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Passive sampling and molecular networking for surveillance of lipophilic marine biotoxins along the south coast of Korea

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ABSTRACT

Lipophilic marine biotoxins (LMTs) are increasingly reported in coastal ecosystems, posing risks to seafood safety and public health. This study investigated the spatial and seasonal distributions of LMTs along the south coast of Korea in 2022 by integrating phytoplankton and mussel sampling with passive solid-phase adsorption toxin tracking (SPATT) and molecular networking-based high-resolution mass spectrometry. Diatoms dominated the phytoplankton community, but toxin-producing dinoflagellates such as *Dinophysis acuminata* and *Gonyaulax spinifera* appeared during seasonal peaks in spring and summer. Four LMTs, including pectenotoxin-2 (PTX2), PTX1, yessotoxin (YTX), and homo-YTX (hYTX), were detected in phytoplankton, whereas mussels accumulated only hYTX, peaking later, consistent with prolonged retention in bivalves. SPATT captured a broader toxin spectrum, including okadaic acid (OA), dinophysistoxin-1 (DTX1), azaspiracid-2 (AZA2), and domoic acid (DA), and often detected toxins earlier than biological samples. The combined application of SPATT and molecular networking further revealed putative PTX-related features based on MS/MS spectral similarity that were not observed in phytoplankton or mussels. Dietary exposure assessment of commercially distributed shellfish based on detected free toxin forms indicated hazard quotients and indices well below regulatory thresholds, suggesting low acute health risk. Repeated detection of LMTs in domestic seafood raises concerns about chronic exposure. Integrating SPATT with molecular networking proved highly effective for early biotoxin surveillance, underscoring the necessity of sustained, multi-matrix monitoring to ensure seafood safety.

1. Introduction

Marine biotoxins are natural secondary metabolites produced by harmful microalgae. Their occurrence has become a growing concern due to their association with harmful algal blooms (HABs), which negatively impact marine ecosystems, seafood safety, and coastal economies (Griffith and Gobler, 2020; Hallegraeff et al., 2021). More than 200 marine biotoxins have been described to date (Gerssen et al., 2011), many of which accumulate in filter-feeding organisms such as shellfish and fish, thereby posing risks to human health through trophic transfer (Liu et al., 2019; Zhao et al., 2022). Among the different groups, lipophilic marine biotoxins (LMTs) are most frequently reported in monitoring programs. This group includes okadaic acid (OA), dinophysistoxins (DTXs), pectenotoxins (PTXs), yessotoxins (YTXs), and azaspiracids (AZAs), all of which are known to bioaccumulate in bivalves and compromise seafood safety (Lane et al., 2010; Li et al., 2014).

In Korean coastal waters, particularly along the southern coast, where aquaculture activities are concentrated, occurrences of LMTs such as PTXs and YTXs have been repeatedly documented in both phytoplankton and shellfish (Kim et al., 2022, 2023, 2025). Known toxin producers, including *Dinophysis acuminata, D. fortii*, and *Gonyaulax spinifera*, are regularly recorded in Korean waters (Kim et al., 2023, 2024), whereas *Protoceratium reticulatum* and *Lingulodinium polyedrum* have only been detected occasionally (Paz et al., 2007; Pizarro et al., 2008). The latter taxa, however, are well-established YTX producers in other regions and therefore remain relevant for toxin surveillance in Korea.

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Despite these reports, the frequency and intensity of toxin events remain highly variable, and conventional grab sampling often fails to capture their sporadic occurrence or low-level presence.

To overcome these limitations, passive sampling devices such as solid-phase adsorption toxin tracking (SPATT) have been developed. SPATT continuously adsorbs dissolved toxins during deployment, producing a time-integrated profile of toxin occurrence that parallels bioaccumulation in filter-feeding organisms. This method increases detection sensitivity, minimizes matrix interferences, and captures episodic contamination events that conventional monitoring may miss (MacKenzie et al., 2004; Rundberget et al., 2009; Zendong et al., 2016). Consequently, SPATT has become a valuable complementary tool for investigating the occurrence of LMTs in coastal waters.

In addition to advances in field monitoring, analytical developments have further expanded the scope of LMT detection. High-resolution mass spectrometry (HRMS), coupled with molecular networking analysis, offers an untargeted approach to characterizing toxin diversity. Molecular networking organizes tandem mass spectral data into clusters based on fragmentation similarity, enabling the identification of both known toxins and structurally related analogs often overlooked in targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) workflows (Olivon et al., 2017; Wang et al., 2016). Applications to environmental samples containing *Dinophysis* and *Prorocentrum* have revealed a wide variety of OA and DTX derivatives with distinct structural features, demonstrating the potential for uncovering cryptic analogs in complex matrices (Sibat et al., 2021; Wu et al., 2020).

Despite these methodological advances, the regulatory framework for LMTs in South Korea remains limited compared to those of the European Union and the United States. Current domestic standards primarily address diarrhetic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), and amnesic shellfish poisoning (ASP) toxins (MFDS, 2009), while thresholds for other major LMTs, such as YTXs, PTXs, and AZAs, have not yet been established. This regulatory gap is of particular concern, given that the southern coast is a key region for aquaculture production and seafood supply. Moreover, climate change is driving the poleward expansion of subtropical and tropical dinoflagellates, thereby increasing the likelihood of new or previously unregulated toxins entering Korean coastal ecosystems (Gobler et al., 2017; Kim et al., 2011).

Taken together, these issues highlight the need for more comprehensive monitoring strategies that can detect both recognized and emerging LMTs with improved temporal and analytical resolution. In this study, we examined the seasonal and spatial distribution of LMTs in phytoplankton, wild mussels, and dissolved-phase samples collected using SPATT devices from the southern coast of Korea in 2022. By integrating quantitative LC-MS/MS with HRMS-based molecular networking, we aimed to broaden the characterization of LMT diversity and explore structurally related analogs that might otherwise remain undetected. This combined approach strengthens the framework for monitoring toxin dynamics and provides critical baseline data for safeguarding seafood safety and protecting coastal ecosystems in Korea.

2. Materials and methods

2.1. Sample collection and water quality measurements

From January to December 2022 (excluding September and November), 13 stations along the south coast of Korea were surveyed for biological and water quality parameters (Fig. 1). Phytoplankton samples (n = 127) were collected with a 20-μm mesh plankton net and sieved through a 200-μm mesh to remove large zooplankton and debris. The 20–200 μm fraction was transferred onto 20-μm nylon mesh filters (Millipore, Merck, Darmstadt, Germany), flash-frozen on site, and stored at –20 °C until toxin analysis. Mussel specimens (n = 106) were collected simultaneously at the same stations. At least ten individuals were pooled per site to obtain composite samples, which were frozen, homogenized, and stored.

At each station, temperature, salinity, pH, and dissolved oxygen (DO) were measured in situ with a multi-parameter sonde (YSI 6600v2, YSI Inc., Yellow Springs, OH). Additional water samples were taken for

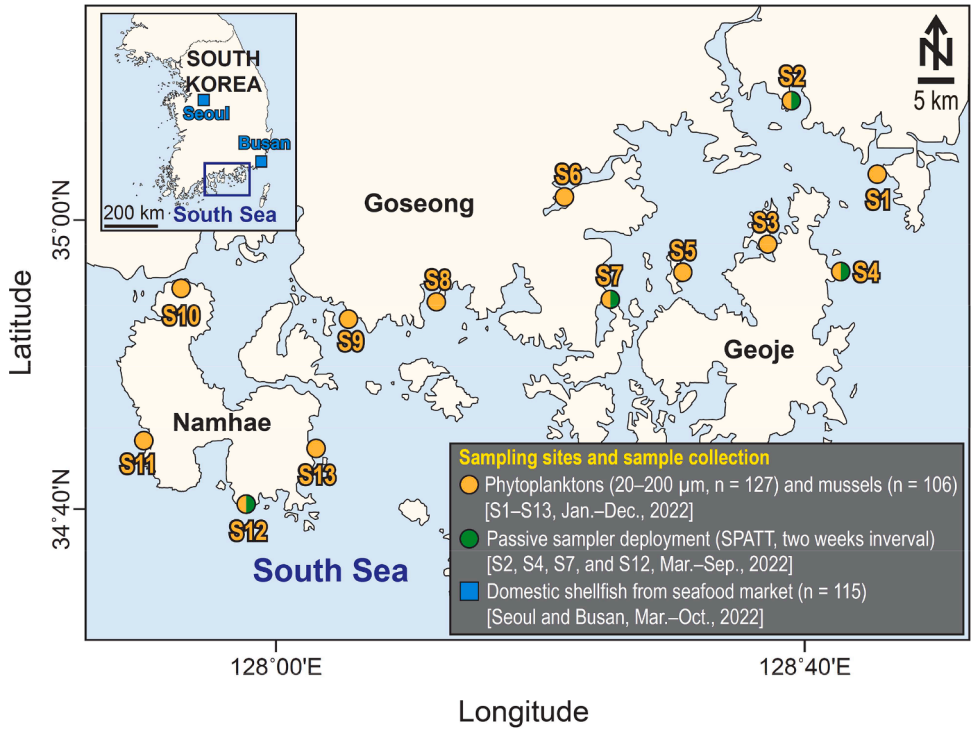

Fig. 1. Study area and sampling design along the south coast of Korea in 2022. Thirteen stations were investigated for phytoplankton (20–200 μm SPM), wild mussels, and surface seawater between January and December. SPATT devices were deployed biweekly at four sites (S2, S4, S7, and S12) from March to August. Commercial shellfish were obtained from seafood markets in Seoul and Busan between March and October.

nutrient analysis (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, and SiO_2) using a flow-injection analyzer (Lachat QuikChem 8000, Hach Co., Loveland, CO). A summary of water quality parameters is provided in Table S1. In addition, 115 domestic shellfish products were purchased from seafood markets in Seoul and Busan between March and October 2022. These samples were collected to evaluate potential LMT exposure via commercial consumption and were transported frozen for toxin analysis (Table S2).

2.2. SPATT preparation and on-site deployment

Passive samplers (SPATT) were prepared by enclosing 3 g of Diaion HP20 resin (Sigma-Aldrich, St. Louis, MO) between two layers of 30-μm nylon mesh and mounting them in stainless steel frames, giving an adsorption surface area of 47.5 cm^2 per side. The resin was preconditioned in 100% methanol for 48 h, rinsed with Milli-Q water, and sonicated to remove residual solvent. Conditioned samplers were kept in Milli-Q water at 4 °C until deployment.

SPATT units were deployed at four stations (S2, S4, S7, and S12) along the south coast of Korea. Deployments were conducted every two weeks between March and August 2022, with an exposure duration of about 14 d. Surface seawater was collected at each deployment and retrieval for comparison with SPATT extracts. In total, 12 SPATT deployments and retrievals were carried out during the study period. All samples were transported on ice and stored at –20 °C until analysis.

2.3. Phytoplankton identification and quantification

Seawater samples (500 mL) were preserved with acidified Lugol's iodine solution for taxonomic analysis. Samples were concentrated to ~50 mL by gravitational settling, and the supernatant was gently removed. Concentrates were stored in the dark at room temperature until examination. For microscopic observation, aliquots were placed in a Sedgewick–Rafter counting chamber and allowed to settle for at least 10 min to ensure homogeneous cell distribution. Depending on cell density, 100–400 μL of the concentrate was analyzed under an optical microscope at 200 × and 400 × magnifications. Phytoplankton were identified to the lowest possible taxonomic level based on morphological characteristics (Lim et al., 2019; Kim et al., 2025).

2.4. Extraction and cleanup of lipophilic marine biotoxins

The extraction of LMTs followed established protocols, with biological and environmental samples processed according to the validated LC-MS/MS method described by Gerssen et al. (2010), and SPATT resins treated following the approaches outlined by Fux et al. (2009) and Zendong et al. (2016). For shellfish, pooled soft tissue samples were homogenized, and 2 g subsamples were extracted with 9 mL of methanol (MeOH). After sonication for 15 min and centrifugation at 3500 rpm for 10 min, the supernatants were combined and adjusted to 20 mL. Extracts were purified using Strata-X SPE cartridges (30 mg, 3 mL, Phenomenex, Torrance, CA) preconditioned with MeOH and Milli-Q water. Diluted samples were loaded, rinsed with 15% MeOH, and eluted with MeOH containing 1% ammonium hydroxide. The eluates were evaporated under nitrogen and reconstituted in 1 mL of MeOH with 1% ammonium hydroxide, then stored at −20 °C.

For phytoplankton, the 20–200 μm size fraction collected on nylon filters (20 μm, Millipore) was thawed at room temperature and extracted with 3 mL of MeOH by vortexing for 30 s, followed by 15 min sonication. After centrifugation (3500 rpm, 10 min), the supernatant was collected, and the procedure was repeated twice. The combined extracts (~10 mL) were filtered through a 0.22 μm PTFE syringe filter (Advantec, Tokyo, Japan) and stored at −20 °C. For seawater, samples were filtered through GF/F (47 mm, Whatman, Maidstone, UK) to remove suspended particles. One liter of the filtrate was passed through preconditioned Oasis HLB cartridges (500 mg, 6 cc, Waters, Milford, MA), rinsed with water, and eluted with MeOH containing 1% ammonium hydroxide. The eluates were evaporated and processed as described for shellfish samples. For SPATT, HP20 resin was removed from the holders, rinsed with Milli-Q water, and transferred into empty SPE cartridge tubes (Supelco, Bellefonte, PA). Resin was extracted with 24 mL of MeOH under gentle agitation, and the eluates were evaporated under nitrogen at 40 °C. Dried extracts were reconstituted in 1 mL of pure MeOH and stored at −20 °C. All sample matrices, including phytoplankton, SPATT extracts, and shellfish, were analyzed without alkaline hydrolysis, and only free toxin forms were targeted in the present study.

2.5. Quantification of lipophilic marine biotoxins

A total of 18 LMTs and domoic acid (DA) were quantified. Certified reference materials for OA, DTX1, DTX2, YTX, homo-YTX (hYTX), PTX2, AZA1, AZA2, AZA3, gymnodimine A (GYM), 13-desmethyl spirolide C (SPX), and DA were obtained from the National Research Council (NRC, Ottawa, ON, Canada). Additional standards, including PTX11 (Sigma-Aldrich), AZA4 and AZA5 (Cifga, Lugo, Spain), and brevetoxins (BTX1, BTX2, BTX3, and BTX5; LKT Laboratories, St. Paul, MN), were used to ensure coverage of the targeted toxin groups. Analyses were conducted with an Agilent 1290 Infinity II HPLC coupled to an Agilent 6470 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. Chromatographic separation was achieved using a Waters X-Bridge C18 analytical column (3.0 × 150 mm, 5 μm particle size) maintained at 25 °C. The mobile phase consisted of (A) ultrapure water and (B) 90% acetonitrile in water (v/v), both containing 0.05% ammonium hydroxide, delivered in gradient mode. Detection and quantification were performed in multiple reaction monitoring (MRM) mode, targeting characteristic precursor-to-product ion transitions for each analyte. Retention times, MRM transitions, and compound-specific settings are provided in Tables S3 and S4.

2.6. Quality control

Analytical performance was evaluated in terms of calibration linearity, sensitivity, extraction efficiency, and reproducibility (Table S5). Calibration curves for LMTs and DA were prepared over 1–50 ng mL^{-1}, and BTXs over 5–200 ng mL^{-1}. Samples above these ranges were diluted and reanalyzed. Limits of detection (LOD) and quantification (LOQ) were defined as 3.143 and 10 times the standard deviation, respectively, based on replicate analyses ($n = 7$) of blank matrices spiked with certified reference standards. The same spiking levels (1 ng for LMTs and DA; 5 ng for BTXs), calculation criteria, and analytical procedures were applied to mussel tissue, phytoplankton samples, and SPATT resins to ensure matrix-to-matrix comparability. Recovery was evaluated by spiking blank matrices with 25 ng of target analytes ($n = 4$), followed by identical extraction and cleanup procedures for all matrices. The method showed linear calibration ($R^2 \geq 0.99$), recoveries >85%, and low variability among replicates. These results confirm that the LC-MS/MS workflow provides reliable and comparable quantification of LMTs and DA across biological and SPATT matrices.

2.7. HRMS-based molecular networking analysis

To explore putative LMT-related features based on MS/MS spectral similarity, HRMS data from the south coast of Korea were processed using molecular networking on the Global Natural Products Social Molecular Networking (GNPS) platform. LC-HRMS data were acquired in full-scan MS and information-dependent acquisition (IDA) modes using an Agilent 1290 Infinity II LC system coupled to a QTOF mass spectrometer (TripleTOF 5600, AB Sciex, Framingham, MA). Detailed LC-QTOFMS parameters are provided in Table S6. Raw MS/MS files were converted to the mzXML format with MSConvert (ProteoWizard) and uploaded to GNPS via WinSCP. GNPS-based molecular networking was applied as an exploratory screening and visualization approach to group MS/MS features based on spectral similarity and to examine the

chemical diversity of putative LMT-related features, rather than for definitive structural elucidation, as adopted in previous studies (Wang et al., 2016; Sibat et al., 2021). Network construction used a cosine similarity score ≥ 0.7, a minimum of six matched fragment ions, and retention of the top 10 fragment peaks per spectrum. These criteria enhanced spectral matching reliability and facilitated the annotation of structurally related features. The resulting networks were visualized in Cytoscape (v.3.10.2), where each node represented a unique molecular feature, with node size scaled to relative abundance. Pie-chart attributes indicating sample-specific distributions across phytoplankton, mussel, and SPATT extracts.

2.8. Statistical analysis

Normality of variables was tested with the Shapiro–Wilk test in R (version 4.4.1). As most variables did not satisfy normality assumptions, non-parametric methods were applied. Prior to correlation analysis, concentration and environmental data were square-root transformed to reduce skewness and stabilize variance. Spearman's rank correlation was conducted to evaluate associations among LMT concentrations, environmental parameters (temperature, salinity, pH, DO, and nutrients), and phytoplankton abundances. Pairwise correlation matrices were generated in R using the '*vegan*' and '*dplyr*' packages, and statistical significance was assessed at the 0.05 probability level. For consistency, toxin concentrations below the LOD were replaced with half the LOD value.

2.9. Risk assessment of LMTs in commercial shellfish

Human health risk assessment was conducted following the approach of Kim et al. (2025), using LMT concentrations detected in commercially available shellfish (mussels and pen shells). Estimated daily intake (EDI, μg kg^{-1} d^{-1}) for each toxin was calculated by multiplying the highest detected concentration (μg g^{-1} ww) by the daily consumption amount (g $person^{-1}$ d^{-1}) and adjusting for an average adult body weight of 60 kg. Consumption data were obtained from the 7th Korea National Health and Nutrition Examination Survey (KNHANES, 2016–2018) (KDCA, 2020). Four intake scenarios were applied: (S1) average daily intake across the general population, (S2) average daily intake among shellfish consumers, (S3) 95th percentile intake across the general population, and (S4) 97.5th percentile intake representing high consumers. EDI values were compared to the acute reference dose (ARfD) of 25 μg kg^{-1} bw d^{-1} established by the EFSA for yessotoxins (EFSA, 2008). For each shellfish type and intake scenario, hazard quotients (HQs) were calculated as (EDI/ArfD) × 100. Cumulative exposure was assessed using hazard indices (HIs), obtained by summing HQs from mussels and pen shells within each scenario.

3. Results and discussion

3.1. Phytoplankton community and seasonal occurrence of LMTs-producing microalgae

In 2022, the phytoplankton assemblage along the south coast of Korea was dominated by Bacillariophyceae, accounting for about 65% of the total biomass (Fig. S1 and Table S7). Cryptophyceae (21.2%), Dinophyceae (10.0%), and Dictyochophyceae (0.2%) followed, while minor taxa collectively contributed less than 5%. This distribution reflects a relatively stable taxonomic composition, consistent with previous surveys in the region (Kim et al., 2023). Seasonal changes showed that the relative contribution of dinoflagellates increased in late spring and early summer, reaching 12.7% in May and 13.0% in June. Because dinoflagellates include toxin-producing genera, these seasonal increases suggest a higher likelihood of LMTs contamination in shellfish during warmer months.

Among recognized toxin producers, *D. acuminata, G. spinifera*, and *Karenia* spp. were repeatedly observed and considered potential contributors to PTXs, YTXs, and BTXs, respectively (Fig. 2a, Fig. S2, and Table S8). *D. acuminata* first appeared in April with a peak density of 4400 cells L^{-1}, followed by *G. spinifera* at 1000 cells L^{-1} and *Karenia* spp. at 800 cells L^{-1}. The temporal separation in peak densities among these dinoflagellates likely reflects their thermal preferences: *D. acuminata* typically blooms in cooler spring conditions, whereas *K. brevis* is associated with warmer temperatures and often proliferates in late summer to early autumn (Vargo, 2009; Kamiyama et al., 2010; Fiorendino et al., 2020). *G. spinifera*, with broad thermal tolerance, was present over a longer period throughout the sampling season (Kim et al., 2023). Collectively, elevated cell densities of these species between April and August suggest a higher potential for LMT accumulation in filter-feeding shellfish, identifying this interval as a possible high-risk period.

Compared with 2020 and 2021, both total phytoplankton biomass and the abundance of toxin-producing genera were lower in 2022 (Kim et al., 2022, 2023). This reduction may be linked to decreased rainfall and freshwater discharge, which limited the inputs of terrestrially derived nutrients that support phytoplankton growth. Recent high-resolution observations along the Chinese coast have shown that rainfall-driven freshwater influxes significantly increase nutrient concentrations and trigger phytoplankton blooms after a lag of several days, highlighting the strong link between precipitation events and algal proliferation in coastal systems (Wang et al., 2024). These findings indicate that rainfall and freshwater inputs strongly influence the seasonal occurrence of potential LMT producers. Long-term monitoring that integrates environmental variables with phytoplankton dynamics will be required to clarify these relationships.

3.2. Spatial and temporal distributions of LMTs in phytoplankton

In 2022, four LMTs, such as PTX2, PTX11, YTX, and hYTX, were detected in phytoplankton samples (Fig. 2b and Table S9). Maximum concentrations were 390 ng g^{-1} ww for PTX2, 43 ng g^{-1} ww for PTX11, 33 ng g^{-1} ww for YTX, and 302 ng g^{-1} ww for hYTX. PTXs appeared earlier in the season, peaking in April–May, whereas YTXs and hYTX became more prevalent later in spring and early summer. These temporal patterns broadly followed the succession of key dinoflagellates such as *D. acuminata* and *G. spinifera* (Fig. 2a), indicating a strong link between phytoplankton composition and LMTs profiles. Overall, LMT concentrations were lower than those reported in 2020 and 2021, likely reflecting the reduced abundance of causative species during 2022 (Kim et al., 2022, 2023).

Correlation analysis supported these findings (Fig. 3a). PTX concentrations were positively and significantly correlated with *D. acuminata* ($r = 0.36$, $p < 0.05$), confirming its role as a primary PTX source. PTX levels were also associated with members of the *Gyrodinium* genus ($r = 0.36$, $p < 0.05$), which have not been previously recognized as PTX producers in Korean coastal waters. Similar statistical associations between *Gyrodinium* occurrence and LMT concentrations have been reported in previous studies conducted in Korean coastal waters (Kim et al., 2025), indicating that this genus may be ecologically linked to PTX variability. However, *Gyrodinium* spp. is not interpreted here as a PTX-producing taxon, and the observed association likely reflects indirect or unresolved ecological mechanisms rather than direct toxin production. In this context, previous studies have reported toxic or neurotoxic effects associated with *Gyrodinium* spp. in bioassays (da Costa et al., 2005), as well as PSP events linked to unidentified *Gyrodinium* species in the Adriatic Sea (Pavela-Vrančić and Marasović, 2004).

In contrast, YTX concentrations showed no significant correlation with *G. spinifera* ($p > 0.05$). Although *G. spinifera* is widely recognized as a representative YTX-producing dinoflagellate and was broadly distributed across the study area, the absence of a significant statistical relationship indicates that YTX variability along the southern coast of Korea cannot be attributed primarily to this species based on the present dataset. This suggests that additional YTX-producing taxa or alternative

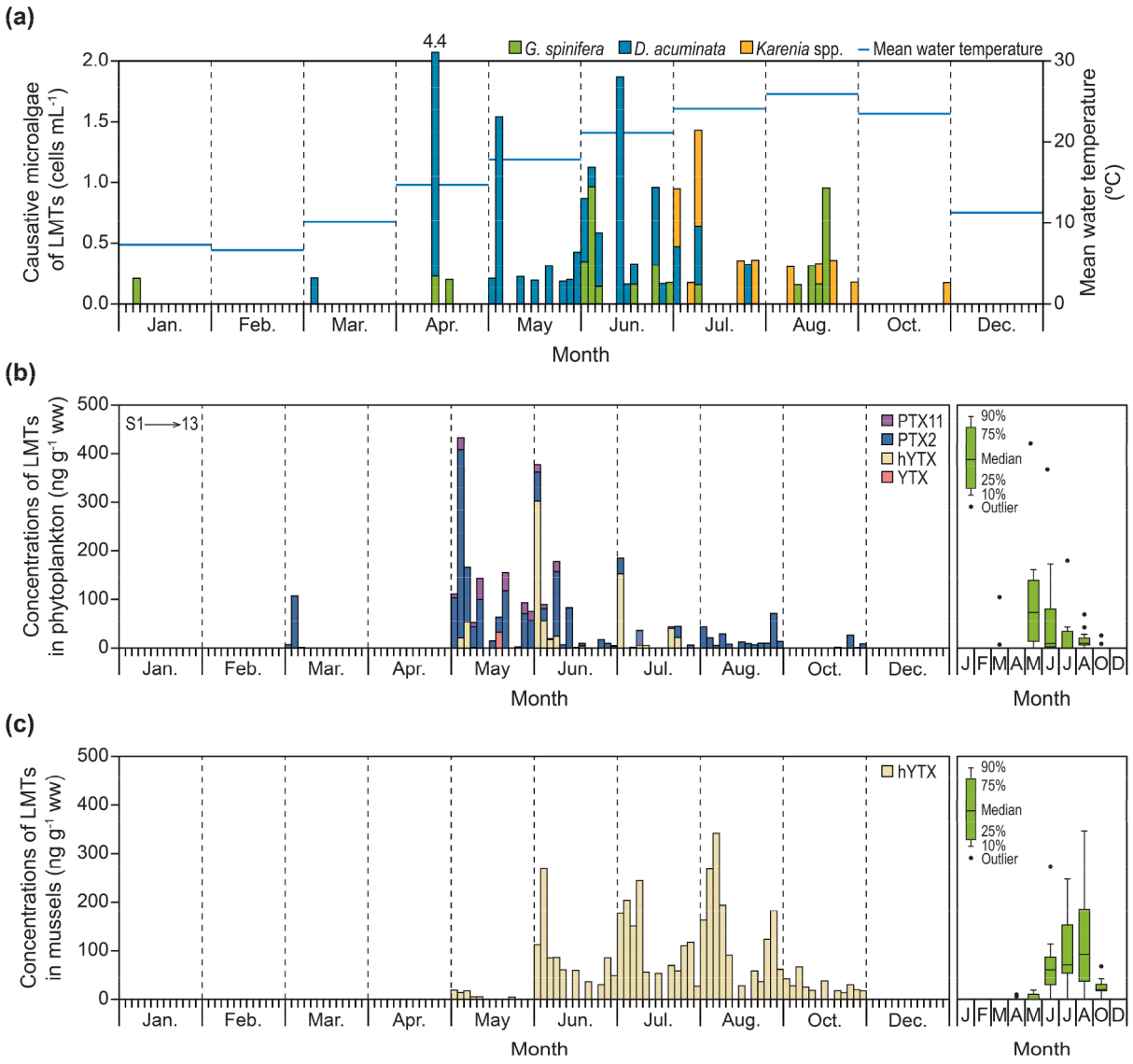

Fig. 2. Seasonal distributions of LMT-producing microalgae and corresponding LMT concentrations along the south coast of Korea in 2022. **(a)** Monthly cell densities of *Dinophysis acuminata* (blue), *Gonyaulax spinifera* (green), and *Karenia* spp. (orange); mean surface seawater temperature is shown as a blue line. **(b)** Monthly concentrations of LMTs in phytoplankton samples and **(c)** wild mussels collected from the south coast of Korea.

ecological processes, including contributions from other known producers such as *P. reticulatum* and L. *polyedrum* (Liu et al., 2019), may also contribute. Although *Karenia* spp. were detected in phytoplankton samples, BTXs were not observed. This absence may be related to low cell abundance and environmental conditions unfavorable for BTXs production, but may also reflect uncertainty in species-level identification based on light microscopy and strain-specific variability in toxin production within the genus *Karenia* (Reynolds et al., 2020; Sunda et al., 2013).

These results show that LMT is present in phytoplankton and closely follows the seasonal occurrence of major producers, while also highlighting a previously unreported association with *Gyrodinium*. Continued monitoring that integrates environmental variables, such as rainfall, nutrients, and temperature, will be crucial for clarifying the ecological factors that drive toxin dynamics and for assessing the potential roles of less common taxa. Because these seasonal patterns have been observed consistently in recent years, special attention should be given to this period, when filter-feeding shellfish are more likely to accumulate LMTs and pose a greater risk to seafood safety.

3.3. Bioaccumulation patterns of LMTs in mussels

Analysis of mussel samples showed that among the target LMTs, only hYTX was detected along the south coast in 2022 (Fig. 2c and Table S10). Concentrations increased from May, peaked at 350 ng g^{-1} ww in August, and then gradually declined, with no detections during winter. This temporal profile indicates that mussels accumulated toxins later than phytoplankton, reflecting a lag between toxin production in the water column and uptake by shellfish. Similar seasonal patterns have been observed in recent years, and consistent with the lower abundances of causative dinoflagellates, mussel toxin concentrations in 2022 were also comparatively low (Kim et al., 2022, 2023).

Correlation analysis revealed that hYTX levels in mussels were strongly and positively associated with those in phytoplankton, whereas no significant relationships were observed between YTXs and individual dinoflagellate taxa (Fig. 3b, $p > 0.05$). These findings highlight the capacity of mussels to integrate toxin exposure over time, even when causative algae are present at low densities. Although *G. spinifera* occurred at relatively low abundances, it produces YTX at high cellular quotas (176–200 pg $cell^{-1}$), which are 20 to 600 times greater than those

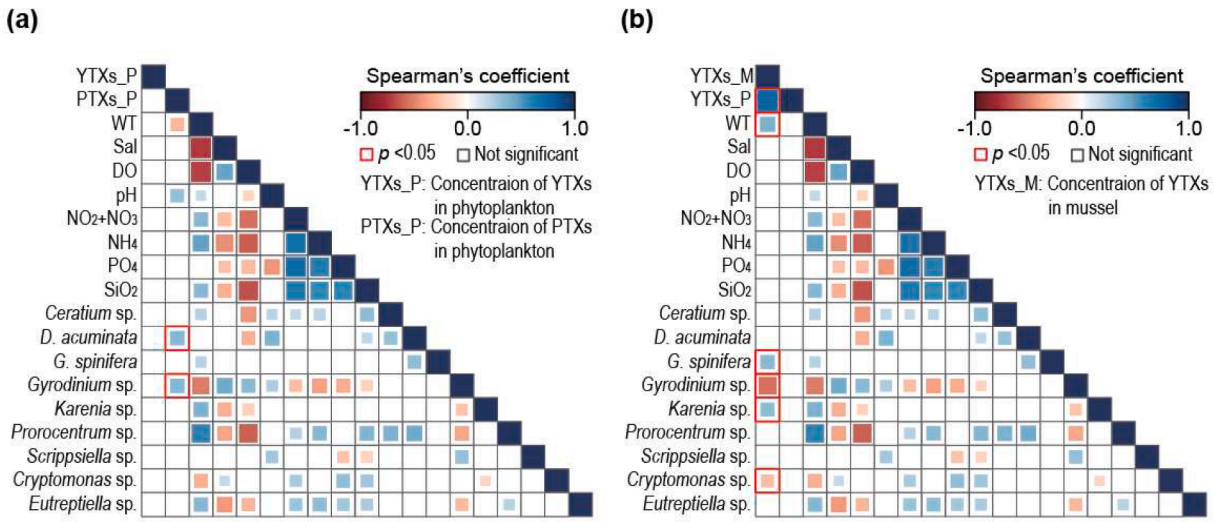

Fig. 3. Spearman's rank correlation matrices of LMT concentrations, environmental parameters, and phytoplankton taxa along the south coast of Korea in 2022. **(a)** Correlations among LMTs in phytoplankton, environmental variables, and key phytoplankton taxa. **(b)** Correlations between LMTs in mussels and the same environmental and biological variables. Significant correlations ($p < 0.05$) are outlined in red.

reported for *P. reticulatum* or L. *polyedrum* (Caron et al., 2010; Paz et al., 2004; Rhodes et al., 2006). Given the high filtration capacity of mussels, even low cell abundances can result in measurable accumulation of YTXs in tissues, as continuous filtration over time allows uptake rates to exceed metabolic transformation and elimination, leading to net toxin retention. In this study, shellfish samples were analyzed without alkaline hydrolysis; thus, the reported OA and DTX concentrations reflect free toxin forms only. Esterified OA/DTX, which may contribute to total toxin equivalents in shellfish, were not assessed under the present analytical conditions.

The toxin composition in mussels differed from that in phytoplankton. While PTX2 dominated in plankton samples, PTXs were not detected in mussels, likely due to rapid metabolism and excretion. Previous studies have shown that PTX2 is readily converted to PTX2-seco acid in bivalves, with a short retention time (Miles et al., 2004; Suzuki et al., 2001). In addition, PTX2-seco acid can be further metabolized into fatty acid ester derivatives in bivalves, which have been reported to occur at substantially higher concentrations than free analogues in shellfish tissues (Wilkins et al., 2006). As esterified forms were not included in the present analysis and PTXs are known to be unstable under alkaline hydrolysis conditions commonly used for ester cleavage (Doucet et al., 2007), the presence of esterified PTX metabolites may partly explain the absence of detectable free PTXs in mussel tissues. In contrast, YTXs, including hYTX, persist longer in shellfish tissues (Aasen et al., 2005; Nielsen et al., 2016). This difference explains the distinct time lag between toxin peaks in phytoplankton (May) and mussels (August). LMTs in shellfish can therefore remain at detectable levels long after plankton blooms have subsided, as consistently observed in earlier studies (Kim et al., 2022, 2023). These findings suggest that monitoring programs limited to bloom periods may overlook critical exposure windows. Because mussels retain toxins for extended periods, seafood harvested after the visible bloom has declined can still pose risks to consumers. Effective management should therefore encompass pre-bloom buildup and post-bloom retention periods to ensure seafood safety and accurate risk assessment.

3.4. Compositions of LMTs in SPATT and seawater

To characterize dissolved toxin dynamics, SPATTs were deployed along the south coast of Korea during the study period. Eight compounds were detected in SPATT extracts, including OA, DTX1, YTX, hYTX, PTX2, PTX11, AZA2, and DA. Maximum concentrations reached 98 ng g^{-1} for OA, 204 ng g^{-1} for DTX1, 12 ng g^{-1} for YTX, 454 ng g^{-1} for hYTX, 523 ng g^{-1} for PTX2, 19 ng g^{-1} for PTX11, 2.2 ng g^{-1} for AZA2, and 262 ng g^{-1} for DA (Fig. 4a and Table S11). OA, DTX1, AZA2, and DA were found only in SPATT and not in phytoplankton or mussel samples, highlighting the sensitivity of passive samplers for detecting toxins at low concentrations or in dissolved form (MacKenzie, 2010).

The toxin composition in SPATT extracts reflected the seasonal succession of causative dinoflagellates, indicating close correspondence between toxin occurrence and algal dynamics. In particular, PTX2 and OA followed the seasonal distribution of *Dinophysis* species, whereas YTXs mirrored patterns of *Gonyaulax*-type taxa. These associations are consistent with findings from other coastal environments where SPATT toxin profiles tracked the succession of LMT-producing algae (García-Altares et al., 2016; Hattenrath-Lehmann et al., 2018). Another notable observation was that SPATT often detected peak toxin levels earlier than either phytoplankton or mussel samples. This suggests that dissolved toxins can be detected before algal cell densities rise sufficiently to be reflected in plankton counts or before significant accumulation occurs in shellfish. By integrating exposure over its deployment period, SPATT can capture short-lived or low-level events that may be missed by conventional grab sampling, making it a valuable early-warning tool for harmful algal bloom monitoring and seafood safety programs.

Although OA and DTXs were detected in SPATT extracts, SPATT functions as a passive sampler that integrates dissolved-phase toxins over time and reflects their presence in the surrounding water column rather than bioaccumulation in shellfish tissues (MacKenzie et al., 2004; Fux et al., 2009; Zendong et al., 2016). Consequently, SPATT detections indicate environmental occurrence and temporal trends of dissolved toxins but do not directly represent bioavailable doses or accumulated concentrations in shellfish. Accumulation in bivalve tissues is governed by multiple biological and physiological factors, including species-specific uptake, metabolism, depuration capacity, exposure duration, and toxin form, and therefore does not necessarily correspond directly to dissolved toxin occurrence in seawater (Ochoa-Esteso et al., 2024).

In seawater collected during SPATT deployments, seven toxins were detected: OA, DTX1, YTX, hYTX, PTX2, PTX11, and DA (Fig. 4b and Table S12). Among these, PTX2 accounted for the largest proportion, likely due to its hydrophobicity, which enables persistence in the water column despite adsorption onto suspended particles (Wu et al., 2019). In contrast, OA and DA, both more hydrophilic, were primarily present in

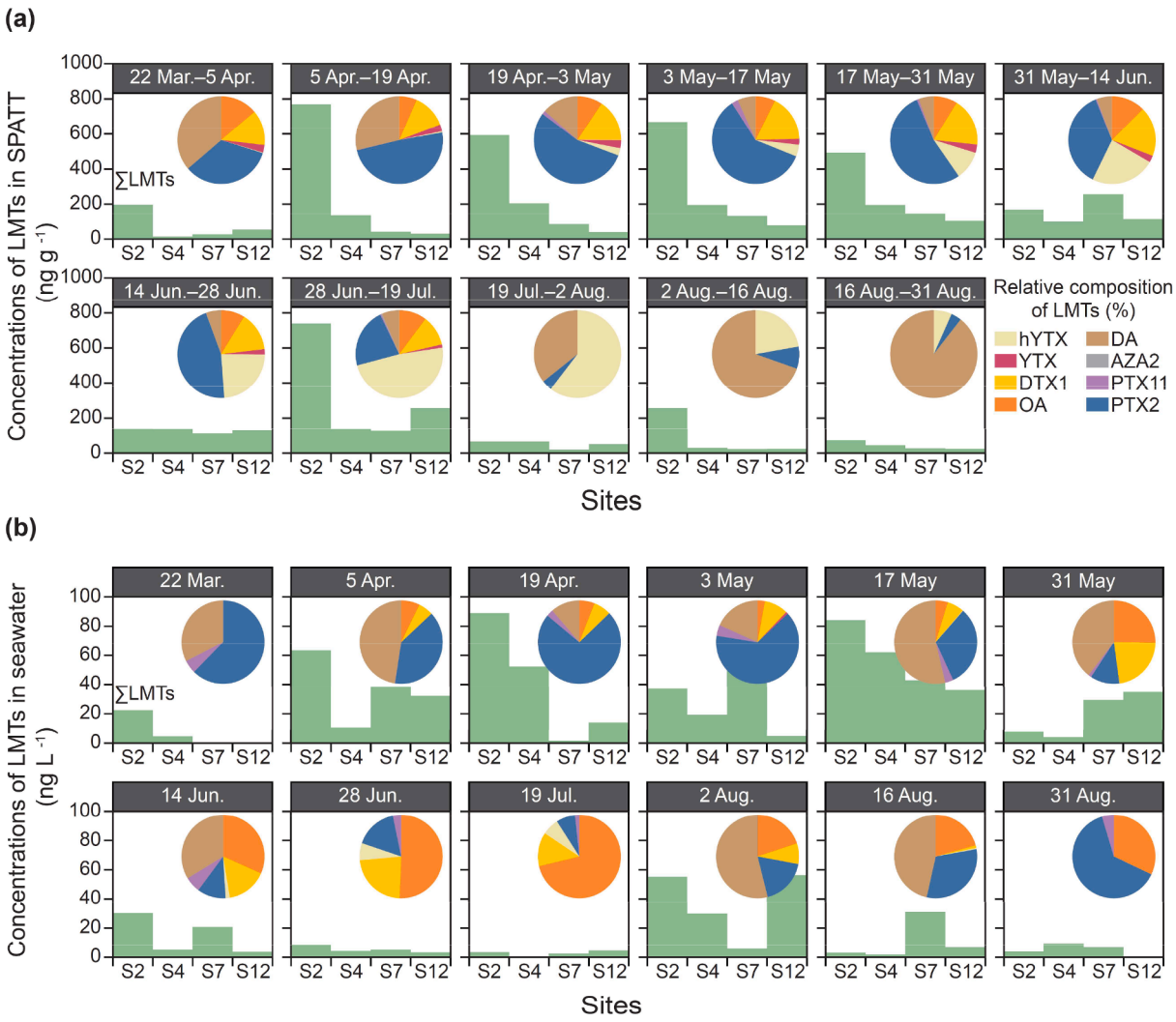

Fig. 4. **(a)** LMT concentrations in SPATT samples from four stations (S2, S4, S7, and S12) during biweekly deployments. Green bars indicate total concentrations of LMTs, and pie charts show the average composition of individual LMTs across the deployment period. **(b)** LMT concentrations and compositions in seawater collected at the time of SPATT retrieval. Pie charts represent the relative proportions of LMT analogs, and bar graphs indicate total LMT concentrations at each station.

dissolved form rather than in phytoplankton or mussels, contributing approximately 25% and 30% of the total LMTs during the deployment period, respectively. The relatively high proportion of DA suggests that this compound remains largely dissolved instead of accumulating in organisms. LMTs are produced within algal cells and released into seawater following cell lysis or senescence. Once dissolved, they may undergo microbial degradation, adsorption onto particles, or dilution, with persistence determined by chemical properties and environmental conditions (Shetty et al., 2010). These findings demonstrate the importance of combining SPATT, seawater, and organism-based monitoring. Such an integrated approach captures both particulate and dissolved toxin fractions, improves bloom forecasting, and strengthens seafood safety measures.

3.5. *Molecular networking of putative LMT-related features*

High-resolution molecular networking was applied to SPATT, phytoplankton, and mussel extracts to explore the diversity and MS/MS spectral similarity patterns of LMT-related features (Fig. 5). Clear differences were observed among the three matrices, with SPATT exhibiting the most complex network. The SPATT-derived network contained dense clusters and multiple nodes connected to PTX2 and PTX11 standards (blue and green nodes), suggesting the presence of PTX-related features with similar MS/MS fragmentation patterns that were not detected in biological samples. This observation is consistent with previous reports of diverse PTX derivatives in *Dinophysis* spp. revealed by molecular networking (Sibat et al., 2021). In contrast, OA- and DTX-related standards (orange and yellow nodes) showed no direct links to SPATT-derived features, suggesting limited or no detection of these compounds in SPATT extracts under the present analytical conditions. Phytoplankton and mussel samples showed comparatively simpler networks (Fig. 5b and 5c). Few nodes were connected to toxin standards, likely reflecting lower concentrations or limited detectability rather than complete absence. This reduced network complexity supports the interpretation that SPATT, by integrating dissolved toxins over time, is more effective in capturing PTX-related spectral features prior to bioaccumulation in organisms.

Previous studies have utilized GNPS-based molecular networking to identify toxin analogs in biological matrices such as phytoplankton or shellfish (Liu et al., 2023; Sibat et al., 2021; Tonon et al., 2020; Wu et al., 2020). However, none have combined GNPS with passive sampling techniques. In the present study, integration of SPATT with GNPS enabled visualization of dissolved-phase LMT-related MS/MS similarity patterns that are not readily evident in organism-based samples. This approach provides complementary information to conventional monitoring and supports the use of molecular networking as a surveillance

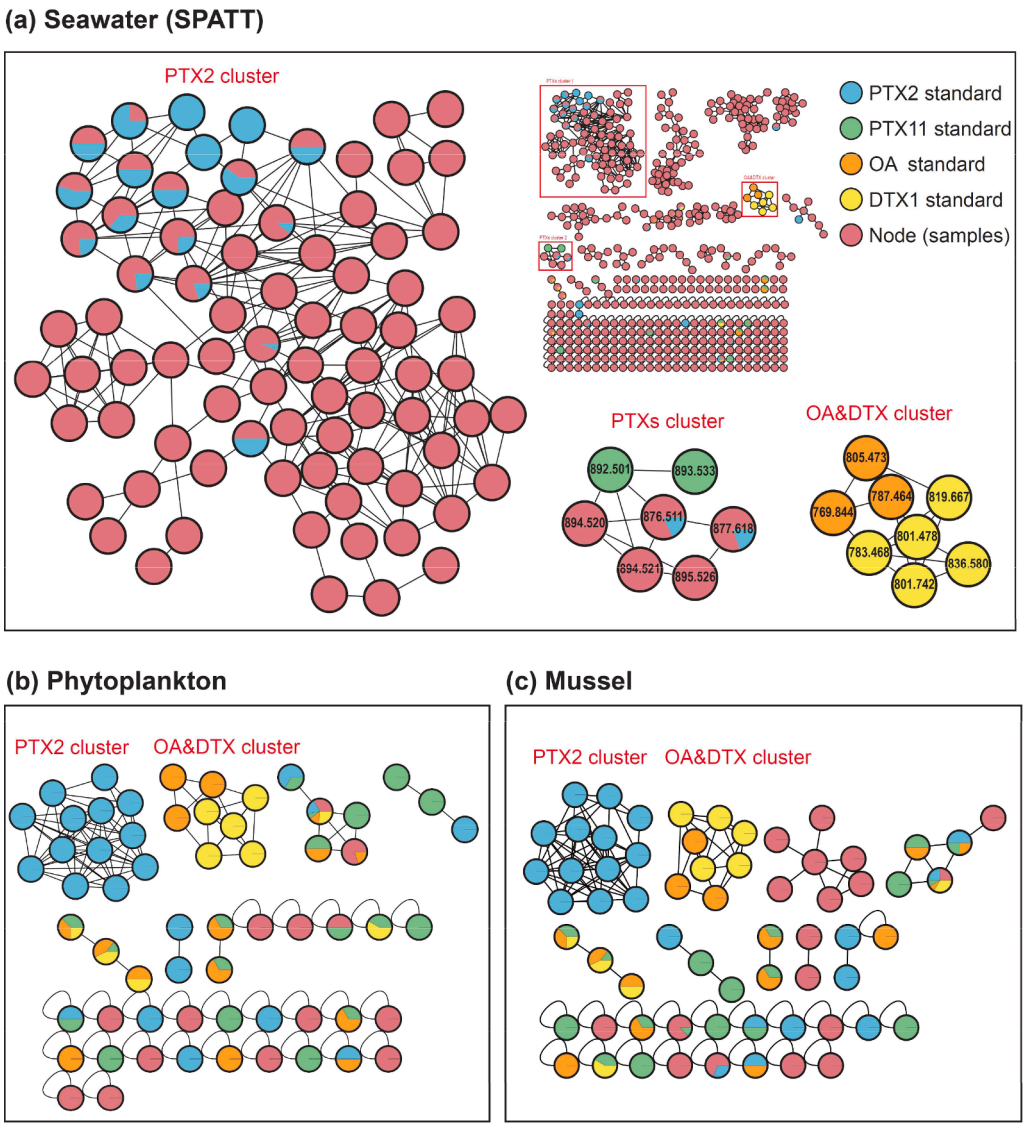

Fig. 5. Molecular networking of LMTs in **(a)** SPATT, **(b)** phytoplankton, and **(c)** mussel extracts from the south coast of Korea in 2022. Each node represents a unique MS/MS feature, and edges indicate cosine similarity based on fragmentation patterns. Standard compounds (PTX2, PTX11, OA, and DTX1) are highlighted in color, and clusters associated with these standards are annotated. Node size reflects relative ion abundance.

tool for characterizing LMT diversity in coastal systems. Nevertheless, a key limitation is that putative features observed in molecular networks were not structurally confirmed, as GNPS does not provide definitive chemical identification. Future studies should incorporate complementary techniques such as nuclear magnetic resonance (NMR), high-resolution isolation, and structural elucidation workflows to verify these compounds and expand our understanding of their toxicological significance. Such efforts will help determine whether these analogs represent novel bioactive metabolites or degradation products, thereby clarifying their roles in toxin dynamics and potential risks to marine food webs.

3.6. Potential risk of LMTs from consumption of commercial shellfish

LMTs were detected in commercially distributed shellfish purchased from seafood markets in Korea. Among the twelve species examined, detectable levels were found only in pen shells and mussels, consistent with previous reports that identified these species as frequent carriers of LMT contamination across multiple years (Kim et al., 2022, 2025). Specifically, only hYTX was detected in these samples, and no OA-, DTX-, PTX-, AZA-, or other YTX-group analogues were observed. Maximum concentrations reached 45 ng g^{-1} ww in mussels and 38 ng g^{-1} ww in pen shells. Both species originated from aquaculture areas in Yeosu and Myeongji along the south coast of Korea, where LMT-producing phytoplankton have been recurrently reported. Compared with field-collected mussels from the same region, market samples generally contained lower LMT concentrations. This pattern is consistent with earlier findings that biotoxin levels in cultured shellfish can decline rapidly after harvest, possibly due to depuration in cleaner water or reduced exposure to toxin-producing algae (Kim et al., 2025). Nevertheless, the seasonal trend in toxin presence, peaking in spring and summer, broadly mirrored patterns observed in phytoplankton and mussels during previous field surveys (Kim et al., 2022, 2023). These observations suggest that commercial bivalves remain influenced by environmental toxin inputs from the south coast.

Despite the relatively low concentrations, a human health risk assessment was conducted using dietary exposure estimates (Table 1 and Table S13). In the present study, dietary exposure and risk assessment were conducted based on free toxin concentrations measured in shellfish samples, as alkaline hydrolysis was not applied during sample preparation. Consequently, the calculated HQs and HIs reflect exposure to free toxin forms only and do not account for esterified OA/DTX (e.g., DTX3),

Table 1
Hazard quotients (HQ) and hazard index (HI) for YTX based on four intake scenarios (S1–S4) using the highest YTX concentrations detected in mussels and pen shells.

Shellfish	Maximum concentrations of YTX (ng g^{-1} ww)	Intake scenarios (S1–S4)	Daily intake (g d^{-1})	EDI (ng kg^{-1} d^{-1})	HQ (%)	HI
Mussel	45	S1	1.10	0.0495	0.0033	0.0034
		S2	15.19	0.2336	0.0150	0.0327
		S3	2.02	0.0909	0.0061	0.0063
		S4	9.48	0.4266	0.0284	0.0286
Pen shell	38	S1	0.03	0.0011	0.0001	0.0034
		S2	6.97	0.2649	0.0177	0.0327
		S3	0.075	0.0028	0.0002	0.0063
		S4	0.09	0.0034	0.0002	0.0286

which may contribute to total toxin equivalents in shellfish. HQs for individual shellfish species ranged from 0.0001% to 0.0381%, and HI, reflecting cumulative exposure across species, remained well below the threshold of 1 in all intake scenarios. These results indicate that exposure to free toxin forms under current consumption scenarios is unlikely to pose significant acute health risks (Evans et al., 2015). In addition, all detected concentrations were far below the acute reference dose for YTX (3750 ng g^{-1} ww) established by EFSA, supporting the conclusion that acute toxicity risk under present conditions is negligible. However, the recurrent detection of LMTs in domestic shellfish raises concerns about potential chronic exposure. As LMTs are not currently regulated in Korea despite their recognition as emerging marine contaminants worldwide, the integration of routine monitoring and risk-based regulatory frameworks is urgently needed to ensure seafood safety and protect public health.

4. Conclusion

This study investigated the occurrence of LMT-producing microalgae and the bioaccumulation of LMTs in phytoplankton and mussels along the south coast of Korea. SPATT detected low concentrations of LMTs not captured by conventional biological monitoring, demonstrating its utility for trace toxin surveillance. Molecular networking analysis highlighted MS/MS spectral similarity patterns of putative LMT-related features, suggesting previously unrecognized chemical diversity in the region without definitive structural identification. Peak LMT concentrations occurred in summer, highlighting the need for intensified monitoring during high-risk periods. LMTs were also detected in commercially available shellfish, particularly mussels and pen shells. Although concentrations were low, dietary exposure assessments based on free toxin forms showed that HQs and HIs remained well below international safety thresholds, indicating minimal acute risk under current consumption levels. As Korea currently lacks regulatory limits for LMTs such as YTXs and PTXs, establishing risk-based regulations and incorporating SPATT into routine monitoring would enhance seafood safety and public health protection.

CRediT authorship contribution statement

Mungi Kim: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Young Kyun Lim:** Investigation, Formal analysis, Data curation. **Youngnam Kim:** Investigation, Formal analysis. **Jihyun Cha:** Investigation, Formal analysis. **Xiaowan Liu:** Methodology, Formal analysis. **Leo Lai Chan:** Writing – review & editing, Methodology, Formal analysis. **Kenneth M.Y. Leung:** Writing – review & editing, Methodology, Formal analysis. **Seung-Ho Baek:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Seongjin Hong:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2026.103067.

Data availability

Data will be made available on request.

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