66 |
| April                    | 15 | 7.98 | 47.75 | 168.99 | 715.60 | 796.00 | 715.60 | 796.00 |
| May                      | 14 | 7.45 | 0.66 | 124.60 | 4.54 | 127.30 | 4.54 | 127.30 |
| June                     | 14 | 7.45 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| July                     | 17 | 9.04 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| August                   | 15 | 7.98 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| September                | 16 | 8.51 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| October                  | 16 | 8.51 | 0.00 | 124.16 | 0.00 | 124.16 | 0.00 | 124.16 |
| November                 | 17 | 9.04 | 0.07 | 124.22 | 1.18 | 125.14 | 1.18 | 125.14 |
| December                 | 16 | 8.51 | 15.06 | 136.13 | 78.02 | 181.58 | 78.02 | 181.58 |
| 2020                     |    |    |    |    |    |    |    |    |    |    |
| January                  | 16 | 8.51 | 14.08 | 136.49 | 137.41 | 242.98 | 137.41 | 242.98 |
| February                 | 17 | 9.04 | 6.19 | 129.95 | 49.81 | 172.78 | 49.81 | 172.78 |
day\(^{-1}\) because a wide spectrum of people report shellfish poisoning, and mild illness is readily reversible (FAO/IOC/WHO 2004; Picot et al. 2011; Toyofuku 2006). On the other hand, the European Food Safety Authority (EFSA) established a lower LOAEL (1.5 \(\mu\)g kg\(^{-1}\) bw day\(^{-1}\)) based on ~500 reported PSP cases; an ARfD of 0.5 \(\mu\)g STX eq. kg\(^{-1}\) bw day\(^{-1}\) was proposed subsequently using a safety factor of 3 (European Food Safety Authority (EFSA) 2009). Both ARfDs were used as shellfish acute dietary exposure level limits.

In the results showed in Fig. 5, the highest acute dietary exposure value was 1.84 \(\mu\)g STX eq. kg\(^{-1}\) bw day\(^{-1}\) using the upper bound of the maximum contamination value of *Chlamys nobilis*, which corresponded to approximately 2.6–3.7 times the ARID established by FAO/IOC/WHO (0.7 \(\mu\)g STX eq. kg\(^{-1}\) bw day\(^{-1}\)) and EFSA (0.5 \(\mu\)g STX eq. kg\(^{-1}\) bw day\(^{-1}\)) respectively. In the lower bound scenario, the acute dietary exposure value for *Chlamys nobilis* was 1.65 \(\mu\)g STX eq. kg\(^{-1}\) bw day\(^{-1}\), a value corresponding to 2.4–3.3 times as ARfDs established by FAO/IOC/WHO and EFSA respectively. The acute dietary exposure values of other species were lower than 0.5 \(\mu\)g STX eq. kg\(^{-1}\) bw day\(^{-1}\).

**Chronic exposure assessment**

For the chronic exposure assessment of PSTs in shellfish, both lower and upper bounds (LB, UB) of mean concentrations were used. According to SZFCS 2008, the mean shellfish consumption was 4.82 g day\(^{-1}\). The PST chronic dietary exposure value of all samples ranged from 0.009 (LB) to 0.0107 (UB) \(\mu\)g STX eq. kg\(^{-1}\) bw day\(^{-1}\). Tolerable daily intake (TDI) is considered to be the standard reference for chronic exposure assessment (Shin et al. 2018); however, no such reference has been established by reliable authorities or formal organizations. Therefore, in this study, chronic exposure values were compared to ARfDs by EFSA and FAO/IOC/WHO. Chronic dietary exposure values using mean shellfish consumption data were much lower than recommended ARfD. In the estimated extreme scenario, even the upper-bound chronic dietary exposure value was 0.44–0.62 times the EFSA ARfD. Chronic dietary exposure assessment was also analyzed for different species as summarized in Table 5.

**Discussion**

The present study used mass spectrometry to detect and quantify PSTs in marine species sold for human consumption in Shenzhen city in southern China. Seafood samples included the viscera, human consumption of which was identified in neighboring Hong Kong as the highest risk factor for shellfish poisoning (Chung et al. 2006). We found that most seafood samples had lower concentrations of PSTs than the corresponding limits of detections. Among 13 PST derivatives, C1, C2, GTX1, GTX2, dcGTX2, dcGTX3, GTX5, dcNEO, and dcSTX were detected in seafood samples sold in Shenzhen, with C1 and C2 the most predominant components. These results are similar to those of Liu et al. (2017), who studied PSTs in phytoplankton and shellfish samples from the Bohai Sea in northern China. They found that toxic *Alexandrium spp.* and *Gymnodinium catenatum* were
potential producers of PSTs, and most low-PST-content phytoplankton samples were C1/C2-detectable.

The concentration of PSTs differs in shellfish species (Brieelj et al. 1990) and origins. We found that 8 of 14 species were contaminated, particularly \textit{Chlamys nobilis} (noble scallop) with PST concentration of 715.6 (LB)–796 (UB) μg STX eq. kg$^{-1}$, a value at the edge of the international regulation limit of 800 μg STX eq. kg$^{-1}$ (FAO/IOC/WHO 2004). Based on ARFDs of 0.5–0.7 μg STX eq. kg$^{-1}$ bw day$^{-1}$, we show here that Shenzhen residents are at risk for PSP, especially after ingestion of \textit{Chlamys nobilis} (1.65–1.84 μg STX eq. kg$^{-1}$ bw day$^{-1}$). Similar dietary exposure assessment has been conducted in a nearby Asian country Korea; the highest PSTs concentration was detected in muscles and acute exposure value was 0.3 μg STX eq. kg$^{-1}$ bw day$^{-1}$ (Shin et al. 2018), much lower than our study. Shenzhen residents are at risk for PSP, especially after ingestion of \textit{Chlamys nobilis}. Consumption of other shellfish species on sale in Shenzhen requires ongoing PST monitoring but presently appears to be safe.

The tolerable daily intake (TDI) for PSTs has not been established because chronic toxicity studies are unavailable (Visciano et al. 2016), thus, we used the acute reference dose.

### Table 5 Chronic PST exposure assessment using Shenzhen resident mean and P$_{99}$ shellfish consumption data

| Species                     | Mean shellfish contamination data (μg STX eq. kg$^{-1}$) | Chronic exposure values (μg STX eq. kg$^{-1}$ bw day$^{-1}$) |
|-----------------------------|----------------------------------------------------------|-------------------------------------------------------------|
|                             | LB    | UB    | LB          | UB          | LB          | UB          |
| Total species               | 10.85 | 134.06| 0.0009      | 0.0107      | 0.0251      | 0.3100      |
| \textit{Paphia undulata}    | 1.29  | 125.42| 0.0001      | 0.0100      | 0.0030      | 0.2900      |
| \textit{Chlamys nobilis}    | 50.84 | 170.90| 0.0041      | 0.0137      | 0.1175      | 0.3952      |
| \textit{Crassostrea rivularis} | 5.88  | 129.09| 0.0005      | 0.0103      | 0.0136      | 0.2985      |
| \textit{Perna viridis}      | 1.644 | 125.41| 0.0001      | 0.0100      | 0.0038      | 0.2900      |
| \textit{Meretrix meretrix}  | 0.00  | 124.16| 0.0000      | 0.0099      | 0.0000      | 0.2871      |
| \textit{Atrinapectinate}    | 0.06  | 124.20| 0.0000      | 0.0099      | 0.0001      | 0.2872      |
| \textit{Sinonovacula constricta} | 0.00  | 124.16| 0.0000      | 0.0099      | 0.0000      | 0.2871      |
| \textit{Babylonia areolata} | 6.07  | 129.77| 0.0005      | 0.0104      | 0.0141      | 0.3001      |
| \textit{Ruditapes philippinara} | 0.00  | 124.16| 0.0000      | 0.0099      | 0.0000      | 0.2871      |
| \textit{Patinopecten yessoensis} | 0.00  | 124.16| 0.0000      | 0.0099      | 0.0000      | 0.2871      |
| \textit{Scapharca broughtoni} | 0.27  | 124.36| 0.0000      | 0.0100      | 0.0006      | 0.2876      |
| \textit{Solen gouldi}       | 0.00  | 124.16| 0.0000      | 0.0099      | 0.0000      | 0.2871      |
| \textit{Mytilus edulis}     | 0.00  | 124.16| 0.0000      | 0.0099      | 0.0000      | 0.2871      |
| \textit{Scapharca subcrenata} | 14.61 | 137.27| 0.0023      | 0.0120      | 0.0338      | 0.3174      |

LB means lower bound values, UB means upper bound values.
(ARfD) as a reference limit for chronic dietary exposure assessment. The chronic exposure value ranged from 0.0009–0.0107 μg STX eq. kg\(^{-1}\) bw day\(^{-1}\) using mean shellfish consumption data, a level much lower than the ARfD. To assess the chronic dietary exposure in a more conservative way, we used \(P_{99}\) shellfish consumption data to exaggerate the chronic dietary exposure level, the results of which were 0.0251–0.31 μg STX eq. kg\(^{-1}\) bw day\(^{-1}\), values that were also lower than the recommended ARfDs.

There are limitations in this study, including the limited amount of sample contamination data; samples collected from upper distributor Buji seafood wholesale market instead of retail stores that close to consumers; outdated (SZFCS 2008) local dietary survey data and population data (9.5 million vs. 12.5 million today); uncertainties associated with dietary exposure assessment, such as the accuracy of consumption data; using 0 and 1/2 LOD to substitute ND values as the lower and upper bounds; and the lack of a proper reference for chronic exposure assessment, which could cause the outcomes to be under- or over-estimated.

Ongoing studies should be conducted on the diet, nutrition, and health status of the Shenzhen population, with dietary PST exposure assessments based on contemporary population figures and stratified consumption data. Since species like Solen gouldi and Mytilus edulis are marketed in only specific months, periodic long-term toxin screening studies are needed to develop an in-depth picture of temporal changes in shellfish contamination. Studies to understand the habitats of shellfish species prone to high concentrations of PSTs are also needed.

### Conclusions

We found that none of the seafood samples contained levels of total PSTs that exceeded international regulation limits. However, dietary exposure results indicated that Shenzhen residents are at risk for acute paralytic shellfish toxins poisoning. Local authorities should strengthen the screening of shellfish for PSTs before they are placed on the market, especially in spring and winter, thereby ensuring product quality and control of high-risk species, notably Chlamys nobilis, Crassostrea rivularis, Perna viridis, among other species. Meanwhile, the Shenzhen PST exposure levels determined in this study might serve as a basis for establishing local limit values for shellfish consumption.

**Author contribution** YZ: writing-original draft preparation, visualization, formal analysis. SL: methodology. JZ: data curation. JZ: project administration, methodology. ZW: methodology, data curation. LP: methodology, data curation. BH: conceptualization, methodology. KH: data curation. XC: methodology. QZ: data curation. TJ: methodology, funding acquisition. JL: project administration, conceptualization, methodology, supervision, funding acquisition.

**Funding** This work was supported by the Shenzhen Basic Research Plan [JCYJ20180508152311822], the National Key Research and Development Program of China (2018YF100201, 2019YFC0407900), Shenzhen Key Medical Discipline Construction Fund (SZXK069), and Sanming Project of Medicine in Shenzhen (SZSM201611090).

**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate** No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species. Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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