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Alexandrium catenella (Group I) Causes Higher and Faster Toxicity Than *A. pacificum* (Group IV) in *Mytilus edulis*

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Abstract: Consuming poisoned shellfish can lead to severe health problems and even death. *Alexandrium catenella* (Group I) and *A. pacificum* (Group IV) cause paralytic shellfish poisoning (PSP) in Korea, and PSP is detected more in a wider area. However, the association between toxic dinoflagellates and shellfish poisoning is unclear. Therefore, it is necessary to understand the toxicity, detoxification, and compositional differences in PSP in *Mytilus edulis* exposed to PSP caused by *A. catenella* and *A. pacificum*. High-performance liquid chromatography with post-column oxidation was used to analyze PSP toxicity in poisoned *M. edulis*. PSP in *M. edulis* increased as the *A. catenella* and *A. pacificum* cell density increased. However, the cell density of *A. catenella* peaked faster than that of *A. pacificum*, and a high level of toxicity was detected. In the detoxification experiment, PSP in *M. edulis* decreased rapidly within 24 h in filtered seawater. However, PSP was continuously detected without decreasing below the detection limit until the last day of the experiment. In addition, the carbamate composition (GTX1+4) was detected as the main toxic composition in poisoned *M. edulis*, unlike in vegetative cells. GTX1+4 can poison shellfish quickly when toxic dinoflagellates appear in the marine environment. However, poisoned shellfish take a long time to be completely detoxified. Moreover, if shellfish continuously feed on poisonous dinoflagellates, their toxicity can increase rapidly due to biotransformation. Our results can help identify the mechanisms of shellfish toxicity and detoxification after PSP caused by toxic dinoflagellates.

Keywords: *Alexandrium catenella*; *Alexandrium pacificum*; paralytic shellfish poisoning; *Mytilus edulis*; toxification; detoxification



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1. Introduction

Bivalves feed on particulate organic matter floating in seawater, and toxic plankton can accumulate poison in the shellfish, leading to them being poisoned. Consuming poisoned shellfish can lead to food poisoning and even death [1–7]. Among various shellfish poisons, paralytic shellfish poisoning (PSP) is caused by a toxin produced primarily by the dinoflagellate *Alexandrium* species, *Gymnodinium catenatum*, and *Pyrodinium bahamense*. PSP has been occurring for a long time in coastal environments and has strong heat-resisting properties that are not easily destroyed by general heating and cooking [8–10]. Among the more than 20 *Alexandrium* species, six species are present in Korea (*A. catenella*, *A. affine*, *A. insuetum*, *A. pacificum*, *A. minutum*, and *A. fraterculus*) [11–13]. Moreover, early detection of shellfish toxins accelerates with increasing water temperature, and the area of occurrence also expands [14,15]. Although PSP caused by *A. pacificum* (Group IV), which grows at relatively higher temperatures than *A. catenella* (Group I) [16], has not yet been reported, bivalves in late spring or early summer may be toxified due to climate change.

Aquaculture in the coastal areas of Korea, dominated by seaweed and shellfish, accounts for over 60% of Korea's fishery production [17]. However, during spring in the

southeastern coastal area of Korea, the PSP in shellfish aquaculture caused by *A. catenella* exceeds the regulatory limit ($<80 \mu\text{g}/100 \text{g}$ of tissue) annually. The blue mussel *Mytilus edulis* is the second most productive shellfish after the Pacific oyster (*Crassostrea gigas*) and is produced in large quantities on the southern coast of Korea [18]. *M. edulis* is a filter feeder more easily poisoned than other bivalves under the same environmental conditions [19–21] and has high bioaccumulation [22–24]. Thus, mussels are typical biomarkers that provide an early warning of PSP, as they accumulate PSP and reach high toxicity faster than other species [25].

However, the PSP of bivalves is occasionally unrelated to toxic dinoflagellate exposure according to the increasing and decreasing cell density of toxic dinoflagellates in coastal areas [26] because the maximum toxicity of bivalves does not always match the maximum cell density of toxic dinoflagellates [27,28]. Moreover, PSP in bivalves has been observed even in coastal areas where the cell density of toxic dinoflagellates is extremely low or where appearances have not been reported yet [26,27,29,30]. Therefore, the relationship between toxic dinoflagellates and shellfish poisoning remains unclear.

This study investigated the toxicity and detoxification of *M. edulis* after exposure to *A. catenella* and *A. pacificum* to clarify the environmental conditions contributing to PSP caused by toxic dinoflagellates in the natural environment. We discuss the changes in toxin amount, toxicity, and toxicity composition during toxification and detoxification.

2. Materials and Methods

2.1. Microalgae Culture

A. catenella (JM-1) was collected from the surface seawater of Jangmok Bay in January 2021. The cells were separated with a Pasteur pipette (length 230 mm) and washed with filtered seawater (0.22 μm pore size, Millipore, Burlington, MA, USA) to establish monoclonal cells. *A. pacificum* (LIMS-PS-2611) was obtained from the Library of Marine Samples at the Korea Institute of Ocean Science and Technology. f/2 medium [31] based on the Southern Sea of Korea was used, and the incubation temperature, salinity, and light conditions were 15 °C, 30 psu, and 300 $\mu\text{mol}/\text{m}^2/\text{s}$ (12-h period), respectively, to maintain the *A. catenella* and *A. pacificum* cultures.

Two types of mass cultures were used for the exposure experiments. The batch culture for the initial culture was started in 250 mL of f/2 medium, and the culture conditions were the same as those for the maintenance culture. A mass culture system was then constructed by inoculating the two species in 10 L and 30 L water tanks, respectively, and the exposure experiment was performed by culturing until the late exponential phase.

2.2. Toxification of *M. edulis* by Continuous Exposure to *A. catenella* and *A. pacificum*

M. edulis were collected from the tidal zone of Jangmok Bay. The shell length, height, width, and wet weight were $65.34 \pm 5.93 \text{ mm}$, $24.04 \pm 2.59 \text{ mm}$, $35.86 \pm 2.67 \text{ mm}$, and $5.52 \pm 1.4 \text{ g}$, respectively. *M. edulis* transported to the laboratory were acclimatized ($15 \pm 1 \text{ }^\circ\text{C}$; DBA-075, Daeil Cooler Co., Busan, Republic of Korea) in 10 L filtered (GF/C filter; 1.2 μm pore size) seawater in a 20 L water tank for 3–5 days, and aerated using an aquarium air pump. The same conditions were maintained during the experimental period, and freshly filtered seawater was exchanged once every two days.

Total 18 *M. edulis* individuals was continuously exposed to *A. catenella* and *A. pacificum* for five days. *A. catenella* at cell densities of 10, 100, and 1000 cells/mL and *A. pacificum* at cell densities of 10 and 100 cells/mL were constantly inoculated into the experimental tank containing *M. edulis* under $15 \pm 1 \text{ }^\circ\text{C}$, 30 psu, 100 $\mu\text{mol}/\text{m}^2/\text{s}$ (12-h period), similar to the acclimatization conditions (Figure 1). Three *M. edulis* individuals in the experimental tank were randomly collected at the same time every day, and their whole flesh was analyzed. An aquarium air pump was used to prevent suffocation, and every effort was made to protect the *M. edulis* from external stimuli during the experimental period. Before initiating the experiment, the toxicity of *M. edulis* was below the detection limit (Day 0). Three *M. edulis* individuals in the experimental tank were randomly collected at the same time

every day, and their whole flesh was analyzed. To compare the toxic compositions of *Alexandrium* species and *M. edulis*, the toxic compositions of *Alexandrium* species were analyzed immediately before exposure.

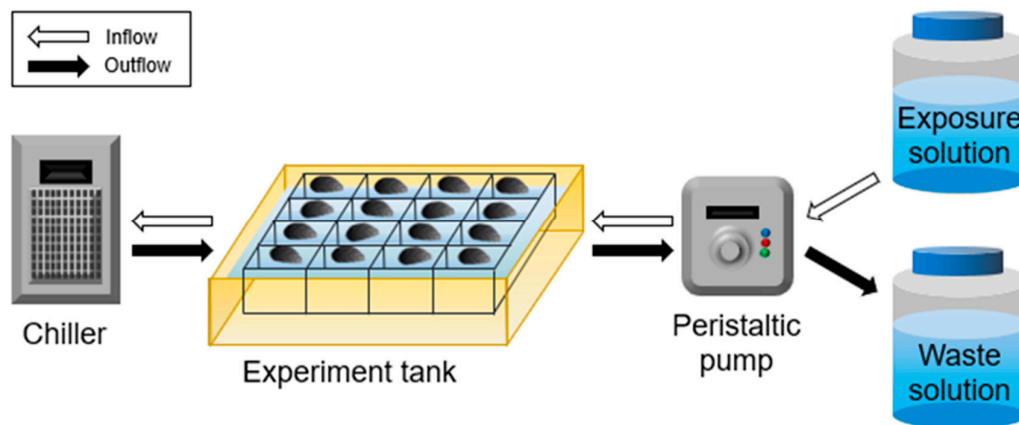


Figure 1. Schematic of the experimental design for continuous exposure of *Alexandrium catenella* (Group I) and *A. pacificum* (Group IV) to *Mytilus edulis*. *A. catenella* at cell densities of 10, 100 and 1000 cells/mL and *A. pacificum* at cell densities of 10 and 100 cells/mL were constantly exposed to the experimental tank containing *M. edulis* under 15 ± 1 °C, 30 psu, and $100 \mu\text{mol}/\text{m}^2/\text{s}$. The exposure solution contained the dinoflagellates. Exposure solution was 10 L, and the experimental tank was 20 L.

2.3. Detoxification of *M. edulis* Poisoned by *A. catenella* and *A. pacificum*

The exposure solution (10 L) containing *A. catenella* and *A. pacificum* was exposed to the 20 L experimental tank containing *M. edulis* (maximum cell densities of *A. catenella* and *A. pacificum* were approximately 2500 and 4500 cells/mL, respectively). Microscopic observations confirmed that *M. edulis* consumed *A. catenella* and *A. pacificum* in the exposure solution for 20 min. The experimental conditions of temperature, salinity, and light intensity were identical to those used for the continuous exposure experiments. For the detoxification analysis, three *M. edulis* individuals were randomly collected from the experimental tank at the same time each day, and their whole flesh was analyzed. The initial toxicity to *M. edulis* (day 0), before exposure, was below the detection limit. One day after the first exposure to *Alexandrium*, the entire volume of seawater in the experimental tank was replaced with freshly filtered seawater. The experiment was conducted over nine days.

2.4. PSP Analysis Using High-Performance Liquid Chromatography

PSP toxicity and toxin composition were measured in the whole flesh of three *M. edulis* individuals collected at 16:00 on the first, second, third, fourth, and fifth days after continuous exposure to *A. catenella* and *A. pacificum*. Fresh samples from three *M. edulis* individuals and two species of *Alexandrium* were immediately pretreated to toxin analysis by the following protocol. The supernatants were removed by centrifuging ($3000 \times g$, 10 min; Combi-514R, Hanmail Scientific Inc., Deajeon, Republic of Korea) using cells in the late exponential growth phase to analyze the PSP of *A. catenella* and *A. pacificum*. Then, 0.5 N acetic acid was added, and after freezing for 24 h (-20 °C), the cells were fragmented using a sonicator, and the supernatant was fractionated through centrifugation ($3000 \times g$, 10 min). The aliquoted supernatant was filtered with an ultrafiltration filter (10,000 MW, Millipore; Merck Millipore, Billerica, MA, USA) and frozen (-20 °C) until analysis.

For *M. edulis* PSP analysis, the same amount of 0.1 N HCl as the weight of the whole flesh was added, and the tissue was fragmented using a sonicator. After boiling at 100 °C for 5 min, the mixture was centrifuged at $3000 \times g$ for 10 min. The supernatant was passed through a Sep-pak C18 cartridge (Waters Associates Inc., Milford, MA, USA), filtered through an ultrafilter (10,000 MW), and frozen (-20 °C) until analysis. All pretreated

samples were subjected to a HPLC (high-performance liquid chromatography) with fluorescence detector (UtiMate 3000, Thermo Fisher Scientific, Sunnyvale, CA, USA) with post-column derivatization and a Hypersil Gold C8 column (250 mm × 4.6 mm i.d., particle size 5 µm) as previously described by Oshima [32]. The toxin content and composition of the samples were determined by comparing the detection time and peak area with those of a standard toxin. The toxicity of the mussels was calculated from the calculated toxin content using the non-toxicity value [32]. Standard toxins (N-sulfocarbamoyl toxin 1 and 2, gonyautoxin 1 and 4, gonyautoxin 2 and 3, gonyautoxin 5 and 6, neosaxitoxin, and saxitoxin) were purchased from the National Research Council Institute for Marine Biosciences. Abbreviations of toxins are as follows: C1+2 = N-sulfocarbamoyl toxin 1+2, GTX 1+4 = gonyautoxin 1+4, GTX 2+3 = gonyautoxin 2+3, GTX 5+6 = gonyautoxin 5+6, neoSTX = neosaxitoxin, STX = saxitoxin.

3. Results

3.1. *A. catenella* Causes Higher Toxicity in *M. edulis* Than *A. pacificum*

When *M. edulis* was exposed to 10 cells/mL of *A. catenella*, the toxin amount and toxicity accumulated in *M. edulis* gradually increased from the first day after exposure (Figure 2a). However, no values exceeding the regulatory limit of 4 MU/g were observed. At 100 cells/mL (Figure 2b), the toxin amount and toxicity increased continually by day 4. However, the toxicity exceeded the regulatory limit from day 1 (4.86 MU/g). On the fifth day, the toxicity was approximately 17.5 times higher than the maximum toxicity (1.51 MU/g) when *M. edulis* was exposed to 10 cells/mL *A. catenella*. After exposing *M. edulis* to 1000 cells/mL (Figure 2c), the accumulated toxin amount in *M. edulis* on the fifth day was the highest at 65.1 nmoL/g. This toxin amount was approximately 35 and 3.76 times higher than that after exposure to 10 and 100 cells/mL, respectively. The toxicity increased rapidly from the second day and exceeded the regulatory limit. On the fifth day, the toxicity was 41.7 and 23.8 times higher than that of exposure to 10 and 100 cells/mL, respectively.

The toxin amounts and toxicities of *M. edulis* exposed to *A. pacificum* were lower than those exposed to *A. catenella* (Figure 3a). When *M. edulis* was exposed to 10 cells/mL of *A. pacificum*, the toxin amount and toxicity increased; however, the values were much lower than those in the 10 cells/mL continuous exposure experiment of *A. catenella*. Toxin amounts and toxicities increased with increased time after exposure to 100 cells/mL and were the highest on day 5 (Figure 3b); however, the toxicity was approximately 5.2 times lower than the maximum toxicity achieved by exposure to 100 cells/mL of *A. catenella*. Considering the *M. edulis* toxicities after continuous exposure to *A. pacificum* at 10 and 100 cells/mL, the toxicities of *M. edulis* continually exposed to 1000 cells/mL of *A. pacificum* may be much lower than those of *A. catenella*, although an exposure of 1000 cells/mL of *A. pacificum* was not tested.

3.2. Fresh Seawater Detoxifies *M. edulis*

The toxin content and toxicity in *M. edulis* exposed to *A. catenella* reached the maximum values on the first day after exposure, exceeding the shipping prohibition toxicity standard value (Figure 4a). On the second day, after the water was replaced with fresh seawater, the toxin content and toxicity decreased sharply to below the shipping prohibition toxicity standard value and continued to maintain a similar level until the ninth day. The maximum toxin content and toxicity were detected on the second day after *A. pacificum* exposure. However, the maximum toxin content and toxicity of *A. pacificum* were lower than those of *A. catenella* even though the cell density of *A. pacificum* was approximately 1.8 times higher than that of *A. catenella* (Figure 4b). The toxin content and toxicity rapidly decreased from the third day, and then gradually decreased until the last day.

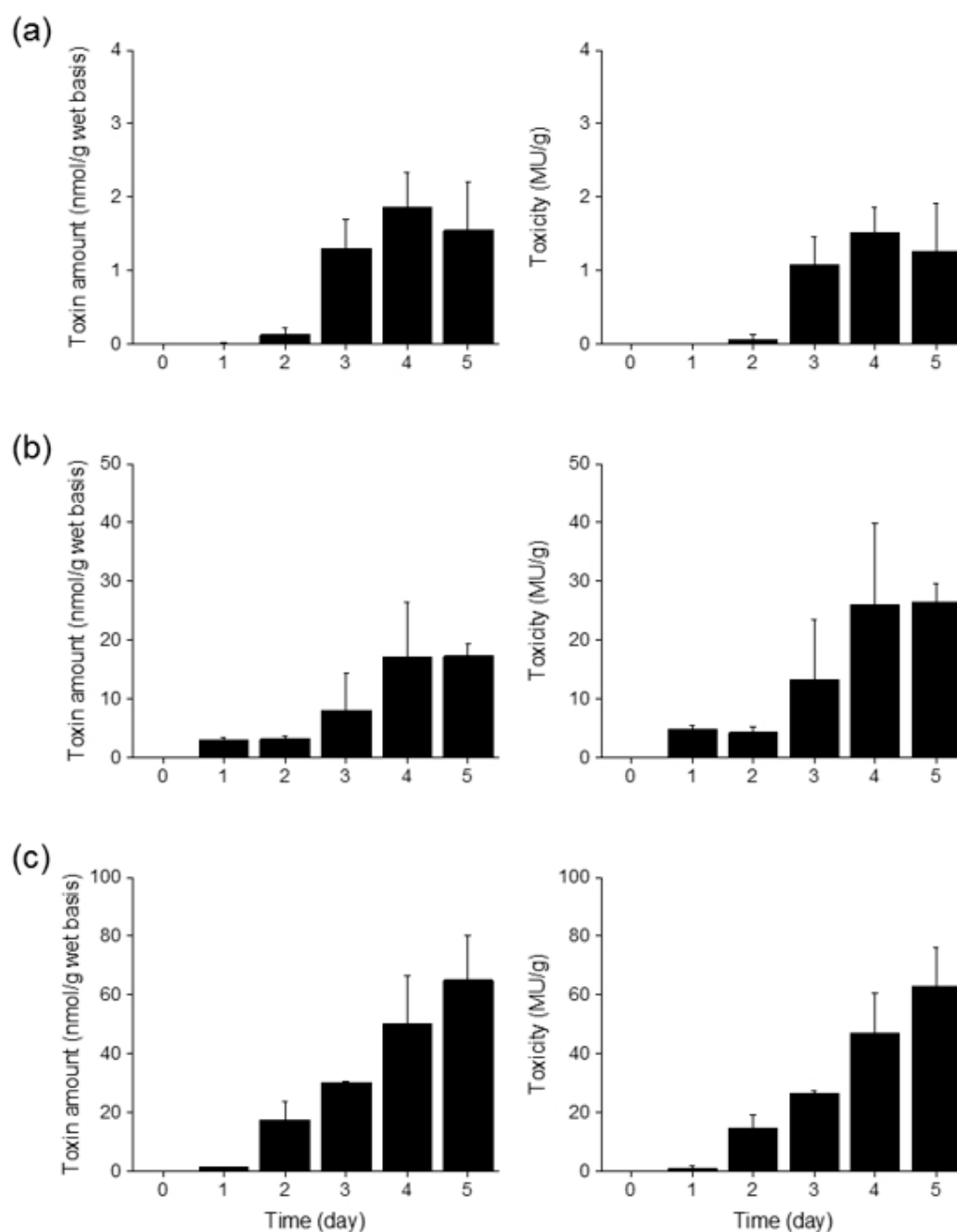


Figure 2. Changes in toxin content and toxicity of *Mytilus edulis* exposed to *Alexandrium catenella* (Group I) at a cell density of 10 (a), 100 (b), and 1000 cells/mL (c).

3.3. GTX1+4 Is the Most Prevalent Composition in *M. edulis* Poisoned by *A. catenella* and *A. pacificum*

Among the toxic compositions of *A. catenella* vegetative cells used in this study, C1+2, GTX1+4, and neoSTX were the main compositions, whereas GTX2+3 and GTX5 were minor compositions (Table 1, Figure 5). The toxic compositions of *A. pacificum* vegetative cells comprised C1+2 and GTX 5 as the main compositions, and neoSTX as the minor composition (Table 1, Figure 6).

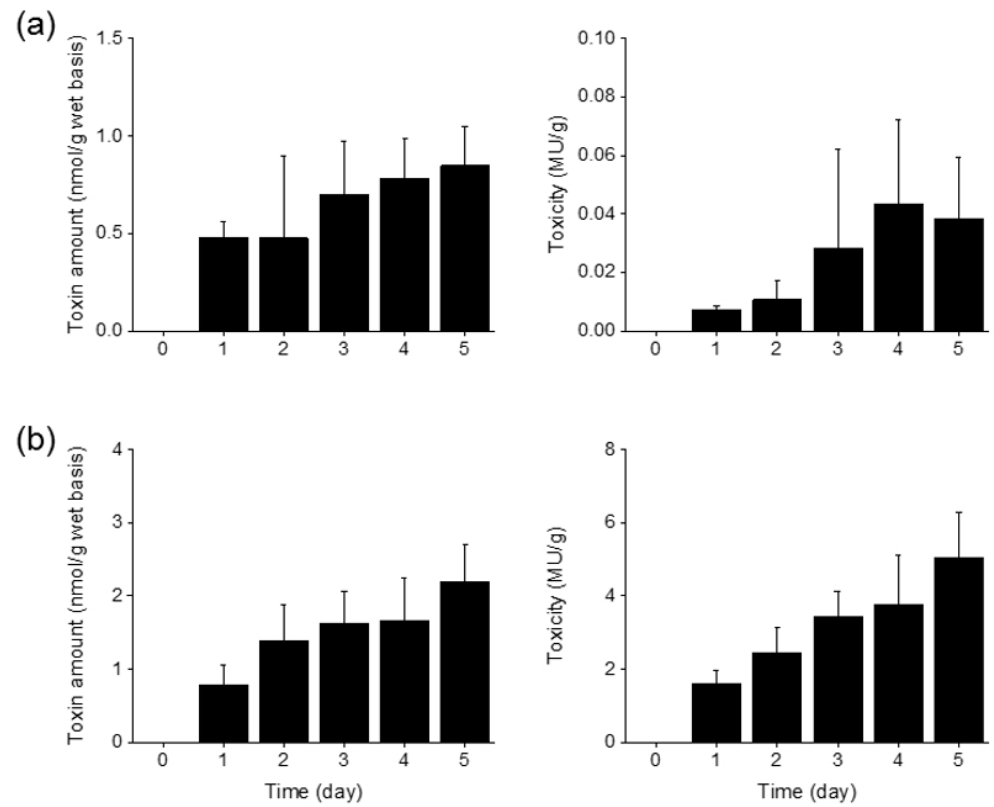


Figure 3. Changes in toxin content and toxicity of *Mytilus edulis* exposed to *Alexandrium pacificum* (Group IV) continuously exposed at a cell density of 10 (a) and 100 cells/mL (b).

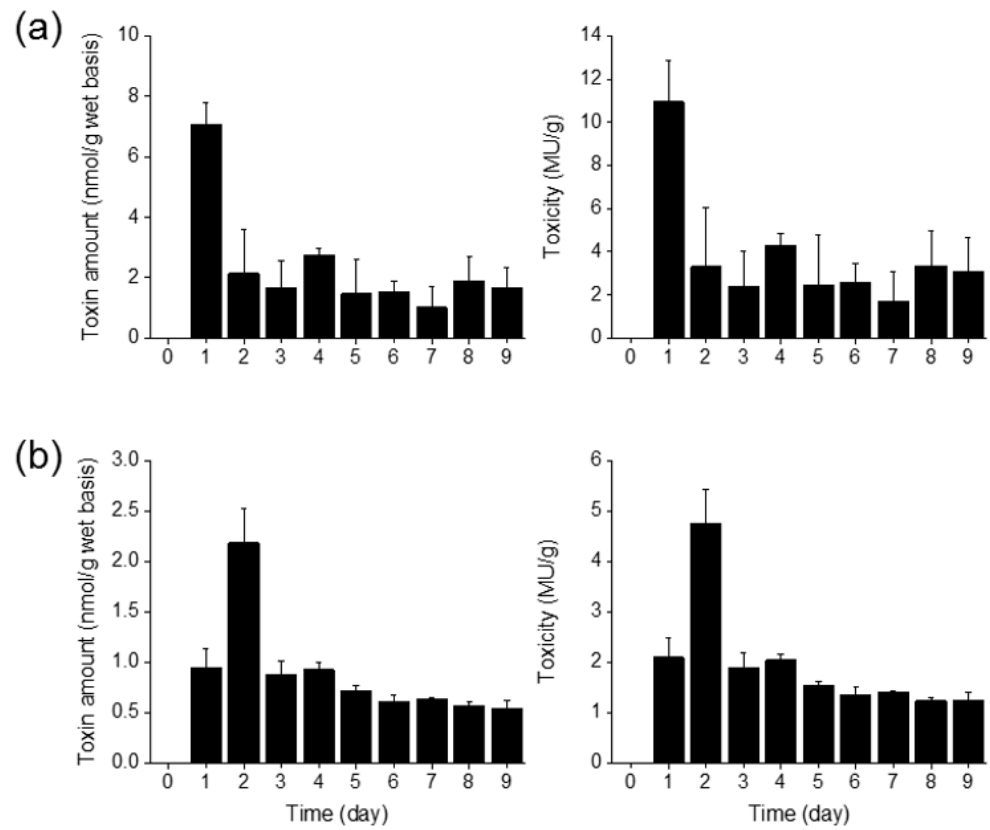


Figure 4. The comparison of the toxin content and toxicity of *Mytilus edulis* after a one-time exposure to *Alexandrium catenella* (Group I) (a) and *A. pacificum* (Group IV) (b).

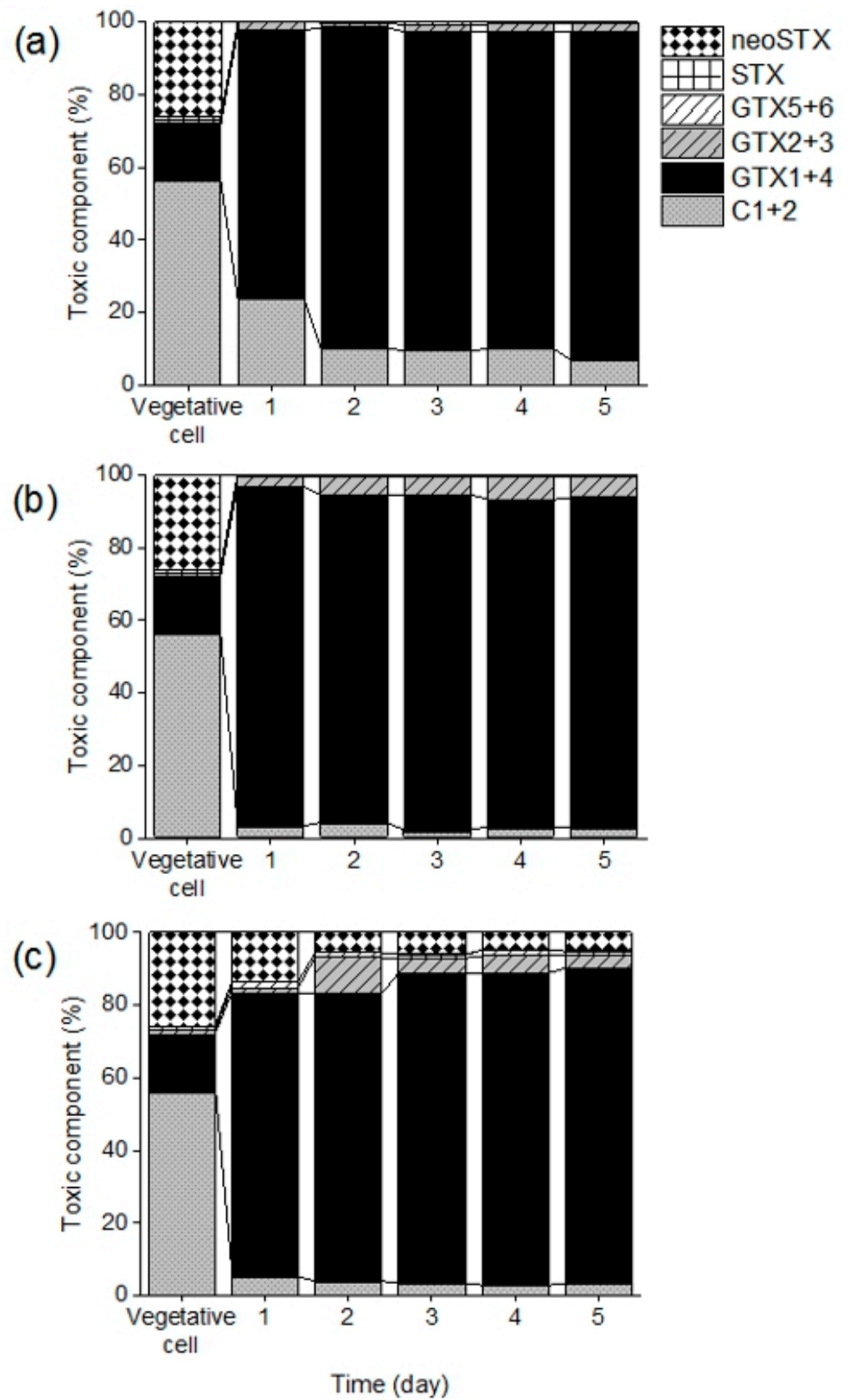
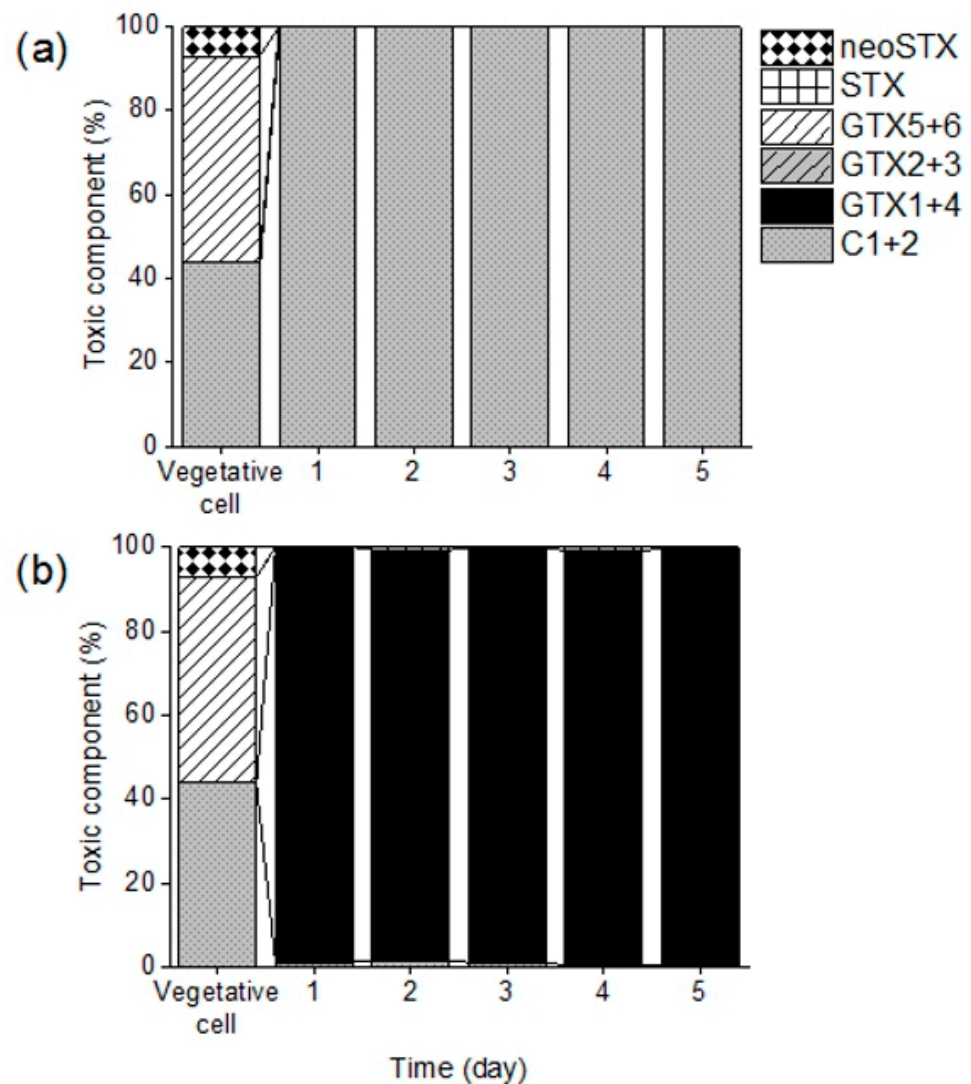


Figure 5. Changes in the toxic compositions of *Mytilus edulis* exposed to *Alexandrium catenella* (Group I) at a cell density of 10 (a), 100 (b), and 1000 cells/mL (c).

Table 1. Toxic contents detected in *Alexandrium catenella* (Group I) and *A. pacificum* (Group IV) in this study.

Toxin	<i>A. catenella</i> (fmol/Cells)	<i>A. pacificum</i> (fmol/Cells)
C1+2	37.91 ± 5.17 *	9.73 ± 1.13
GTX 1+4	10.96 ± 0.30	-
GTX 2+3	0.16 ± 0.02	-
GTX 5	0.72 ± 0.07	10.67 ± 0.26
neo STX	17.49 ± 1.46	1.49 ± 0.21
Total	67.24 ± 6.83	21.89 ± 0.61

Note: * Mean and standard deviation were calculated from cellular toxic cotents in the late exponential phase after 2 times repeatedly incubating to each same strain.

**Figure 6.** Changes in the toxic compositions of *Mytilus edulis* when continuously exposed to *Alexandrium pacificum* (Group IV) at a cell density of 10 (a) and 100 cells/mL (b).

When *M. edulis* was exposed to *A. catenella* at a cell density of 10 cells/mL (Figure 5a), GTX1+4 was the most prevalent composition in *M. edulis*. On the first day of exposure, GTX1+4 and C1+2 accounted for 54.7% and 45.1%, respectively. On the second day, GTX1+4 expression increased substantially and was maintained until the fifth day. When *M. edulis* was exposed to *A. catenella* at a cell density of 100 cells/mL (Figure 5b), GTX1+4 increased considerably from the first day after exposure, unlike that at 10 cells/mL. After that, a

similar trend was observed until the fifth day. When *M. edulis* was exposed to *A. catenella* at a cell density of 1000 cells/mL (Figure 5c), GTX1+4 accounted for 78.1% of the primary composition, similar to that at 100 cells/mL. Unlike in 10 and 100 cells/mL, neoSTX was detected as a minor composition (13.7%). When *M. edulis* was exposed to 10 cells/mL *A. pacificum*, the toxic composition of *M. edulis* was C1+2 from days 1 until 5 (Figure 6a). Unlike in 10 cells/mL *A. catenella*, no carbamate toxins were detected. However, when 100 cells/mL *A. pacificum* was exposed to *M. edulis* (Figure 6b), the toxic composition of GTX1+4 on the first day accounted for approximately 98.8% of the main compositions. C1+2 was detected as a minor composition, and the pattern was similar until the fifth day.

4. Discussion

Paralytic shellfish toxin in shellfish is expected to increase with the abundance of toxic dinoflagellates, including *Alexandrium* species. Continuous exposure to increasing *A. catenella* and *A. pacificum* cell densities rapidly increased the toxin amount and toxicity in *M. edulis*. Similarly, the toxicity of the *Corbicula japonica* clam gradually increased with the *A. tamarense* cell density, and the toxicity showed maximum values one week after exposure to the maximum *A. tamarense* cell density [33]. Furthermore, toxicity in mussels is associated with the abundance of *A. catenella* in Korean coastal areas [34]. Thus, if toxic dinoflagellates appear around bivalve aquaculture sites, the bivalves may be poisoned quickly. However, in some cases, bivalves were observed with PSP below the detection limit, even though the cell density of *A. catenella* and *A. pacificum* was high. Moreover, shellfish toxicity did not decrease, although non-toxic phytoplankton such as diatoms were dominant [35]. Discrepancies in the relationship between the cell density of toxic dinoflagellates and the toxicity of shellfish might have occurred because of biological factors such as temperature, salinity, and nutrients [36–41].

The paralytic shellfish toxin of *M. edulis* rapidly decreased within 24 h in the filtered seawater; however, it was continuously detected without decreasing below the detection limit until the last day of the experiment. The toxicity of low-poisoned (84 µg/100 g) blue mussels was reduced by approximately 36% after 10 days and decreased by approximately 40% after 1 month using a flow water tank, which was continuously supplied with seawater containing free toxic dinoflagellates. However, in highly poisoned (2684 µg/100 g) blue mussels, the toxicity was reduced by approximately 94% after 5 days and decreased by approximately 97% after 1 month [42]. Similarly, the toxicity of poisoned bivalves in a flow-water tank was reduced by 90% after 5 days, and toxicity was not detected after 1 month [43]. The detoxification of poisoned mussels possibly depends on excreting seawater from the gut containing digested or poorly digested toxic dinoflagellate cells in the early stages [44]. In contrast, Sekiguchi et al. [45] speculated that another unknown mechanism in toxin accumulation in the food chain involves toxin accumulation in bivalves. We hypothesized that the rapidly decreasing toxicity of mussels poisoned by *A. catenella* and *A. pacificum* in the early stages could be due to replacing the water with new filtered seawater. However, detoxification above 90% may require considerable time when using only filtered seawater.

Feeding activity is essential to detoxifying poisoned bivalves [46]. In a study on the detoxification rate of oysters exposed to *A. minutum*, the detoxification rate differed by more than two-fold depending on the presence of organic matter, and activating feeding behavior was due to promoting the overall metabolism (i.e., decomposition and excretion) of the intestinal excretion rate [47,48]. The results of our study, conducted under controlled conditions such as filtering seawater, would have differed from those of a natural environment where a large amount of organic matter exists. Therefore, to completely detoxify poisoned shellfish over a short period, continuously feeding non-toxic algae or moving shellfish to sea areas without toxic dinoflagellates rather than in a filtered seawater environment would be more effective. In Canada and the United States, detoxifying shellfish is achieved by moving poisoned shellfish to an area where no toxic plankton have been detected [49–51].

The toxic compounds produced by dinoflagellates remain constant and species-specific despite dramatic variations in toxicity [43,52–56]. Thus, the toxic composition has been

used as a biochemical marker [55,57,58]. Although the toxin compositions of identified *Alexandrium* species in Korean coastal waters have rarely been reported, Kim et al. [57] and Cho and Lee [59] reported that the toxic compositions of *A. tamarense* in the southeastern coastal waters of Korea were C1 and neoSTX as major compositions, and GTX 1+4, GTX 2+3, and dcGTX 2+3 as minor compositions. However, STX and GTX 5 could not be detected in *A. tamarense* strains [59]. In addition, the major toxins produced by *A. catenella* (Group I) were C1+2, and those produced by *A. tamarense* (Group IV) were C1+2 and GTX4 in most isolates from similar coastal areas [60]. Recently, Baek et al. [34] found C1+2, GTX-1, and GTX-2 in *A. catenella* (Group I) bloom samples from the Geoje coast. In contrast, Shin et al. [16] found that GTX 3+4 (~90 mol%) was the dominant PSTs in *A. catenella* (Group I) and *A. pacificum* (Group IV). C2 (7.8 mole%) was the next most toxic compound in both species.

However, toxic compositions vary significantly with the growth phase and under different environmental conditions, such as temperature, salinity, and nutrients [36,38,39,41]. High salinity increases GTX 2+3 in *A. minutum* [36]. Furthermore, variations in toxic composition during exponential growth in batch cultures of *Alexandrium* isolates were also reported. The largest changes in the toxic composition occurred in response to variations in temperature [38]. Furthermore, the response of toxin composition under phosphorous depletion varied with the nitrogen source, such as urea, NH₄, and NO₃ [40]. Changes in the toxic composition of dinoflagellates under different nutrient conditions are strain-specific because apparent regular patterns of toxic composition changing under different nutrient conditions were not observed [39].

Poison in bivalves has a higher carbamoyl toxin than the *N*-sulfocarbamoyl toxin, which differs from the toxicity of ingested swimming cells [47,61,62]. When *Aulacomy ater* was exposed to *A. catenella* swimming cells, the main compositions of C1+2, GTX1+4, and GTX2+3 accounted for most of the primary toxic compositions in the bivalves [63]. In addition, the toxins detected in *A. catenella* swimming cells in the field were C1, GTX1, and GTX2. However, in *M. galloprovincialis* fed with them, toxic compositions were detected in the order of GTX1+4, GTX3, and STX [34]. Toxic dinoflagellates are converted into toxic carbamoyl toxins in shellfish tissues through hydrolysis at low pH and natural reducing agents when many unstable *N*-sulfocarbamoyl toxins are present owing to chemical toxin conversion [64–66]. In bivalves that feed on toxic dinoflagellates, chemical toxin conversion [65,66] through acid hydrolysis and natural reducing agents at low pH and bioconversion [32,47,67] through enzymatic reactions occur. Therefore, even if the toxic dinoflagellates have low toxicity owing to a large amount of *N*-sulfocarbamoyl toxin, it is likely that the toxicity level will be converted into a high-toxicity composition by the ingested dinoflagellates, which will increase the toxicity of shellfish and lead to public health risks. In this study, *A. catenella* required careful monitoring because this toxin conversion phenomenon was evident, and *A. pacificum* was expected to convert to a high level of toxicity in bivalves with continuous exposure.

In conclusion, *M. edulis* was exposed to toxic dinoflagellates, and toxicity was detected after 24 h. PSP accumulated in *M. edulis* was detected at a higher level when fed *A. catenella* than when fed *A. pacificum* and reached its maximum value quickly. The poisoned *M. edulis* was reduced to below the permissible PSP level within a short time in the absence of toxic dinoflagellates; however, it is expected that it will take a considerable amount of time to detoxify *M. edulis* below the detection limit completely. In addition, the *N*-sulfocarbamoyl composition (C1+2) was the main composition responsible for the toxicity of *A. catenella* and *A. pacificum* swimming cells, but the carbamate composition (GTX 1+4) was detected as the main composition because of biotransformation in *M. edulis* fed with it. Our study results can help to clarify the environmental conditions of PSP generation caused by toxic plankton in the future and help establish a preliminary forecast system for PSP. Therefore, continuous monitoring over more than 9 days is necessary to minimize damage. Furthermore, research on the differences in toxic compositions according to numerous *A. catenella* and *A. pacificum*

strains and the changing toxic compositions under different environmental conditions is necessary to understand the PSP outbreak in Korean coastal areas.

Author Contributions: S.J.O. analyzed the data, designed the study and prepared the manuscript. S.Y.J. performed the experiments. M.S. participated in the design of the study and played the role of the corresponding author. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All organisms used for this research were phytoplankton and invertebrate bivalves and as such were not subject to the Korean Institutional Animal Care and Use Committee.

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Edebo, L.; Lange, S.; Li, X.P.; Allenmark, S.; Lindgren, K.; Thompson, R. Seasonal, geographic, and individual variation of okadaic acid content in cultivated mussels in Sweden. *APMIS* **1988**, *96*, 1036–1042. [[CrossRef](#)] [[PubMed](#)]
- Silvert, W.; Rao, D.V.S. Dynamic model of the flux of domoic acid, a neurotoxin, through a *Mytilus edulis* population. *Can. J. Fish. Aquat. Sci.* **1992**, *49*, 400–405. [[CrossRef](#)]
- Taylor, F.J.R.; Fukuyo, Y.; Larsen, J.; Hallegraeff, G.M. Taxonomy of harmful dinoflagellates. In *Manual on Harmful Marine Microalgae*; Hallegraeff, G.M., Anderson, D.M., Cembella, A.D., Eds.; UNESCO: Paris, France, 2003; pp. 389–432.
- Anderson, D.M. Turning back the harmful red tide. *Nature* **1997**, *388*, 513–514. [[CrossRef](#)]
- Indrasena, W.M.; Gill, T.A. Thermal degradation of partially purified paralytic shellfish poison toxins at different times, temperatures, and pH. *J. Food Sci.* **2000**, *65*, 948–953. [[CrossRef](#)]
- Toyofuku, H. Joint FAO/WHO/IOC activities to provide scientific advice on marine biotoxins (research report). *Mar. Pollut. Bull.* **2006**, *52*, 1735–1745. [[CrossRef](#)]
- EFSA. Marine biotoxins in shellfish—Saxitoxin group. *EFSA J.* **2009**, *1019*, 1–76.
- Asakawa, M.; Takagi, M. Quantitative Relationship between PSP Components of *Protogonyaulax tamarensis* and of Scallops, from Funka Bay, Hokkaido. *Bull. Fac. Fish. Hokkaido Univ.* **1983**, *34*, 345–349.
- Takata, K.; Mizuta, M.; Monden, T. Reduction in toxicity of PSP-infested oysters by heat treatment. *Food Hyg. Saf. Sci.* **1994**, *35*, 624–630. [[CrossRef](#)]
- Mizuta, M.; Takata, K.; Monden, T.; Yoneda, T.; Yamauchi, S. Reduction in toxicity of PSP infested oysters during canning process. *Food Hyg. Saf. Sci.* **1995**, *36*, 423–427. [[CrossRef](#)]
- Kim, K.Y.; Matoko, Y.; Yasuwo, F.; Kim, C.H. Morphological observation of *Alexandrium tamarensis* (Lebour) Balech, *A. catenella* (Whedon et Kofoid) Balech and one related morphotype (Dinophyceae) in Korea. *Algae* **2002**, *17*, 11–19. [[CrossRef](#)]
- Oh, S.J.; Park, J.A.; Kwon, H.K.; Yang, H.S.; Lim, W. Ecophysiological studies on the population dynamics of two toxic dinoflagellates *Alexandrium tamarensis* and *Alexandrium catenella* isolated from the Southern Coast of Korea-I. Effects of temperature and salinity on the growth. *J. Korean Soc. Mar. Environ. Energy* **2012**, *15*, 133–141. [[CrossRef](#)]
- Kim, E.S.; Li, Z.; Oh, S.J.; Yoon, Y.H.; Shin, H.H. Morphological identification of *Alexandrium* species (Dinophyceae) from Jinhae-Masan Bay, Korea. *Ocean Sci.* **2017**, *52*, 427–437. [[CrossRef](#)]
- Kim, C.H.; Lee, J.S. Occurrence of toxic dinoflagellates and PSP toxin profiles in *Alexandrium* spp. from Jinhae bay, Korea. In *Harmful and Toxic Algal Blooms*; Yasumoto, T., Oshima, Y., Fukuyo, Y., Eds.; UNESCO: Paris, France, 1996; pp. 61–64.
- Lee, K.J.; Kwon, S.J.; Jung, Y.J.; Son, K.T.; Ha, K.S.; Mok, J.S.; Kim, J.H. Comparison of analytical methods for the detection of paralytic shellfish toxins (PSTs). *Korean J. Fish. Aquat. Sci.* **2017**, *50*, 669–674.
- Shin, H.H.; Li, Z.; Kim, H.J.; Park, B.S.; Lee, J.; Shin, A.-Y.; Park, T.-G.; Lee, K.W.; Han, K.H.; Youn, J.Y.; et al. *Alexandrium catenella* (Group I) and *A. pacificum* (Group IV) cyst germination, distribution, and toxicity in Jinhae-Masan Bay, Korea. *Harmful Algae* **2021**, *110*, 102122. [[CrossRef](#)]
- MOF. *Statistical Yearbook of Oceans and Fisheries*; MOF: Sejong, Republic of Korea, 2021.
- Lee, J.S.; Shin, I.S.; Kim, Y.M.; Chang, D.S. Paralytic shellfish toxins in the mussel, *Mytilus edulis*, caused the shellfish poisoning accident at Geoje, Korea, in 1996. *Korean J. Fish. Aquat. Sci.* **1997**, *30*, 158–160.
- Chun, J.H.; Lee, J.T.; Kim, S.C.; Lee, C.U.; Kim, J.Y.; Kim, B.S.; Paik, N.W. Bioassay on PSP in Some Shellfishes from Pusan and Kyungnam Area. *Korean J. Malacol.* **1986**, *2*, 1–9.
- Jeon, J.K.; Yi, S.K.; Huh, H.T. Paralytic Shellfish Poison of Bivalves in the Korean Waters. *J. Korean Soc. Oceano.* **1988**, *23*, 123–129.

21. Shon, M.B.; Kim, Y.S.; Kim, C.R. Paralytic Shellfish Poisoning of Mediterranean mussels from Jinhae Bay in Korea. *Korean J. Fish. Aquat. Sci.* **2009**, *42*, 366–372. [[CrossRef](#)]
22. Park, M.J.; Lee, H.J.; Lee, T.S.; Son, K.T.; Byun, H.S.; Park, J.H.; Jang, D.S. Comparison of paralytic shellfish poison contents and components in the different bivalve species. *J. Fd. Hyg. Saf.* **2000**, *15*, 293–296.
23. Kim, Y.S.; Shon, M.B.; Kim, C.H. Paralytic Shellfish Poisoning Toxin Accumulation in Four Mussel Species Fed on Toxic *Alexandrium tamarense*. *Korean J. Fish. Aquat. Sci.* **2006**, *39*, 49–54.
24. Mok, J.S.; Oh, E.G.; Son, K.T.; Lee, T.S.; Lee, K.J.; Song, K.C.; Kim, J.H. Accumulation and Depuration of Paralytic Shellfish Poison in Marine Organisms. *Korean J. Fish. Aquat. Sci.* **2012**, *45*, 465–471.
25. Shumway, S.E. A review of the effects of algal blooms on shellfish and aquaculture. *J. World Aquac. Soc.* **1990**, *21*, 65–104. [[CrossRef](#)]
26. Dong, D.S.; Shin, I.S.; Cho, H.R.; Kim, J.H.; Pyeon, J.H.; Park, Y.H. Studies on distribution, characterization and detoxification of shellfish in Korea 1. A study on the distribution of paralytic shellfish poison. *Korean J. Fish. Aquat. Sci.* **1988**, *21*, 113–126.
27. Nishitani, L.; Hood, R.; Wakeman, J.; Chew, K.K. Potential importance of an endoparasite of *Gonyaulax* in paralytic shellfish poisoning outbreaks. In *Seafood Toxin*; Edward, P.R., Ed.; American Chemical Society: Washington, DC, USA, 1984; pp. 139–149.
28. Shin, I.S.; Chang, D.S. Studies on thermal resistance of paralytic shellfish poison in blue mussel. *Korean J. Food Sci. Technol.* **1990**, *22*, 799–801.
29. Prakash, A.; Medcof, J.C.; Tennant, A.D. *Paralytic Shellfish Poisoning in Eastern Canada*; Fisheries Research Board of Canada: Ottawa, ON, Canada, 1971.
30. Hashimoto, Y.; Noguchi, T.; Adachi, R. Occurrence of toxic bivalves in association with the bloom of *Gonyaulax* sp. in Owase Bay. *Nippon Suisan Gakk.* **1976**, *42*, 671–676. [[CrossRef](#)]
31. Culture methods. In *Manual on Harmful Marine Microalgae*; Hallegraeff, G.M.; Anderson, D.M.; Cembella, A.D. (Eds.) UNESCO: New York, NY, USA, 1995; pp. 45–62.
32. Oshima, Y. Post-column derivatization HPLC methods for paralytic shellfish poisons. In *Manual on Harmful Marine Microalgae*; Hallegraeff, G.M., Anderson, D.M., Cembella, A.D., Eds.; UNESCO: Paris, France, 1995; pp. 81–94.
33. Yamamoto, K.; Tsujimura, H.; Nakajima, M.; Harrison, P.J. Flushing rate and salinity may control the blooms of the toxic dinoflagellate *Alexandrium tamarense* in a river/estuary in Osaka Bay, Japan. *J. Oceanogr.* **2013**, *69*, 727–736. [[CrossRef](#)]
34. Baek, S.H.; Choi, J.M.; Lee, M.; Park, B.S.; Zhang, Y.; Arakawa, O.; Takatani, T.; Jeon, J.K.; Kim, Y.O. Change in paralytic shellfish toxins in the mussel *Mytilus galloprovincialis* depending on dynamics of harmful *Alexandrium catenella* (Group I) in the Geoje coast (South Korea) during bloom season. *Toxins* **2020**, *12*, 442. [[CrossRef](#)]
35. Siu, G.K.Y.; Young, M.L.C.; Chan, D.K.O. Environmental and nutritional factors which regulate population dynamics and toxin production in the dinoflagellate *Alexandrium catenella*. *Hydrobiologia* **1997**, *352*, 117–140. [[CrossRef](#)]
36. Hwang, D.F.; Lu, Y.H. Influence of environmental and nutritional factors on growth, toxicity, and toxin profile of dinoflagellate *Alexandrium minutum*. *Toxicon* **2000**, *38*, 1491–1503. [[CrossRef](#)]
37. Hamasaki, K.; Horie, M.; Tokimitsu, S.; Toda, T.; Taguchi, S. Variability in toxicity of the dinoflagellate *Alexandrium tamarense* isolated from Hiroshima Bay, western Japan, as a reflection of changing environmental conditions. *J. Plankton Res.* **2001**, *23*, 271–278. [[CrossRef](#)]
38. Etheridge, S.M.; Roesler, C.R. Effects of temperature, irradiance, and salinity on photosynthesis, growth rates, total toxicity, and toxin composition for *Alexandrium fundyense* isolates from the Gulf of Maine and Bay of Fundy. *Deep-Sea Res. PT II* **2005**, *52*, 2491–2500. [[CrossRef](#)]
39. He, H.; Chen, F.; Li, H.; Xiang, W.; Li, Y.; Jiang, Y. Effect of iron on growth, biochemical composition and paralytic shellfish poisoning toxins production of *Alexandrium tamarense*. *Harmful Algae* **2010**, *9*, 98–104. [[CrossRef](#)]
40. Xu, J.; Ho, A.Y.T.; He, L.; Yin, K.; Hung, C.; Choi, N.; Lam, P.K.S.; Wu, R.S.S.; Anderson, D.M.; Harrison, P.J. Effects of inorganic and organic nitrogen and phosphorus on the growth and toxicity of two *Alexandrium* species from Hong Kong. *Harmful Algae* **2012**, *16*, 89–97. [[CrossRef](#)]
41. Han, M.S.; Lee, H.; Anderson, D.M.; Kim, B. Paralytic shellfish toxin production by the dinoflagellate *Alexandrium pacificum* (Chinhae Bau, Korea) in axenic, nutrient-limited chemostat cultures and nutrient-enriched bath cultures. *Mar. Pollut. Bull.* **2016**, *104*, 34–43. [[CrossRef](#)]
42. Chang, D.D.; Shik, C.S.; Goo, H.Y.; Oh, E.G.; Pyun, J.H.; Park, Y.H. (Studies on distribution, characterization and detoxification of shellfish toxin in Korea. *Bull. Korean Fish. Soc.* **1988**, *21*, 297–302.
43. Oshima, Y.; Hayakawa, T.; Hashimoto, M.; Kotaki, Y.; Yasumoto, T. Classification of *Protogonyaulax tamarensis* from northern Japan into three strains by toxin composition. *Bull. Jpn. Soc. Sci. Fish.* **1982**, *48*, 851–854. [[CrossRef](#)]
44. Suzuki, T.; Ichimi, K.; Oshima, Y.; Kamiyama, T. Paralytic shellfish poisoning (PSP) toxin profiles and short-term detoxification kinetics in mussels *Mytilus galloprovincialis* fed with the toxic dinoflagellate *Alexandrium tamarense*. *Harmful Algae* **2003**, *2*, 201–206. [[CrossRef](#)]
45. Sekiguchi, K.; Sato, S.; Ogata, T.; Kaga, S.; Kodama, M. Accumulation and depuration kinetics of paralytic shellfish toxins in the scallop *Patinopecten yessoensis* fed *Alexandrium tamarense*. *Mar. Ecol. Prog. Ser.* **2001**, *220*, 213–218. [[CrossRef](#)]
46. Qiu, J.; Fan, H.; Liang, X.; Meng, F.; Quiliam, M.; Li, A. Application of activated carbon to accelerate detoxification of paralytic shellfish toxins from mussels *Mytilus galloprovincialis* and scallops *Chlamys farreri*. *Ecotoxicol. Environ. Saf.* **2018**, *148*, 402–409. [[CrossRef](#)]

47. Bricelj, V.M.; Shumway, S.E. Paralytic shellfish toxins in bivalve molluscs: Occurrence, transfer kinetics, and biotransformation. *Rev. Fish. Sci.* **1998**, *6*, 315–383. [[CrossRef](#)]
48. Guéguen, M.; Bardouil, M.; Baron, R.; Lassus, P.; Truquet, P.; Massardier, J.; Amzil, Z. Detoxification of Pacific oyster *Crassostrea gigas* fed on diets of *Skeletonema costatum* with and without silt, following PSP contamination by *Alexandrium minutum*. *Aquat. Living Resour.* **2008**, *21*, 13–20. [[CrossRef](#)]
49. Gibbard, J.; Naubert, J. Paralytic shellfish poisoning on the Canadian Atlantic coast. *Am. J. Public Health Nations Health* **1948**, *38*, 550–553. [[CrossRef](#)] [[PubMed](#)]
50. Jamieson, G.S.; Chandler, R.A. Paralytic shellfish poison in sea scallops (*Placopecten magellanicus*) in the west Atlantic. *Can. J. Fish. Aquat. Sci.* **1983**, *40*, 313–318. [[CrossRef](#)]
51. Shumway, S.E.; Sherman-Caswell, S.; Hurst, J.W. Paralytic shellfish poisoning in Maine: Monitoring a monster. *J. Shellfish Res.* **1988**, *7*, 643–652.
52. Maranda, L.; Anderson, D.M.; Shimizu, Y. Comparison of toxicity between populations of *Gonyaulax tamarensis* of eastern north American waters. *Estuar. Coast. Shelf Sci.* **1985**, *21*, 401–410. [[CrossRef](#)]
53. Boyer, G.L.; Sullivan, J.J.; Anderson, R.J.; Harrison, P.J.; Taylor, F.J.R. Effects of nutrient limitation on toxin production and composition in the marine dinoflagellate *Protogonyaulax tamarensis*. *Mar. Biol.* **1987**, *96*, 123–128. [[CrossRef](#)]
54. Cembella, A.E.; Sullivan, J.J.; Boyer, G.L.; Taylor, F.J.R.; Anderson, R.J. Variation in paralytic shellfish toxin composition within the *Protogonyaulax tamarensis/catenella* species complex: Red tide dinoflagellates. *Biochem. Syst. Ecol.* **1987**, *15*, 171–186. [[CrossRef](#)]
55. Anderson, D.M. Toxin variability in *Alexandrium* species. In *Toxic Marine Phytoplankton*; Graneli, E., Sundstrom, B., Edler, L., Anderson, D.M., Eds.; Elsevier: Amsterdam, The Netherlands, 1990; pp. 41–51.
56. Ishida, Y.; Kim, C.-H.; Sako, Y.; Hirooka, N.; Uchida, A. PSP toxin production is chromosome dependent in *Alexandrium* spp. In *Toxic Phytoplankton Blooms in the Sea*; Smayda, T.J., Shimizu, Y., Eds.; Elsevier: Amsterdam, The Netherlands, 1993; pp. 881–887.
57. Kim, C.-H.; Sako, Y.; Ichida, Y. Comparison of toxic composition between populations of *Alexandrium* spp. from geographically distant areas. *Nippon Suisan Gakk.* **1993**, *59*, 641–646. [[CrossRef](#)]
58. Oshima, Y.; Blackburn, S.I.; Hallegraef, G.M. Comparative study on paralytic shellfish toxin profiles of the dinoflagellate *Gymnodinium catenatum* from three different countries. *Mar. Biol.* **1993**, *116*, 471–476. [[CrossRef](#)]
59. Cho, E.S.; Lee, H.J. Thecal plates, toxin content and growth of five clones of *Alexandrium tamarensis* (Dinophyceae) isolated from Jinhae Bay. Korea. *Phycologia* **2001**, *40*, 435–439. [[CrossRef](#)]
60. Kim, C.J.; Kim, C.H.; Sako, Y. Paralytic shellfish poisoning toxin analysis of the genus *Alexandrium* (Dinophyceae) occurring in Korean coastal waters. *Fish. Sci.* **2005**, *71*, 1–11. [[CrossRef](#)]
61. Oshima, Y.; Fallon, W.E.; Shimizu, Y.; Noguchi, T.; Hashimoto, Y. Toxins of the *Gonyaulax* sp. and infested bivalves on Owase bay. *Bull. Jpn. Soc. Sci. Fish.* **1976**, *42*, 851–856. [[CrossRef](#)]
62. Oshima, Y.; Sugino, K.; Itakura, H.; Hirota, M.; Yasumoto, T. Comparative studies on paralytic shellfish toxin profile of dinoflagellate and bivalves. In *Toxic Marine Phytoplankton*; Graneli, E., Sundstorm, B., Edler, L., Anderson, D.M., Eds.; Academic Press: New York, NY, USA, 1990; pp. 391–396.
63. Krock, B.; Seguel, C.G.; Cembella, A.D. Toxin profile of *Alexandrium catenella* from the Chilean coast as determined by liquid chromatography with fluorescence detection and liquid chromatography coupled with tandem mass spectrometry. *Harmful Algae* **2007**, *6*, 734–744. [[CrossRef](#)]
64. Shimizu, Y.; Yoshioka, M. Transformation of paralytic shellfish toxins as demonstrated in scallop homogenates. *Science* **1981**, *212*, 547–549. [[CrossRef](#)] [[PubMed](#)]
65. Cembella, A.; Shumway, S.E.; Lewis, N. A comparison of anatomical distribution and spatio-temporal variation of paralytic shellfish toxin composition in two bivalve species from the Gulf of Maine. *J. Shellfish Res.* **1993**, *12*, 389–403.
66. Oshima, Y. Chemical and enzymatic transformation of paralytic shellfish toxins in marine organisms. In *Harmful Marine Algal Blooms*; Lassus, P., Arzul, G., Erard-Le Denn, E., Gentien, P., Marcaillou-Le Baut, C., Eds.; Lavoisier Science Publishers: Paris, France, 1995; pp. 475–480.
67. Sullivan, J.J.; Iwaoka, W.T.; Liston, J. Enzymatic transformation of PSP toxins in the littleneck clam (*Protothaca staminea*). *Biochem. Biophys. Res. Commun.* **1983**, *114*, 465–472. [[CrossRef](#)] [[PubMed](#)]

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