



## Baseline

## Spatiotemporal distribution characteristics of yessotoxins and pectenotoxins in phytoplankton and shellfish collected from the southern coast of South Korea



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## ABSTRACT

The distribution characteristics of lipophilic marine biotoxins (LMTs), such as yessotoxins (YTXs) and pectenotoxins (PTXs) in phytoplankton, mussels, and commercial seafood were determined for the southern coast of South Korea. *Gonyaulax spinifera* and *Dinophysis acuminata*, which are the causative microalgae of YTXs and PTXs, were recorded during summer. Homo-YTX and PTX-2 were predominantly detected in phytoplankton (max: 5.7  $\mu\text{g g}^{-1}$  ww), whereas only YTXs were detected in mussels (max: 1.1  $\mu\text{g g}^{-1}$  ww). LMT concentrations in mussels were positively correlated with those in phytoplankton. However, there was a 1-month time gap in maximum LMT concentrations between mussels and phytoplankton. Homo-YTX was detected in commercial seafood, including red scallop and comb pen shell. However, homo-YTX concentrations in shellfish were below the recommended value of the European Food Safety Authority (3.75 mg YTX equivalents  $\text{kg}^{-1}$ ); thus, the consumption of this seafood was not considered to be a significant risk for human health.

Harmful algal blooms (HABs), which can adversely affect marine ecosystems, occur globally, and are increasing in frequency, associated with climate change (Gobler et al., 2017), eutrophication, and expansion of coastal aquaculture (Hallegraeff et al., 2021). Some harmful microalgae naturally produce marine biotoxins that accumulate in filter-feeding organisms, such as bivalves (Liu et al., 2019; Reguera et al., 2012). Marine biotoxins can be transformed to produce many toxic metabolites in bivalves (Yasumoto, 2005), which threaten human health when contaminated shellfish are consumed.

Marine biotoxins are generally classified into hydrophilic and lipophilic biotoxins according to their chemical properties, such as polarity (Chen et al., 2017). More than 200 marine biotoxins have been identified so far (Gerssen et al., 2011), and more than 90% of marine biotoxins belong to lipophilic marine biotoxins (LMTs) (Wang et al., 2015). Representative LMTs include okadaic acid (OA), dinophysistoxins (DTXs), yessotoxins (YTXs), pectenotoxins (PTXs), brevetoxins (BTXs), and azaspiracids (AZAs). Hydrophilic marine biotoxins include domoic acid (DA) and saxitoxin (STX). LMT poisoning of shellfish has been

reported in the coastal areas of various countries, including China, Italy, and Norway (Draisci et al., 1999; Liu et al., 2019; MacKenzie et al., 2002; Ramstad et al., 2001). Cases of LMTs contamination in shellfish are continuously increasing, with areas of biotoxin contamination gradually expanding (Hallegraeff et al., 2021). Consequently, the risk of seafood to human health following consumption is of great concern.

Along the southern coast of Korea, HABs have been reported over the last four decades (Baek et al., 2020; Kim et al., 2019). HABs are caused by *Prorocentrum* spp., *Gyrodinium* spp., *Gymnodinium* spp., *Heterocapsa* spp., *Karenia* spp., *Pseudo-nitzschia* spp., *Thalassionema* spp., and *Cryptomonas* spp. (Baek et al., 2020; Kim et al., 2019). In the South Sea, cultured shellfish frequently die due to high seawater temperature and hypoxic conditions. The growth of shellfish is inhibited by changes to the species composition of phytoplankton, resulting in the number of individuals declining (Lee et al., 2019). The South Sea accounts for 95% of shellfish culture production in South Korea, including scallops, oysters, cockles, and mussels (Lee et al., 2019). Monitoring is regularly performed for certain shellfish toxins, including paralytic shellfish

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poisoning (PSP), diarrhetic shellfish poisoning (DSP), and amnesic shellfish poisoning (ASP) (MFDS, 2009). There have been investigations on the contamination status of unmanaged biotoxins in domestic seafood, but cases of seafood contamination from these toxins have not yet been reported on the southern coast of Korea.

YTXs and PTXs are well-known LMTs. The causative microalgae of YTXs are *Gonyaulax spinifera*, *Protoperidinium reticulatum*, and *Lingulodinium polyedrum* (Paz et al., 2007; Satake et al., 1999; Suzuki et al., 2007). In comparison, PTXs are mainly produced by *Dinophysis fortii*, *D. acuminata*, and *D. caudata* (Li et al., 2017; Pizarro et al., 2008; Reguera et al., 2014). YTXs were previously included in the DSP group (Paz et al., 2008); however, recent studies reported that YTXs do not cause diarrhea, but do affect the myocardium (Ferreiro et al., 2016; Paz et al., 2008). PTXs are also hepatotoxins rather than causing diarrhea (Terao et al., 1993). The European Food Safety Authority (EFSA) recommended setting minimum thresholds for YTXs and PTXs, to prevent accidents caused by the ingestion of shellfish contaminated with them (EFSA, 2008, 2009); however, regulations on these toxins have not yet been established in South Korea. At present, as the sea surface temperature increases due to global climate change, the distribution range of subtropical harmful microalgae is extending (Gobler et al., 2017). Consequently, exotic microalgae might appear in Korean coastal waters, and unmanaged shellfish toxins could occur. However, to date, LMTs (such as YTXs and PTXs) are rarely monitored in Korea. As a result, their distribution characteristics are largely unknown.

In the present study, we investigated the spatiotemporal distributions of YTXs and PTXs along the southern coast of South Korea. The specific objectives were to: i) identify the causative microalgae from summer to winter, ii) determine concentrations of YTXs and PTXs in phytoplankton and field mussels, iii) investigate YTXs and PTXs contamination of various commercial shellfish, and iv) assess the potential risk of YTXs and PTXs to human health through seafood consumption.

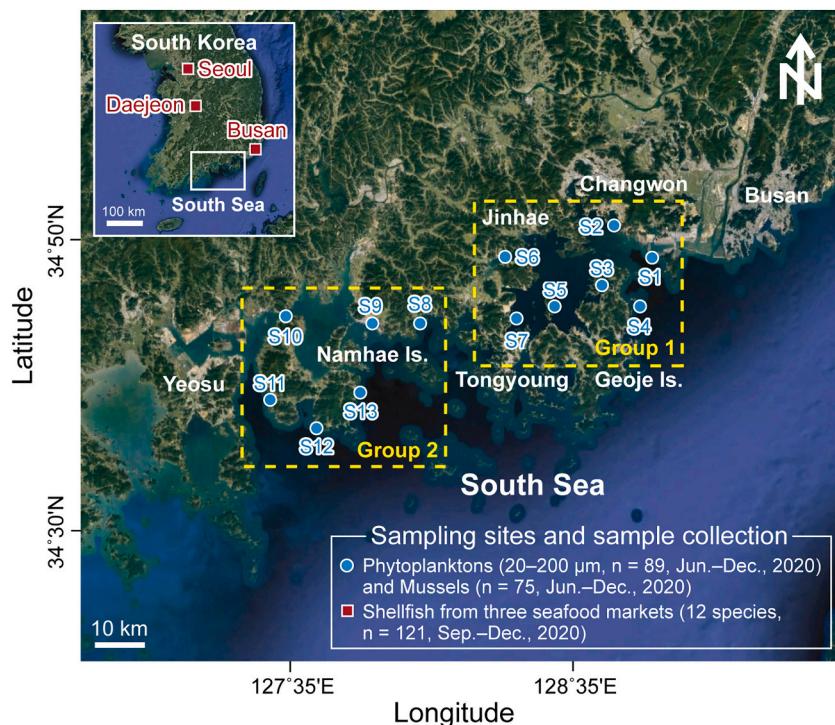
The field investigation and sampling were conducted monthly at 13 sites (Group 1: S1–S7; Group 2: S8–S13) along the southern coast of

Korea from June to December 2020 (Fig. 1). Water quality parameters were measured in situ using a multi-sensor (YSI 6600v2, YSI Inc., Yellow Springs, OH), and included water temperature (WT), salinity, pH, and dissolved oxygen (DO). Dissolved inorganic nutrients ( $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , and  $\text{SiO}_2$ ) were analyzed using an automatic nutrient analyzer (LACHAT Quikchem 8000, Hach Company, Loveland, CO). The concentration was quantified using reference materials for nutrients in seawater (KANSO Technos Co., Osaka, Japan). Chlorophyll-a (Chl.a) was extracted with 90% acetone in the dark for 24 h, and was measured using a Turner-designed fluorometer (Turner BioSystems, Sunnyvale, CA) (Lim et al., 2019).

To analyze the density and species composition of phytoplankton, 500 mL of surface seawater was collected and immediately fixed with Lugol solution (final concentration 3%). The fixed sample in the laboratory was concentrated to 50 mL, transferred to the Sedgewick-Rafter Chamber, and allowed to settle for 10 min. Phytoplankton species were identified using a light microscope. Morphologically distinct species were identified at the species level, and indistinguishable species were identified at the genus level.

Phytoplankton samples (20–200  $\mu\text{m}$  suspended particulate matter (SPM),  $n = 89$ ) were collected using a 20  $\mu\text{m}$  mesh net. Zooplankton and large suspended solids were removed using a 200  $\mu\text{m}$  mesh net. Then, the concentrated phytoplankton (about 150 mL) was filtered with nylon net filters (20  $\mu\text{m}$ , 47 mm, Millipore, Merck, Darmstadt, Germany), and the samples were stored at  $-20^\circ\text{C}$  until analysis. To analyze YTXs and PTXs in phytoplankton, the frozen filter was thawed at room temperature, cut with scissors, and placed in a 15 mL conical tube. The sample was extracted with 3 mL methanol, and was vortex-mixed for 1 min (Liu et al., 2017). After sonication for 5 min, the supernatant was collected by centrifugation at 3500 rpm for 10 min. The same process was repeated two more times, and the extracts were combined (final volume 10 mL). The extract was filtered through a 0.22  $\mu\text{m}$  syringe filter before instrumental analysis.

Mussels ( $n = 75$ ) were collected monthly from the same field sites (S1–S13) as the phytoplankton. More than 20 mussels were collected



**Fig. 1.** Sampling sites of phytoplankton, mussels, and shellfish. Phytoplankton (20–200  $\mu\text{m}$  SPM) and mussels were collected from Sites S1–S13 along the southern coast of South Korea (June to December 2020). Shellfish samples were collected from three seafood markets located in Seoul, Daejeon, and Busan (September to December 2020).

from each site at each sampling event. The mussels were transferred to the laboratory in a cool box, and were stored at  $-20^{\circ}\text{C}$  until analysis. Domestic seafood samples ( $n = 121$ ) were collected monthly from three seafood markets located in Seoul, Daejeon, and Busan from September to December 2020 (Fig. 1). Twelve species of commercial shellfish were collected; namely ark shell, red shell, mussel, corb shell, surf clam, hard clam, purplish clam, marsh clam, comb pen shell, red scallop, manila clam, and oyster. The scientific names and sample information of commercial shellfish are present in Table S1 of the Supplementary Materials. The collected shellfish were transferred to the laboratory, and were stored at  $-20^{\circ}\text{C}$  until analysis.

After removing the shell of the bivalves, the soft tissue was collected and homogenized. More than 20 individuals per sample were pooled. Two grams of the homogenized sample were extracted with 9 mL of 100% methanol and vortex-mixed for 1 min (Orellana et al., 2014). The extract was sonicated in ice water for 10 min, and was then centrifuged at 3500 rpm for 10 min to collect the supernatant. The same process was repeated twice, and the final volume was made up to 20 mL. Solid-phase extraction (SPE) cartridge (Strata-X, 30 mg, 3 mL, Phenomenex, Torrance, CA) was used to remove interfering substances. The SPE cartridge was activated with 3 mL methanol and 3 mL deionized water. After diluting the methanol content of the extract to 20% or less, it was loaded into the cartridge. After loading the sample, the cartridge was washed with 3 mL of 15% methanol and dried under vacuum for 3 min. YTXs and PTXs were eluted with 3 mL methanol containing 1% ammonium hydroxide, and were concentrated to 1 mL under  $\text{N}_2$  gas.

Authentic standards for YTX, homo-YTX, PTX-2, and PTX-11 were obtained from the National Research Council of Canada (Ottawa, ON, Canada) and Sigma-Aldrich (St. Louis, MO). LMTs were analyzed using an Agilent 1290 Infinity II LC system (Agilent Technologies, Santa Clara, CA) coupled with an Agilent 6470 triple quadrupole mass spectrometer MS/MS system (Agilent Technologies). LMTs were separated using a Waters X-Bridge C18 column (3.0 mm  $\times$  150 mm, 5.0  $\mu\text{m}$ ). The mobile phase consisted of (A) 0.05% ammonium hydroxide in water and (B) 0.05% ammonium hydroxide in 90% acetonitrile in water. Target LMTs were analyzed using multiple reaction monitoring (MRM) mode. YTXs and PTXs were detected in negative and positive modes, respectively. Details on MRM transitions and mobile phase gradient conditions are provided in Tables S2 and S3.

A matrix-spiked calibration curve, in the range of 1.0 to 50 ng  $\text{mL}^{-1}$  (six levels), was used to quantify LMTs ( $R^2 > 0.996$ ). Limit of detection (LOD) and limit of quantification (LOQ) were calculated by analyzing a mussel spiked with standard materials (1 ng,  $n = 7$ ). LOD and LOQ were calculated by multiplying the standard deviation of the spiked mussel extract response by 3.143 and 10, respectively. LOD and LOQ ranged from 1.6 to 3.3 ng  $\text{g}^{-1}$  wet weight (ww) and 5.1 to 10.5 ng  $\text{g}^{-1}$  ww, respectively. The recovery rate of each LMT was analyzed for mussels were spiked with standard materials (25 ng), following the procedure described above ( $n = 4$ ). Recoveries of the spike test ranged from 91 to 98% (details in Table S4).

For statistical analysis, IBM SPSS Statistics 26 (Armonk, NY) and R software (Version. 3.6.3) were used. Shapiro-Wilk's normality test was performed to verify the normality of the data, and the data obtained from the present study did not satisfy a normal distribution. The Mann-Whitney test was conducted to confirm the difference in LMT concentrations between Group 1 and Group 2. Spearman's rank correlation and principal component analysis (PCA) were carried out to evaluate significant relationship between LMT concentrations, water quality parameters, and the abundance of causative microalgae. All significance levels were set at 0.05 ( $p$ -value). Values with LMT concentrations below LOD were analyzed using LOD/2. Redundancy analysis (RDA) was performed using CANOCO version 4.5 to investigate the relationship between environmental variables and phytoplankton dynamics. Environmental variables (e.g., DO, pH, WT, salinity, and nutrients) were used in the analysis. Data used for RDA were square root converted and analyzed.

Exposure of YTXs and PTXs was assessed based on the consumption of commercial shellfish. The consumption data of shellfish were estimated from the 7th period of the Korean National Health and Nutrition Examination Survey (KNHANES, 2016–2018) conducted by the Korean Disease Control and Prevention Agency (KDCA, 2020). Consumption data of bivalves were assessed using a 24-h dietary recall questionnaire. Daily food intake was analyzed based on bivalve group. The consumption of red scallops and comb pen shells was calculated. For the exposure assessment of YTX and homo-YTX by intake of bivalves with acute reference dose (ARfD) values, we established four consumption scenarios with the highest detected concentration according to FAO/WHO guidance (FAO/WHO, 2011) and the EFSA report (EFSA, 2008), as follows (Eqs. (1)–(4)):

$$\text{Scenario 1 : Highest detection concentration} \times \text{mean consumption} \quad (1)$$

$$\text{Scenario 2 : Highest detection concentration} \times \text{high consumption I} \quad (2)$$

$$\text{Scenario 3 : Highest detection concentration} \times \text{high consumption II} \quad (3)$$

$$\text{Scenario 4 : Highest detection concentration} \times \text{high consumption III} \quad (4)$$

where mean consumption indicates food consumption by all participants. High consumption I, II, and III indicate food consumption by only consumers, 95th level of food consumption by all participants, and 97.5th level of food consumption by all participants, respectively.

The hazard quotient (HQ%) and hazard index (HI%) were calculated to estimate the exposure level by comparing the estimated daily intake (EDI) with the ARfD value. Based on a previous report (EFSA, 2008), 25  $\mu\text{g}$  YTX equivalent (EQ)  $\text{kg}^{-1}$  body weight was applied as the ARfD for YTX and homo-YTX, because the toxic equivalence factor of YTX and homo-YTX is 1. By considering the Korean mean body weight (60 kg) suggested in the KNHANES, the health-based guidance value (HbGV) of 1500  $\mu\text{g}$  YTX EQs/person/day was utilized. As the sum of HQs for each contaminated bivalve, the HI% for each compound was calculated using Eq. (5) in accordance with the method for mixture-risk assessment of chemical risk factors (Juan-Borrás et al., 2016).

$$\text{HI\%} = \left( \sum_{n=1}^i \text{HQ}_n \% \right) \left[ \text{HQ\%} = \left( \frac{\text{EDI}}{\text{HbGV}} \right) \times 100 \right] \quad (5)$$

The water quality parameters of the southern Korean coast from June to December are shown in Table S5. Notable parameters of seawater quality included variation in salinity and nutrients. In August, salinity was less than 30 psu at all sites. The concentrations of nutrients showed large spatial and temporal variation, with relatively high concentrations being observed in July and August. The average precipitation one week before the sampling campaign was observed to be 30 mm per day, indicating that rainwater had an influence. In addition, freshwater discharge from the Yangtze River reached a maximum of 82,000 tons  $\text{s}^{-1}$  in July 2020. The South Sea is affected by the Tsushima Warm Current during summer and fall, and is affected by freshwater from the Yangtze River after the summer monsoon (Chang, 2003; Isobe, 1999). In addition, nutrient concentrations in seawater are affected by the formation of stratification and inflow of runoff after heavy rainfall (Baek et al., 2015; Lee et al., 2018). These factors seem to be related to variation in water quality. This phenomenon occurs annually in the South Sea of Korea (Lim et al., 2019).

The species composition and density of phytoplankton, as well as LMT concentrations in organisms, were interpreted by dividing the sampling sites into the eastern region near Jinhae Bay (Group 1: S1–S7) and the western region near Namhae Is (Group 2: S8–S13) (Fig. 1). At all sites, phytoplankton included Bacillariophyceae (57  $\pm$  16%), Cryptophyceae (28  $\pm$  21%), Dinophyceae (13  $\pm$  5%), Raphidophyceae (0.49  $\pm$  1.2%), and Dictyochophyceae (0.26  $\pm$  0.34%), supporting previous studies conducted in the South Sea (Baek et al., 2019; Lim et al., 2019, 2021). In general, *Skeletonema* spp. and *Pseudo-nitzschia* spp. occupied

cold seawater with rich nutrient conditions, whereas dinoflagellates occupied warmer water (Fig. S1). The succession from diatoms to dinoflagellates was previously reported to occur in the South Sea during summer (Lim et al., 2019). In the current study, the succession from diatoms to dinoflagellates also occurred during summer; however, the timing of succession differed between Group 1 and Group 2. For example, the relative contribution of Dinophyceae in Group 1 increased from 10% in June to 29% in July. In contrast, in Group 2, its contribution increased from 8.5% in July to 28% in August. In the previous study, phytoplankton communities between Group 1 and Group 2 were statistically distinguished (Baek et al., 2020). The succession of diatoms and dinoflagellates was closely related to the vertical structure of nutrients in the water column (Lim et al., 2019; Baek et al., 2019). In this study, as other environmental factors were similar between the two groups, nutrients were considered to be the major factor controlling the succession of diatoms and dinoflagellates.

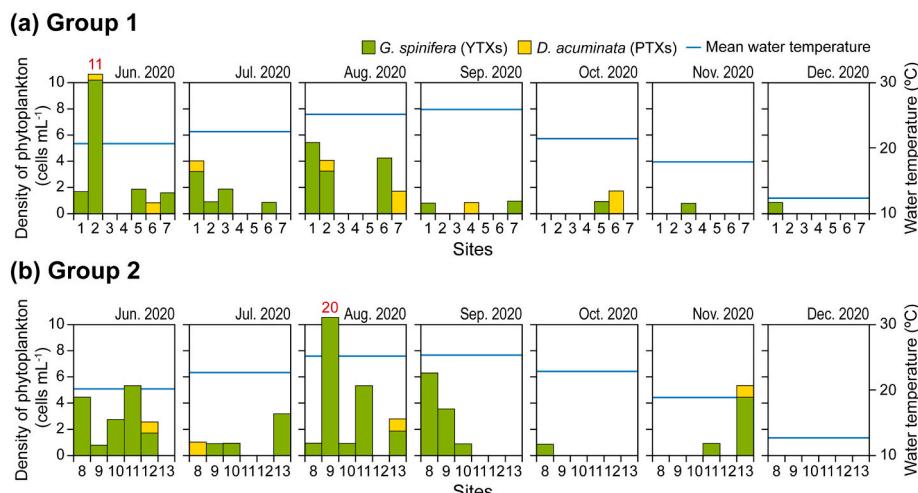
The density of phytoplankton in Group 1 and Group 2 ranged from 52 to 5230 cells  $\text{mL}^{-1}$  and 48 to 2670 cells  $\text{mL}^{-1}$ , respectively (Table S6). There was no significant difference in the density of phytoplankton between the two groups ( $p > 0.05$ ). The maximum density of both groups occurred during summer. After September, the average density decreased to below 1000 cells  $\text{mL}^{-1}$  in both groups. This result was similar to a previous study (Baek et al., 2019). In the South Sea, the species composition and density of phytoplankton showed seasonal variability, and the timing of succession appeared to differ with region. To identify major environmental factors controlling the composition of phytoplankton, statistical analysis was performed on the top 15 species identified in this study. RDA showed that diatoms and dinoflagellates were positively correlated with nutrients. In contrast, salinity was negatively correlated with nutrients (Fig. S2). This result might be attributed to the inflow of nutrient-rich freshwater in summer causing salinity to decrease and phytoplankton levels to increase. Chl.a and water temperature were strongly correlated with certain diatoms, including *Chaetoceros* spp. and *Pseudo-nitzschia* spp., which might be associated with high biomass during summer. Baek et al. (2020) also previously showed a strong positive relationship of these two species with Chl.a, which is a biomass indicator of phytoplankton in the South Sea.

*Gonyaulax spinifera* and *D. acuminata* are known causative microalgae of YTXs and PTXs, respectively, and were found on the southern coast of Korea (Fig. 2 and Table S7). The presence of YTX-producing microalgae has not been previously reported in the coastal waters of Korea; however, it has been confirmed that they exist in the form of cysts in sediments of the South Sea (Yoon and Park, 2017). *Gonyaulax spinifera*

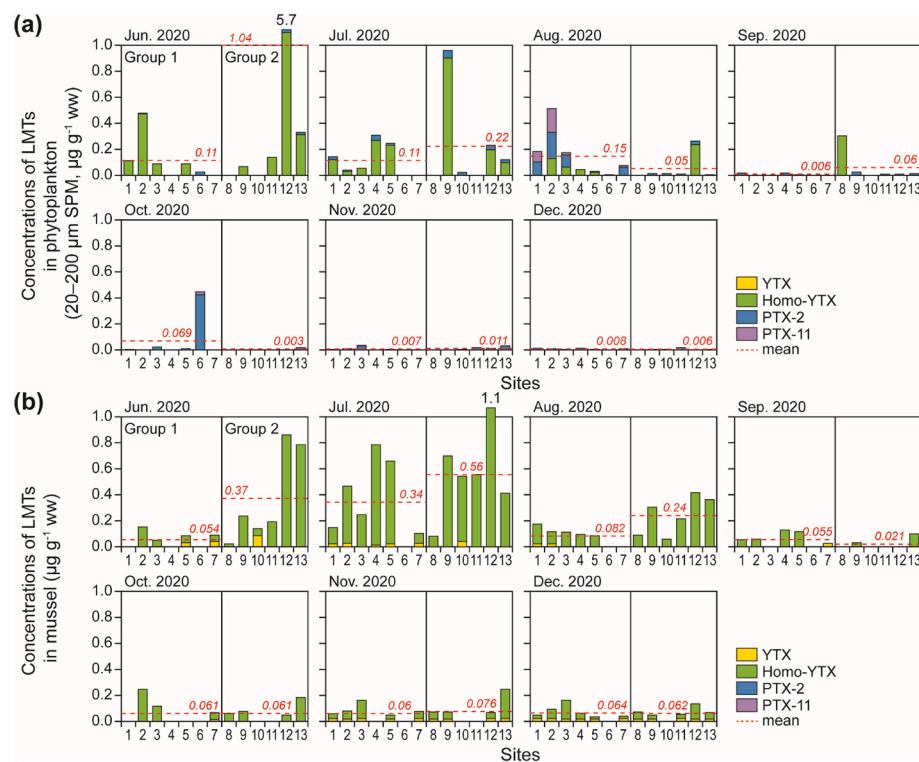
exhibited no significant difference in density between the two groups ( $p > 0.05$ ). Its density peaked during June and August in Group 1 and Group 2, respectively. The density of *G. spinifera* was relatively higher in summer, and tended to decline after August. The density of *D. acuminata* was lower compared to *G. spinifera*; however, its density was also relatively higher during summer. In contrast, Kim et al. (2010) recorded much lower densities of *D. acuminata* in Group 1 (1 cell  $\text{mL}^{-1}$ ). Overall, relatively high densities of LMT-producing microalgae were documented in the southern sea during summer in our study, which is a concern for the poisoning of shellfish. In addition, although domoic acid could not be analyzed in this study, the causative algae, *Pseudo-nitzschia* spp., was present at high densities ( $>10,000$  cells  $\text{L}^{-1}$ ); thus, further investigation on domoic acid poisoning in shellfish is needed (Table S7).

Homo-YTX, PTX-2, and PTX-11 were detected in phytoplankton (20–200  $\mu\text{m}$  SPM) along the southern coast of Korea during the sampling period. The concentrations of these toxins varied across sites, indicating heterogeneous distributions (Fig. 3a and Table S8). The concentrations of homo-YTX, PTX-2, and PTX-11 in Group 1 were  $<\text{LOD}$ –0.48  $\mu\text{g g}^{-1}$  ww,  $<\text{LOD}$ –0.43  $\mu\text{g g}^{-1}$  ww, and  $<\text{LOD}$ –0.18  $\mu\text{g g}^{-1}$  ww, respectively. In Group 2, the concentrations of homo-YTX and PTX-2 were  $<\text{LOD}$ –5.7  $\mu\text{g g}^{-1}$  ww and  $<\text{LOD}$ –0.058  $\mu\text{g g}^{-1}$  ww, respectively. There was no significant difference in LMT concentrations between the two groups, except for June ( $p > 0.05$ ), and PTX-11 was only detected in Group 1. This result might be attributed to *D. acuminata*, the causative microalgae of PTXs, occurring at relatively high densities in Group 1. To date, the detection of homo-YTX and PTX-11 in phytoplankton along the southern coast has not been reported (this study provides the first report). However, small concentrations of PTX-2 were detected in the region of Group 1 by Kim et al. (2010). In both groups, relatively high concentrations of homo-YTX were recorded in summer (June–August) compared to fall and winter, with maximum concentrations occurring during June. LMT concentrations declined after August, and were not detected at most sites of Groups 1 and 2 from September to December. These results were similar to that recorded by Liu et al. (2021), in which relatively high concentrations of homo-YTX were detected in phytoplankton in southern China during summer.

In mussels collected from field sites, LMTs were only detected in YTX and homo-YTX, whereas PTXs were not detected in any samples (Fig. 3b and Table S9). In Group 1, the concentrations of YTX and homo-YTX were  $<\text{LOD}$ –0.041  $\mu\text{g g}^{-1}$  ww and  $<\text{LOD}$ –0.77  $\mu\text{g g}^{-1}$  ww, respectively. In Group 2, the concentrations of YTX and homo-YTX were  $<\text{LOD}$ –0.083  $\mu\text{g g}^{-1}$  ww and  $<\text{LOD}$ –1.1  $\mu\text{g g}^{-1}$  ww, respectively. Similar to LMT concentrations in phytoplankton, LMT concentrations in mussels were higher in Group 2 compared to Group 1. However, there was no



**Fig. 2.** Density of causative microalgae of YTXs and PTXs in (a) Group 1 and (b) Group 2 on the southern coast of South Korea from June to December 2020. Blue lines represent mean water temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

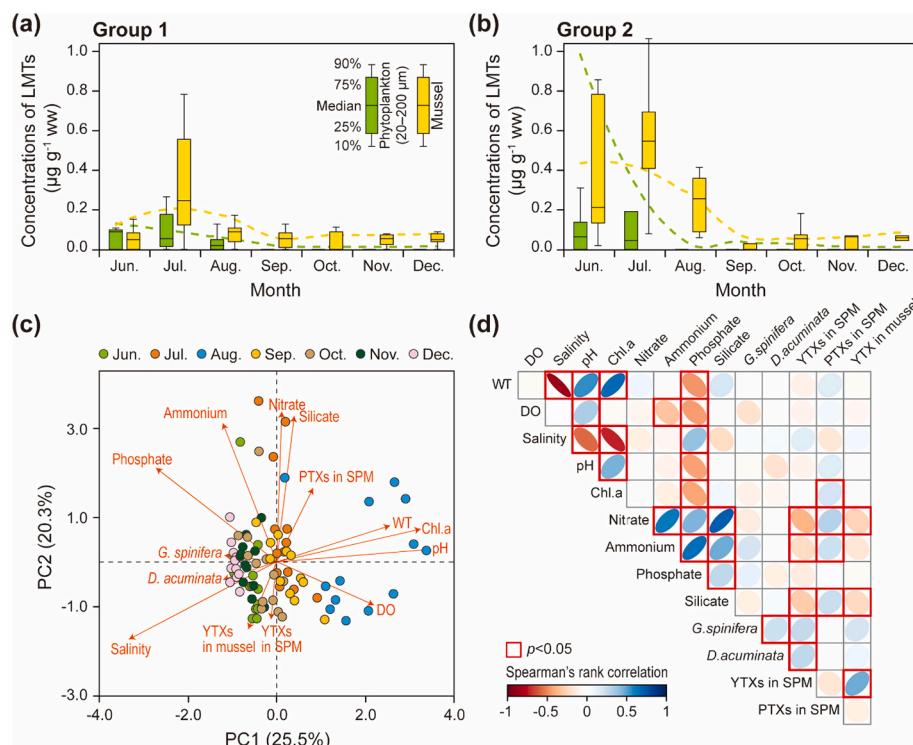


**Fig. 3.** Concentrations of YTXs and PTXs in (a) phytoplankton (20–200 µm SPM) and (b) mussels on the southern coast of South Korea from June to December 2020.

significant difference between the two groups ( $p > 0.05$ ). LMT concentrations in mussels collected in the present study were higher compared to a previous study conducted in the South Sea (Kim et al., 2010). Only YTX and homo-YTX were detected in mussels, contrasting with LMT concentrations in phytoplankton, which might be due to mussels having a different biological half-life for LMTs. The half-life of PTXs in mussels

was reported to be 2.9 d, which was relatively short compared to those of YTXs (range: 20 to 24 d) (Aasen et al., 2005; Nielsen et al., 2016). Although PTXs existed in phytoplankton, they were not detected in mussels because they were easily metabolized and excreted in mussels.

Similar to LMT concentrations in phytoplankton (20–200 µm SPM), those in mussels had a seasonal trend, with relatively higher



**Fig. 4.** Boxplot showing the concentrations of LMTs in phytoplankton (20–200 µm SPM) and mussels in (a) Group 1 and (b) Group 2 on the southern coast of South Korea from June to December 2020. Dotted lines are the fitted lines of the box plot. Fitted lines were created using the ggplot2 package in R software. (c) Results of the principal component analysis (PCA) based on environmental parameters, density of causative microalgae, and LMT concentrations. (d) Spearman's rank correlation between environmental parameters and LMT concentrations in organisms.

concentrations in summer (June–August), which declined after August. This result was comparable to that obtained by Kim et al. (2010). This phenomenon might be explained by seasonal variation in the presence of causative microalgae. For instance, *G. spinifera*, the microalgae causing YTXs, occurred at higher densities in summer, and then decreased, with very low densities ( $<1$  cell  $\text{mL}^{-1}$ ) after September. Compared to LMT concentrations in phytoplankton, those in mussels peaked later (Fig. 4a and b). For instance, LMT concentrations in phytoplankton and mussels peaked in June and July, respectively, at site S12, showing a 1-month difference. Li et al. (2017) reported that the densities of causative microalgae, *Dinophysis* spp. and *Phalacroma rotundatum*, peaked during summer, with LMT accumulation in shellfish being detected 1–2 weeks later (Li et al., 2017). Thus, LMTs, such as YTXs, appear to accumulate for longer in shellfish compared to phytoplankton, with the biological half-life of biotoxins playing a key role.

The relationship between environmental factors and LMT concentrations in phytoplankton (20–200  $\mu\text{m}$  SPM) and mussels was analyzed statistically. In both groups, environmental factors showed no significant relationship with LMT concentrations in organisms (Figs. 4c and d, S3,  $p > 0.05$ ). LMT concentrations in phytoplankton were significantly correlated to the densities of causative microalgae, not environmental factors. Homo-YTX concentrations in phytoplankton were significantly related to *G. spinifera* densities (Fig. 4d). The toxin concentration and causative microalgae density showed similar seasonal variation. Similar to *G. spinifera*, the concentrations of *D. acuminata* also showed a seasonal trend, which was highest in summer. Kim et al. (2010) reported that PTX concentrations in phytoplankton closely corresponded with the density of the causative microalgae. However, in the current study, PTX concentrations in phytoplankton showed no significant relationship with *D. acuminata* densities (Fig. 4d,  $p > 0.05$ ). Thus, other causative microalgae of PTXs might exist on the southern coast of South Korea. *Dinophysis acuminata* could have been misidentified, because it is morphologically very similar to *D. sacculus* and *D. ovum*, which belong to the *D. acuminata* complex (Raho et al., 2008; Zingone et al., 1998). Thus, the causative microalgae of PTXs need to be investigated in detail in the future. Although no significant relationship was found between the density of causative microalgae and LMT concentrations of mussels in this study, a significant relationship was observed between LMT concentrations in phytoplankton and mussels. The concentration of YTX-producing microalgae is 0–200  $\text{pg cell}^{-1}$  (Howard et al., 2008), with mussels having filtration rates of 0.26–2.3  $\text{L h}^{-1}$  (Denis et al., 1999). Thus, more YTX could be accumulated in mussels, even at low densities (i.e.,  $<5$  cells  $\text{mL}^{-1}$ ). Overall, microalgae that produce LMTs were recorded on the southern coast of Korea during summer; consequently, LMTs accumulated in bivalves, raising concerns about the safety of aquatic products in this region.

Among the four target LMTs, homo-YTX was only detected in commercial shellfish samples. Homo-YTX was detected in seven samples (only for two species, red scallops and comb pen shells) out of a total of 121 samples (total 12 shellfish species) (Table 1). Concentrations of homo-YTX in red scallops and comb pen shells were  $<\text{LOD}$ –73 ng  $\text{g}^{-1}$  ww and  $<\text{LOD}$ –124 ng  $\text{g}^{-1}$  ww, respectively. Both red scallops and comb

pen shells, in which homo-YTX were detected, were cultured in Yeosu and Tongyeong, which are located on the southern coast of South Korea. Previous studies reported that scallops accumulate relatively large amounts of biotoxins over relatively long time periods (Lee et al., 2012; Liu et al., 2019). This phenomenon might be attributed to scallops being larger than other shellfish, and due to higher feeding rates on phytoplankton (Li et al., 2015). In this study, the commercial shellfish samples in which LMTs were detected showed a temporal distribution (LMT concentrations were higher during summer), similar to those recorded in mussels collected from the southern coast. This similarity might be because shellfish are cultured in the South Sea, where samples were collected. In shellfish samples, LMTs (such as homo-YTX) were only detected in samples collected in September and October, and were not detected in any samples after October.

The maximum concentrations of YTXs were 73 ng YTX EQ  $\text{g}^{-1}$  ww and 124 ng YTX EQ  $\text{g}^{-1}$  ww in red scallops and comb pen shells, respectively. The concentrations of YTXs detected in seafood were lower than the 3.75 mg YTX EQ  $\text{kg}^{-1}$  ww suggested by EFSA (2008). To date, there has been no report of YTXs in domestic seafood, and regulation of YTXs has not been established in Korea. The exposure of YTXs to humans was calculated to evaluate the potential risks posed by the consumption of red scallops and comb pen shells. The mean and high consumption values of two contaminated bivalves by YTX and homo-YTX in the Korean database are shown in Table S10. The 95th and 97.5th percentile consumption rates of red scallops and comb pen shells were not available; instead, 2.5 and 3.0 times the mean of all participants were used to assess scenarios 3 and 4, respectively, following WHO guidance for dietary exposure assessment of chemical risk factors in food (WHO, 1985, 2020). Results indicated that no potential risk to human health was identified in the dietary exposure assessment of the two contaminated bivalve species (red scallops and comb pen shells) that had the highest homo-YTX concentrations (Table 2).

Comparison of the HQ% among scenarios revealed that food consumption is an important factor in estimating exposure levels. Even if the highest concentrations of homo-YTX in the comb pen shell are higher compared to red scallop, the consumption level of red scallop is around 8-fold higher compared to comb pen shell in South Korea. The range of dietary assessment on human consumption of these two contaminated

**Table 2**

Exposure to YTXs associated with the consumption of shellfish for the four tested scenarios in the human population of South Korea.

Species	Scenario	Dietary exposure ( $\mu\text{g d}^{-1}$ )	HQ (%)	HI (%) <sup>a</sup>
Red scallop	S1	0.017	0.0012	0.0014
	S2	1.7	0.11	0.17
	S3	0.044	0.0029	0.0035
	S4	0.061	0.0041	0.005
Comb pen shell	S1	0.0037	0.0002	0.0014
	S2	0.86	0.058	0.17
	S3	0.0093	0.0006	0.0035
	S4	0.013	0.0009	0.005

<sup>a</sup> HI (%): sum of HQ for the two shellfish species in each scenario.

**Table 1**

Concentrations of LMTs in domestic shellfish collected from seafood markets. Out of 121 samples of 12 shellfish species, only those in which LMTs were detected were included in this table. A list of all tested samples is provided in Table S1 of the Supplementary Material.

Month	Seafood market	Origin	Species	YTX ( $\text{ng g}^{-1}$ ww)	Homo-YTX ( $\text{ng g}^{-1}$ ww)	PTX-2 ( $\text{ng g}^{-1}$ ww)	PTX-11 ( $\text{ng g}^{-1}$ ww)
September	Seoul	Tongyoung	Red scallop	$<\text{LOD}^a$	73	$<\text{LOD}$	$<\text{LOD}$
	Daejeon	Yeosu	Comb pen shell	$<\text{LOD}$	121	$<\text{LOD}$	$<\text{LOD}$
	Busan	Tongyoung	Red scallop	$<\text{LOD}$	37	$<\text{LOD}$	$<\text{LOD}$
October	Seoul	Yeosu	Red scallop	$<\text{LOD}$	65	$<\text{LOD}$	$<\text{LOD}$
		Tongyoung	Red scallop	$<\text{LOD}$	63	$<\text{LOD}$	$<\text{LOD}$
		Yeosu	Comb pen shell	$<\text{LOD}$	124	$<\text{LOD}$	$<\text{LOD}$
	Busan	Tongyoung	Red scallop	$<\text{LOD}$	57	$<\text{LOD}$	$<\text{LOD}$

<sup>a</sup>  $<\text{LOD}$ : below limit of detection.

bivalve species was 0.037–1.7  $\mu\text{g d}^{-1}$ , and an HQ% of over 100% was not observed in any scenario. Thus, the potential risk of consuming bivalves contaminated with YTXs is low in South Korea. In addition, because the HI% was below 1, exposure to toxic levels that could affect the health of consumers was low (Evans et al., 2015). Because there is a risk of chronic exposure to marine biotoxins, continuous monitoring for these toxins is required. Of importance, unmanaged marine biotoxins were detected in both coastal organisms on the southern coast and commercial shellfish in seafood markets.

In this study, YTXs and PTXs-producing phytoplankton were observed at noticeably high densities along the southern coast of Korea during summer. YTXs and PTXs were detected in both phytoplankton and mussels, and concentrations were also greater during summer. In addition, homo-YTX was detected in domestic seafood, but not at a level considered to cause risk to human health. To date, there are no safety guidelines for LMTs, such as YTXs and PTXs, in seafood in Korea. Since LMT-producing microalgae frequently appear along the southern coast of Korea in summer, shellfish contamination by LMTs and chronic human exposure is possible. Therefore, continuous monitoring of LMTs is required, and preemptive management of these toxins.

#### CRediT authorship contribution statement

**Mungi Kim:** Conceptualization, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft. **Seongjin Hong:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition, Supervision. **Young Kyun Lim:** Investigation, Formal analysis, Data curation. **Jihyun Cha:** Investigation, Formal analysis, Data curation. **Jiyun Gwak:** Investigation, Formal analysis, Data curation. **Youngnam Kim:** Investigation, Formal analysis, Data curation. **Seong-Ah An:** Investigation, Data curation. **Hee-seok Lee:** Investigation, Data curation, Writing – review & editing. **Seung Ho Baek:** Conceptualization, Investigation, Formal analysis, Data curation, Writing – review & editing.

#### 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.

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#### Appendix A. Supplementary data

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