A case of paralytic shellfish poisoning caused by consumption of visceral balls from geoduck Panopea japonica in Japan

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Abstract:

In the end of March 2018, an unprecedented food poisoning incident due to ingestion of the visceral balls of geoduck *Panopea japonica* occurred in Japan. The patient, presented with symptoms of numbness on the lips and general weakness, was diagnosed as paralytic shellfish poisoning (PSP). The patient immediately treated with the mechanical ventilation recovered and left the hospital after 3 days treatment. Saxitoxins (STXs) in the plasma and urinary samples collected from the patient on the first and second day after hospitalization were analyzed by ultra high-performance liquid chromatograph coupled with tandem mass spectrometer (UHPLC/MS/MS) and liquid chromatograph with post-column fluorescent detector (LC/FLD). The STXs levels of 499.1 and 6.0 μg/L of STX dihydrochloride equivalent (STX·2HCl eq.) were quantitated by LC/FLD in the urinary samples on the first and second day, respectively. In addition, geoducks harvested from the same areas of the PSP causative specimens after the incident were analyzed by LC/FLD, and the results showed the level of STXs in their whole bodies of the geoducks exceeding 0.8 mg STX·2HCl eq./kg which is the maximum levels of STX in CODEX STAN 292-2008. Prominent toxins in STXs that detected in urinary and geoduck samples and identified by UHPLC/MS/MS and LC/FLD were gonyautoxin-1+4 (GTX1+4). These results concluded that the incident was the food poisoning due to STXs accumulated in the geoducks. This is the first PSP case caused by consumption of geoducks in Japan. This is also the first PSP case that causative toxins are detected in urinary samples of patients involved in PSP in Japan.

Keywords: paralytic shellfish poisoning, saxitoxins, geoduck, UHPLC/MS/MS, LC/FLD, urine

1. Introduction

Saxitoxins (STXs) are a kind of marine biotoxins with neurotoxicity (Wiese et al., 2010, Watanabe et al., 2013), which are produced by certain dinoflagellates (Beppu et al., 2008; Natsuike et al., 2017a, 2017b) and then accumulated by filter-feeder bivalves and/or their predators (Okumura et al., 1994; Bricelj and Shumway, 1998; Oikawa et al., 2004, 2005; Numano et al., 2019). Consumption of bivalves contaminated with STXs over a certain level can cause reversible binding of the toxins to voltage-gated sodium channels, impeding the transmission of the nerve signals, causing paralytic symptoms at various sites of the body, occasionally even resulting in fatal (Cestèle and Catterall, 2000).

Monitoring programs for marine biotoxins in shellfish in each country including Japan have well worked and, as far as we know, the paralytic shellfish poisoning (PSP) by consumption of commercially available bivalves has hardly been reported in recent years in Japan, although STXs have been detected from marketed bivalves through international trade (Campbell et al., 2013; CFIA, 2020). However, several cases of PSP have occurred by consumption of contaminated bivalves collected in recreational clamming. Most of the food poisoning incidents in Japan had been disclosed through the website of local authorities (Akaeda et al., 1998; Toda et al., 2012; Inoue et al., 2015; Yamada et al., 2019).

Besides mouse bioassay (MBA) (AOAC, 1995) which is the official testing method for STXs in many countries, various methods have been developed and used to verify STXs in clinical specimens such as urine, plasma, and the causative foods left over. For example, enzyme linked immunosorbent assay (ELISA) has been applied as an initial screening of PSP diagnosis in Portugal (Carvalho et al., 2019). Liquid chromatography (LC) coupled with pre- or post-column fluorescent detection (LC/FLD) had widely been applied for shellfish and/or clinical matrices in the food poisoning incidents (Gessner et al., 1997; Akaeda et al., 1998; García et al., 2004, 2005; DeGrasse et al., 2014; Coleman et al, 2018; Carvalho et al., 2019). Recently, triple quadrupole tandem mass spectrometry coupled to ultra-high

83 performance liquid chromatography (UHPLC/MS/MS) using a column with a hydrophilic interaction
84 (HILIC) for simultaneous detection of STXs and tetrodotoxin (TTX) has been developed (Boundy et
85 al., 2015) and validated for fresh bivalve molluscs in single- and multi-laboratories (Turner et al., 2015,
86 2020). One of the LC/MS/MS methods was modified and validated for detection of gonyautoxins
87 (GTXs) in human urine (Coleman et al., 2017).
88 An unprecedented food poisoning due to ingestion of geoducks occurred in the western part of
89 Japan in March 2018. We hypothesized that the unprecedented geoduck food poisoning was caused
90 by STXs, and plasma and urine samples of the patient were analyzed by the UHPLC/MS/MS and
91 LC/FLD to identify the causative STXs in the geoducks. In addition, geoducks collected from the same
92 coastal area of causing geoducks harvested were also analyzed to determine the toxins responsible for
93 the food poisoning incident.
94
95 **2. Materials and methods**
96 *2.1. Chemical Reagents*
97 Acetonitrile (MeCN) of LC/MS grade was purchased from Kanto Chemical Co., inc (Tokyo,
98 Japan). Phosphoric acid (85%), acetic acid (AcOH) and 0.1 M hydrochloric acid (HCl) were from
99 FUJIFILM wako pure chemicals (Osaka, Japan). Ion-pairing reagent: 1-heptanesulfonate sodium salt
100 was from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Ammonium hydroxide (25%) and
101 graphitized carbon cartridges of supelclean ENVI-Carb 250 mg/3 ml were purchased from Sigma-
102 Aldrich Japan (Tokyo, Japan). Milli-Q water with a resistance value of 18.2 MΩ·cm and a TOC value
103 of 3 ppb or less was prepared by Milli-Q reference system (Merck, Darmstadt, Germany). In-house
104 standards of C1+2, GTX-1-5, decarbamoylgonyautoxin-2+3 (dcGTX2+3), neosaxitoxin (NEO),
105 decarbamoylsaxitoxin (dcSTX), and STX were used. Concentrations of these toxins were calibrated
106 using the standard prepared by Oshima (Tohoku University, Japan) with elemental analysis (Oshima,

1995).

2.2. Biological specimens

Five individuals of geoducks *Panopea japonica* were provided from a local fisherman on 10 days after the food poisoning incident. These live bivalves were collected between 26th to 28th March 2018 from the same coastal area as the causing specimens were harvested and were kept in a tank with free-flowing natural seawater until sending to our laboratory. The causative dinoflagellate *Alexandrium catenella* (Group I) (former *A. tamarense*) was observed in the natural seawater from the bivalve harvested area.

Urinary and plasma samples from the patient were provided with the agreement by the patient. On 29th March, body fluids of plasma (4 ml) and urine (10 ml) were collected at 19:00, three hours after ingestion of the causative geoducks. On 30th March, the body fluids were collected at 6:00, fourteen hours after ingestion of the causative geoducks.

2.3. Extraction of STXs and TTX in geoduck, urinary and plasma samples

A whole body of geoducks was dissected to siphons, visceral balls, and the other viscera (Fig.1), and dissected tissues were individually weighed. The siphon tissues were divided into muscles and integuments including mantles. A tissue homogenate of the individual geoduck was mixed with an equivalent volume of 0.1 M HCl aq. and the STXs were extracted according to the AOAC official testing method 959.08 (AOAC, 1995).

Fig. 1. Geoducks *Panopea japonica* collected from the same area as the causative geoducks implicated in the food poisoning (left) and the dissected parts (right)

The toxins in urinary and plasma samples (2 ml each) were extracted by adding an equivalent volume of 0.1 M HCl aq. The pH of the extract was measured being acidic using a pH test paper. After heating at 97 °C for 5 min, the extract was cooled down to room temperature and centrifuged at 3,000 rpm for 10 min. Three ml of the supernatant was used for the analysis by LC/FLD and the remaining supernatant was subjected to the UHPLC/MS/MS analysis.

2.4. LC/FLD analysis for the detection of STXs

STXs in the geoduck's extracts were analyzed using the LC/FLD method reported by Oshima (1995) with slight modifications. Briefly, 3 ml of the supernatant obtained by the AOAC official method 959.08 was passed through Sep-pak C18 plus cartridge (Waters, Milford, MA) preconditioned with 10 ml of methanol and Milli-Q water. After discarding the first 1.5 ml eluate, the following 0.5 ml of the eluate was recovered to a centrifugal filter unit (10 kDa cut-off, regenerated cellulose membrane, Amicon-ultra 0.5 ml). After centrifugation, the filtrate was used for LC/FLD analysis.

2.5. UHPLC/MS/MS analysis for the detection of STXs and TTX

STXs and TTX in the urine and the plasma were extracted and determined using the modified

LC/MS/MS method by Watanabe et al. (2019) which was originally reported by Boundy et al. (2015). Briefly, toxins in 2 ml of urinary samples were extracted by heating with an equivalent volume of 0.1 M HCl aq. Five µl of ammonium hydroxide was added to 1 ml of the extract, and 0.4 ml aliquot were loaded on the graphitized carbon cartridge (ENVI-Carb 250 mg/3 ml) preconditioned with 3 ml of 20 % MeCN containing 1 % AcOH and 3 ml of 0.025 % ammonium hydroxide. The cartridge was washed with 0.7 ml of Milli-Q water and then toxins were eluted with 2 ml of 20 % MeCN containing 1 % AcOH from the cartridge. The eluate was diluted 4-fold with MeCN. The plasma sample was prepared using the same procedure as urinary samples.

The toxins were analyzed by UHPLC/MS/MS; Shimadzu NexeraXR liquid chromatograph equipped with SCIEX QTRAP4500 mass spectrometer. Selected reaction monitoring (SRM) transitions of STXs and TTX were carried out according to those reported by Boundy et al (2015). Due to the lack of reference materials of metabolites of STXs (so-called M-toxins), the metabolites were not analyzed in the present study. The data processing software Analyst 1.6.2 (SCIEX, Framingham, USA) was used for quantitation of the toxins. Toxicity equivalency factors were referred to the molar toxicities reported by Oshima (1995).

The MS/MS product ions of GTX1+4 were obtained using QTRAP4500 instrument with the following MS/MS parameters; Product of *m/z* 412.1 (mass range: *m/z* 50 - *m/z* 450), Resolution Q1: Unit, Scan rate: 10,000 Da/s, Q0 trapping: ON, Collision energy of 29 eV, Ion source temperature of 500 °C. GS1: 70 psi, GS2: 90 psi, Declustering potential: 86 V.

3. Results and discussion

3.1. Case reports

The patient was a 31-year-old male who worked as a staff member of sushi restaurant. On 29th March 2018, the patient ate large quantity of the visceral balls from geoducks for early dinner at 16:00.

The patient declared that he exclusively ate the visceral balls, which had been unoffered as a food stuff and irrevocably discarded in the restaurant, for meals on the day. Subsequently, the patient was hospitalized because of numbness in the perioral region and general weakness were developed around 19:00. In the hospital, progress of paralysis and pronounced respiratory difficulty were recognized and the patient was immediately intubated and controlled by mechanical ventilator. On 30th March 2018, the paralysis in the patient was improved, and the tube and the ventilator were withdrawn. On 4 days after admission, the patient was discharged.

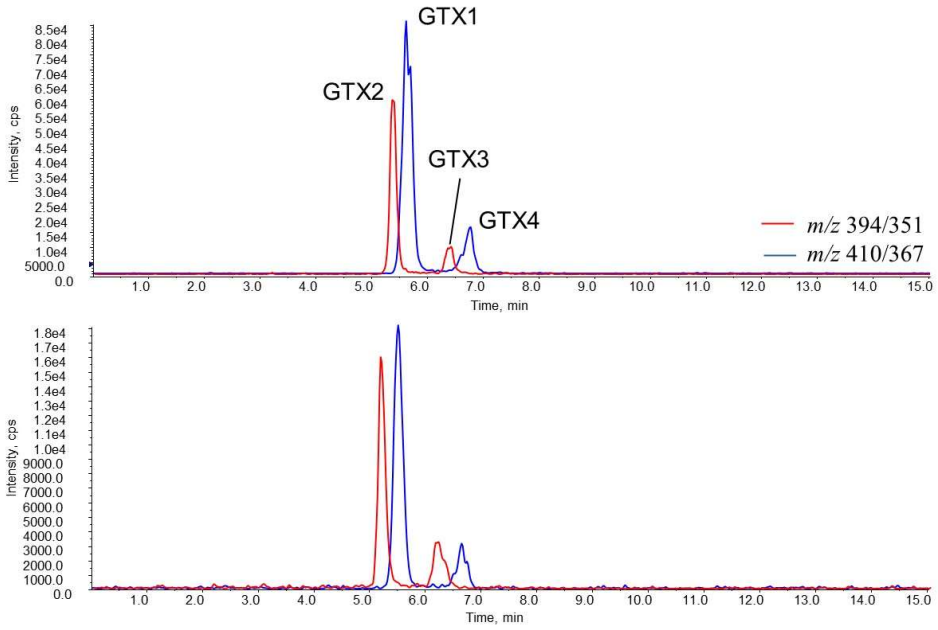
3.2. STXs and TTX in the urinary and plasma samples from the patient

The levels of STXs and TTX in urinary and plasma samples from the patient were quantitated by UHPLC/MS/MS and LC/FLD analyses (Table 1). The toxin content in the urinary sample on 29th March quantified by LC/FLD and UHPLC/MS/MS was 499.1 and 476.3 μg/L of STX dihydrochloride equivalent (STX diHCl eq.), respectively. The dominant toxins identified by LC/FLD and UHPLC/MS/MS (Fig. 2, 3) were GTX1+4.

Table 1 Toxin levels (μg/L of STX diHCl eq.) and ratio (%) of urinary samples from the patient.

Toxins	LC/FLD		UHPLC/MS/MS	
	29th March	30th March	29th March	30th March
C1	1.0 (0.2)	0.0 (0.0)	0.8 (0.2)	0.0 (0.0)
C2	4.7 (0.9)	0.0 (0.0)	3.7 (0.8)	0.0 (0.0)
GTX1	310.8 (62.3)	0.0 (0.0)	287.8 (60.4)	0.0 (0.0)
GTX2	53.5 (10.7)	2.4 (40.0)	63.6 (13.3)	1.8 (100.0)
GTX3	47.5 (9.5)	3.6 (60.0)	49.6 (10.4)	0.0 (0.0)
GTX4	78.4 (15.7)	0.0 (0.0)	70.6 (14.8)	0.0 (0.0)
GTX5	0.0 (0.0)	0.0 (0.0)	0.2 (0.1)	0.0 (0.0)
dcGTX2	2.4 (0.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
dcGTX3	0.8 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
neoSTX	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
dcSTX	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
STX	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
TTX	N.T.	N.T.	0.0 (0.0)	0.0 (0.0)
Total	499.1 (100.0)	6.0 (100.0)	476.3 (100.0)	1.8 (100.0)

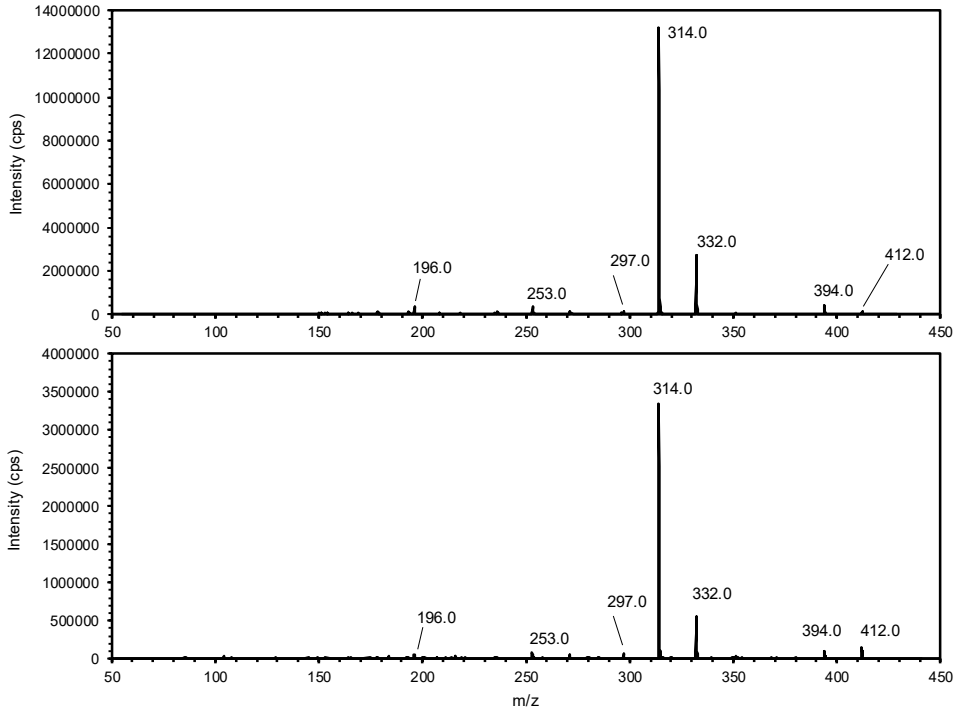
N.T.: not tested



188

189 **Fig. 2**. Selected-reaction monitoring (SRM) chromatograms of GTX group's standards on negative

190 ion mode (upper) and urinary sample on the first day after hospitalization (bottom).



191

192 **Fig. 3**. MS/MS spectra of GTX4 standard by Enhanced Product Ion scan (upper) and urinary sample

193 on the first day after hospitalization (bottom). Collision energy (CE); 29 eV.

194

The sum of GTX1-4 of the urine sample collected on 29th March quantified by LC/FLD accounted for 75.0 mol % and 98.3 % of the total toxicity, respectively. Among them, the total amounts of GTX1+4 of the same sample quantified by LC/FLD accounted for 49.0 mol % and 78.1 % of the total toxicity, respectively. The results of UHPLC/MS/MS analysis were similar to those obtained by LC/FLD. TTX was not detected in the samples. Therefore, GTXs, especially GTX1+4, were determined as the causative toxins in this food poisoning incident. The toxicity level in the urinary sample collected on the next day after hospitalization was drastically decreased to 6.0 μg/L of STX diHCl eq in LC/FLD and 1.8 μg/L of STX diHCl eq in UHPLC/MS/MS analysis. In contrast to the urinary samples, STXs were not detected in the plasma samples by both LC/FLD and UHPLC/MS/MS methods.

To our knowledge, instrumental detection of STXs in human body fluids, especially urinary sample, was firstly accomplished by using continuous-flow fast-atom bombardment mass spectrometry (CF/FAB/MS, Mirocha et al., 1992) with the detection limit of 200 pg of STX. Since the CF/FAB/MS method was developed for quantitative analysis rather than for qualitative, Gessner et al. (1997) reported application of LC/FLD for clinical use, together with sodium channel blocking activity using rat skeletal muscle membranes. Since then, the LC/FLD method have been applied to clinical specimens for qualitative/quantitative analysis (García et al., 2004, 2005; DeGrasse et al., 2014; Carvalho et al., 2019). Since HPLC/MS/MS method was firstly applied for urinary sample by Johnson et al. (2009) utilizing isotopic labelled STXs, several studies using HPLC/MS/MS methods for clinical samples have been reported (Knaack et al., 2016; Coleman et al., 2017, 2018). In the present study, the causative toxins of the food poisoning incident due to ingestion of geoduck was identified using both instrumental analyses of LC/FLD and UHPLC/MS/MS. The UHPLC/MS/MS method used in the study with a graphitized carbon cartridge as pretreatment of sample has been validated for live bivalve molluscs (Boundy et al., 2015; Turner et al., 2015, 2020). In this study, we applied the UHPLC/MS/MS

219 method for clinical purpose. As results, the data obtained by the method agreed well with that by
220 LC/FLD, demonstrating that this method is applicable to clinical use for urinary sample.
221
222 *3.3. STXs distributed in the body of geoducks*
223 The weight of each tissue of the geoducks is shown in Table 2. The whole-body weights ranged
224 from 152 g to 246 g, and the visceral balls comprised 27% (as mean) of the whole-body weights.
225 Siphon tissues are a main edible part of geoducks and accounted for over 50% of the total weights.
226
227 Table 2 Weights (g) of various tissues from geoducks.

Individuals	Siphon		Visceral	The other	Whole
	Muscle	Integument	ball	viscera	body
1	76.2	21.9	44.4	10.3	152.8
2	116.4	21.1	65.6	19.2	222.3
3	141.0	25.1	66.6	13.7	246.4
4	114.1	17.1	49.0	19.7	199.9
5	100.5	18.0	45.3	18.0	181.8
Mean	109.6	20.6	54.2	16.2	200.6
s.d.	23.7	3.2	11.0	4.0	36.1

228
229 The toxin concentrations (μg/kg of STX diHCl eq.) in various tissues of geoducks were
230 quantitated by LC/FLD (Table 3). The toxin levels in whole bodies were far over the regulatory limit
231 (800 μg/kg of STX diHCl eq.) in CODEX STAN 292-2008. Among tissues, the other viscera gave the
232 highest toxin level (59,032 μg/kg of STX diHCl eq.) as mean value, followed by visceral balls (28,031
233 μg/kg of STX diHCl eq.) and siphon tissues (1,140 μg/kg of STX diHCl eq. as the mean of muscle
234 and integument).
235
236

Table 3 Contamination levels (μg/kg of STX diHCl eq.) of saxitoxins in various tissues from the geoducks determined by LC/FLD.

Individuals	Siphon		Visceral ball	The other viscera	Whole body
	Muscle	Integument			
1	1,691	789	26,548	68,253	13,272
2	1,729	1,249	23,207	80,954	14,858
3	1,204	761	22,486	53,654	9,830
4	718	1,000	34,719	57,481	14,667
5	1,397	862	33,196	34,816	12,580
Mean	1,348	932	28,031	59,032	12,931

Total toxin amounts in various tissues from geoducks were calculated by multiplying the toxin concentrations in μg/kg of STX diHCl eq. by the corresponding tissue weight (Table 4). The total toxin amounts in whole bodies were reached to 2 - 3 mg STX diHCl eq., which corresponds to human fatal levels for an adult individual. The visceral balls gave the highest toxin amounts in average (1,481 μg STX diHCl eq.), followed by the other viscera (950 μg STX diHCl eq.) and siphon (82 μg STX diHCl eq.). As the patient declared to eat large quantity of visceral balls, it can be expected that the toxin amounts could be reached to fatal levels (2 - 3 mg STX diHCl eq.) in human.

Table 4 Amounts of saxitoxins (μg STX diHCl eq.) and ratio (%) in various tissues from the geoducks determined by LC/FLD.

Individuals	Siphon		Visceral ball	The other viscera	Total
	Muscle	Integument			
1	129 (6)	17 (1)	1,179 (58)	703 (35)	2,028 (100)
2	201 (6)	26 (1)	1,522 (46)	1,554 (47)	3,303 (100)
3	170 (7)	19 (1)	1,498 (62)	735 (30)	2,422 (100)
4	82 (3)	17 (1)	1,701 (58)	1,132 (39)	2,932 (100)
5	140 (6)	16 (1)	1,504 (66)	627 (27)	2,287 (100)
Mean	144 (6)	19 (1)	1,481 (58)	950 (36)	2,594 (100)

252 Table 5 Toxin profiles (nmol/g) and ratio (%) of various tissues from the geoducks determined by
253 LC/FLD.

Toxins	Siphon		Visceral ball	The other viscera
	Muscle	Integument		
C1	0.6 (11.2)	0.6 (10.6)	9.3 (8.9)	1.2 (0.5)
C2	0.7 (11.8)	0.6 (9.6)	1.8 (1.7)	1.5 (0.6)
GTX1	2.1 (37.2)	0.5 (8.0)	53.2 (51.1)	56.5 (23.7)
GTX2	0.4 (6.6)	2.4 (42.2)	18.6 (17.9)	80.7 (33.7)
GTX3	0.2 (3.8)	1.5 (26.2)	4.7 (4.5)	36.9 (15.5)
GTX4	1.7 (29.4)	0.2 (3.3)	13.5 (13.0)	39.9 (16.7)
dcGTX2	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	1.1 (0.5)
dcGTX3	0.0 (0.0)	0.0 (0.0)	0.4 (0.4)	0.4 (0.2)
neoSTX	0.0 (0.0)	0.0 (0.0)	1.7 (1.7)	13.0 (5.5)
dcSTX	0.0 (0.0)	0.0 (0.0)	0.3 (0.3)	0.1 (0.1)
STX	0.0 (0.0)	0.0 (0.0)	0.6 (0.6)	7.6 (3.2)
Total (n=5)	5.7 (100.0)	5.8 (100.0)	104.2 (100.0)	238.9 (100.0)

254

255 The toxin profiles (nmol/g) and ratio (%) of various tissues from geoducks obtained by LC/FLD
256 are shown in Table 5. A group of GTX was found to be highly contributed to potential toxicity. Among
257 the toxins detected in the urinary sample from the patient, GTX1+4 were the dominant components
258 (Table 1), demonstrating that causative food implicated in the PSP case was the visceral balls of
259 geoduck considering from the similar toxin profiles between the urinary and the geoduck visceral ball
260 samples.

261 The toxin profiles of integuments in siphon tissue and the other viscera, where GTX2+3 were the
262 dominant toxins, were different from that of the siphon muscle and visceral ball, where GTX1+4 were
263 the dominant toxins. The results suggest that GTX1+4 would be chemically or enzymatically
264 converted to GTX2+3 in the process of toxin transportation between muscle and integument, or the
265 bivalve might have some functions of the selective accumulation of GTX2+3 in the integument. As
266 one of other pathways, GTX1+4 might be metabolized and discharged faster than GTX2+3 through
267 the hepatic metabolism pathway (Qiu et al., 2018).

268 Generally, bivalves accumulate most of the toxins in the digestive glands, except for specific
269 species like Alaskan butter clam *Saxidomus giganteus* (Schantz et al., 1975; Smolowitz and Doucette,

1995) which can retain the toxins in a siphon tissue. Geoducks are known to accumulate less amounts of PSP toxins in a siphon tissue compared to other viscera including digestive gland. It is surprising that the toxin levels in siphon tissues were beyond the regulatory level.

In the present study, we identified STXs as causative toxins in the food poisoning incident due to ingestion of visceral balls of geoduck *Panopea japonica* in Japan. Japanese littleneck clam *Ruditapes philippinarum* (Hashimoto et al., 1950), Akazara-gai, a kind of scallop, *Chlamys farreri Akazara* (Kawabata et al., 1962; Iioka et al., 1962), cultured and wild oysters *Crassostrea gigas* (Onoue et al., 1980; Akaeda et al., 1998; Inoue et al., 2015), ascidian *Holocynthia roretzi* (Nagashima et al., 1984), juvenile scallop *Patinopecten yessoensis* (Inoue et al., 1992), and wild mussels *Mytilus galloprovincialis* (Yamada et al., 2019) have been reported as the causative marine organisms for the past PSP incidents in Japan. The food poisoning due to ingestion of geoducks is an unprecedented case in Japan.

Shellfish has been contaminated by the bloom of toxic dinoflagellates such as *Alexandrium* spp. or *Gymnodinium catenatum* in worldwide. Generally, bivalves like scallops, clams and mussels accumulate the toxins exclusively in their digestive glands. Contamination levels of adductor muscles as edible part, mantles, or feet etc. are far lower than those in the digestive glands. Terrazas et al. (2017) investigated several marine organisms from Chilian coastal waters and reported that the feet in gastropods (loco and top shell) retain the toxins with high concentrations. Additionally, Yamamoto and Oikawa (2024) studied the distribution of toxins in several tissues of the arch shells *Anadara broughtonii* and Japanese cockles *Fulvia mutica* exposed to toxic dinoflagellate *Alexandrium catenella.* They showed mantles and adductor muscles in the arch shells exhibited high toxicities, followed by viscera or muscles, and gills. Differences in toxin profiles between the causative microalgae and bivalves were also observed, suggesting the presence of hemo-proteins catalyzing the reductive conversion of GTX1+4 to GTX2+3 (Sato et al., 2014).

294 Medina-Elizalde et al. (2018) reported transformation and depuration of STXs in the geoduck
295 clam *Panopea globosa* from the northern Gulf of California, along with occurrence of toxic
296 dinoflagellate *Gymnodinium catenatum*. According to the report, the visceral mass of the geoduck
297 clam had significantly higher toxicity than the siphon tissue, which tended to accumulate and retain
298 the toxins for a long time. Moreover, Medina-Elizalde et al. (2018) reported that the toxicity in the
299 siphon will exceed the regulatory limit when the toxicity in the visceral mass reaches 18,424 μg/kg of
300 STX eq. The results in this study (Table 3) well agreed with the results reported by Medina-Elizalde
301 et al. (2018).
302
303 **4. Conclusions**
304 We identified the causative toxins of the unprecedented food poisoning due to contaminated
305 geoduck *Panopea japonica* using patient's urinary and plasma samples. The dominant toxins in the
306 urinary sample were demonstrated to be GTX1+4. Saxitoxins were distributed in all tissues. The
307 highest concentrations of STXs were found in the other viscera including heart and kidney etc. while
308 the greatest amounts of STX were found in visceral balls due to occupying the largest weight of the
309 tissues. STXs exceeding the regulatory limit was detected from geoduck from the affected coastal area
310 and intake of the toxin by the patient being close to fatal. Due to the lack of reference materials of
311 metabolites of STXs (so-called M-toxins), these metabolites were not analyzed in the present study.
312 Since the presence of M-toxins in geoducks has already been reported by Medina-Elizalde et al. (2018),
313 we are planning to analyze M-toxins in the contaminated geoduck samples once we have obtained
314 reliable reference materials.
315
316 **Author contributions**
317 Conceptualization; R.W., H.O., T.T., K.M., T.S., Data curation; R.W., H.O., K.M., M.O., H.U.,

S.N., R.M., Investigation; R.W., H.O., T.T., Methodology; R.W., H.O., Project administration; T.S., T.T., Resources; T.T., K.M., Supervision; H.O., Roles/Writing - original draft; R.W., H.O., T.S., and Writing – review & editing; All authors. All authors have approved the submission of the manuscript to the journal.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement

Patient consent was obtained from the patient for publication of the case report. Patient's privacy is respected in all.

Data availability

All data that supports the findings of this study is available within the article.

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