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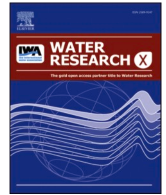

# The ciliate *Euplotes balteatus* is resistant to Paralytic Shellfish Toxins from *Alexandrium minutum* (Dinophyceae)

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ABSTRACT

Research on interactions between grazers and toxigenic algae is fundamental for understanding toxin dynamics within aquatic ecosystems and developing biotic approaches to mitigate harmful algal blooms. The dinoflagellate *Alexandrium minutum* is a well-known microalga responsible for paralytic shellfish toxins (PSTs) contamination in many coastal regions worldwide. This study investigated the impact of the ciliate *Euplotes balteatus* on cell density and PSTs transfer in simulated *A. minutum* blooms under controlled conditions. *E. balteatus* exhibited resistance to the PSTs produced by *A. minutum* with a density of up to 10,000 cells/mL, sustaining growth and reproduction while eliminating algal cells within a few days. The cellular PSTs content of *A. minutum* increased in response to the grazing pressure from *E. balteatus*. However, due to the substantial reduction in density, the overall toxicity of the algal population decreased to a negligible level. Most PSTs contained within algal cells were temporarily accumulated in *E. balteatus* before being released into the water column, suggesting unclear mechanisms for PSTs excretion in unicellular grazers. In principle, the grazing of *E. balteatus* on *A. minutum* promotes the transfer of the majority of intracellular PSTs into extracellular portions, thereby mitigating the risk of their accumulation and contamination through marine trophic pathways. However, this process also introduces an increase in the potential environmental hazards posed by extracellular PSTs to some extent.

## 1. Introduction

Paralytic shellfish toxins (PSTs) are a group of neurotoxic alkaloids, including saxitoxin (STX) and over fifty analogs, characterized by a trialkyl tetrahydropurine skeleton (Mons et al., 1998; Morse, 1977; Wang et al., 2016). They are extensively studied among various marine biotoxins due to their high prevalence and risk consequences in related food webs (Anderson et al., 1996; Etheridge, 2010; Gu et al., 2013; Hallegraeff et al., 1991; Leal and Cristiano, 2022; Llewellyn et al., 2006; McFarren et al., 1961). PSTs cascade in filter-feeding shellfishes and subsequently intoxicate animals within higher trophic levels through the blockage of the voltage-gated sodium channel in their nerve and muscular fibers, resulting in neuromuscular paralysis and metabolic stress (Li et al., 2019; Shumway, 1990; Velez et al., 2001; Wang, 2008). Incidents of fish, birds and mammal deaths, including humans, due to the consumption of PSTs-contaminated organisms have been occasionally reported (Cadaillon et al., 2024; Coulson et al., 1968; Negri et al., 1995).

In the marine environment, PSTs are mainly produced by the dinoflagellates *Gymnodinium catenatum* (Mee et al., 1986), *Pyrodinium bahamense* (Harada et al., 1982), and several species of the genus *Alexandrium* (Hansen et al., 2003). *Alexandrium minutum* is a widespread producer of PSTs that accounts for poisoning events across the world. Occurrences of PSTs contamination caused by *A. minutum* have been reported in the Mediterranean Sea (Bravo et al., 2008; Zingone et al., 2021), Irish coastal waters (Rathaille and Raine, 2011), the Bay of

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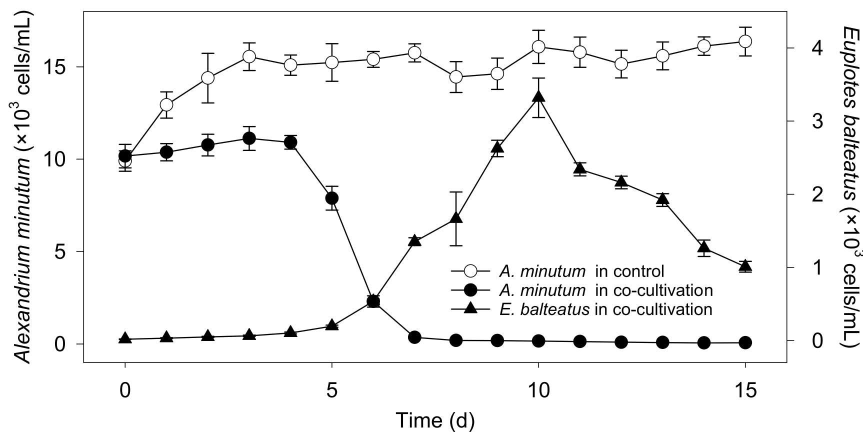


**Fig. 1.** Changes in cells density of *A. minutum* and *E. balteatus* within different treatment groups.

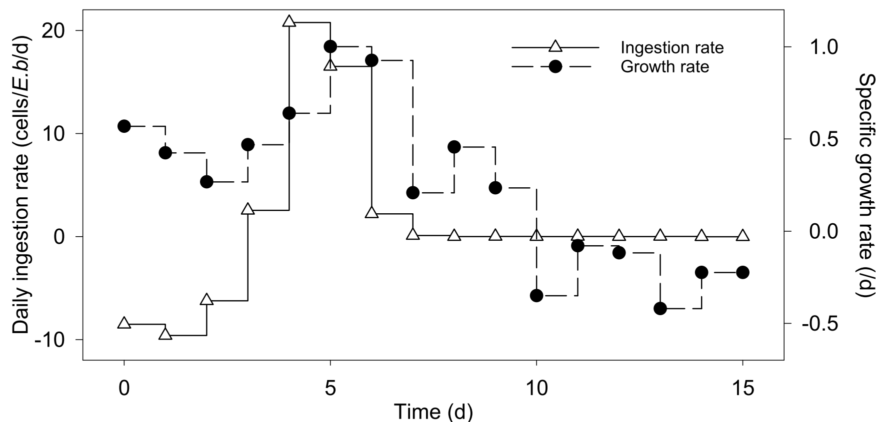


**Fig. 2.** The daily ingestion rate of *E. balteatus* on *A. minutum* and the specific growth rate of *E. balteatus*.

Plenty, New Zealand (Chang et al., 1997), the Cape Town Harbour, South Africa (Pitcher et al., 2007), Greek coastal waters (Ignatiades et al., 2007), Chinese coastal waters (Gu et al., 2022), Spanish coastal waters (Ben-Gigirey et al., 2020), and elsewhere (Lewis et al., 2018).

Grazers can be a substantial loss factor for harmful algal blooms (HABs) and possible cause of their termination (Calbet et al., 2003; Jeong et al., 2008; Martin et al., 1973; Watras et al., 1985). However, grazing pressure and/or grazer-induced lipids have been reported to significantly affect, primarily increasing, toxin production of toxigenic algae (Ger et al., 2016; Ives, 1987; Ryderheim et al., 2021; Selander et al., 2015, 2006). Regarding *A. minutum*, cultures induced by the copepod grazer *Acartia tonsa* contained up to 2.5 times more cellular toxins than controls (Selander et al., 2006). *A. minutum* displays remarkable sensitivity to copepodamides at concentrations ranging from pico- to nanomolar levels, resulting in a 20-fold increase in PSTs production (Selander et al., 2015). Nonetheless, due to limitations such as quantification methods, studies have primarily focused on variations in intracellular toxin levels, with minimal attention given to changes in extracellular toxin content.

In the pursuit of potential grazers to mitigate HABs, *Euplotes balteatus* displayed grazing and removal capacity for *Karenia mikimotoi* and several other toxigenic algal species, including *A. minutum* (under review). *Euplotes* is a genus of ciliates in the class Spirotrichea, commonly found in various aquatic environments, with a predominant presence in marine and brackish water areas (Syberg-Olsen et al., 2016). The genus has been extensively studied for its mating types, sexual pheromones (Beale, 1990; Vallesi et al., 2014), intracellular bacterial symbioses (Boscaro et al., 2019; Fokin and Serra, 2022; Heckmann et al., 1983; Vannini et al., 2014), and its role in exerting grazing pressure on microalgae, serving as a model ciliate (Long et al., 2007; Ng et al., 2017; Wang et al., 2015). Several characteristics of *Euplotes* indicate its potential as a promising candidate for HABs mitigation, including: (1) ecological positioning that overlaps with regions prone to HABs outbreaks (Syberg-Olsen et al., 2016); (2) as a protist, *Euplotes* has a higher growth rate compared to larger multicellular zooplankton, typically; (3) *Euplotes* is a recognized dietary component for initial feeding in various fish larvae, contributing positively to aquaculture and fishery resources (Wan-Mohtar et al., 2022); and (4) the genus exhibits a robust nature, enabling its preparation in environmentally challenging conditions.

However, it remains inconclusive how the grazing pressure and removal process of *E. balteatus* on *A. minutum* affect the production, accumulation, and transformation of PSTs. Considering the notable risk potential of PSTs in aquatic ecosystems and the lack of related research, this study investigated the mitigation effect of *E. balteatus* regarding algal cell density and PSTs transfer in a simulated *A. minutum* bloom. Toxin compositions of the algae, the ciliate, and the water column were compared to the control group within 15 days under laboratory conditions.

## 2. Results

### 2.1. Impact of E. balteatus on the cell density of A. minutum

The cell density of *A. minutum* and *E. balteatus* over time in different treatment groups are illustrated in Fig. 1. In the control group, the initial density of *A. minutum* was 9900 ± 552 cells/mL. During the 0–3 d period, the cell density in the control group increased rapidly, reaching 15,544 ± 746 cells/mL by the third day. From 3 d to 15 d, *A. minutum*

 

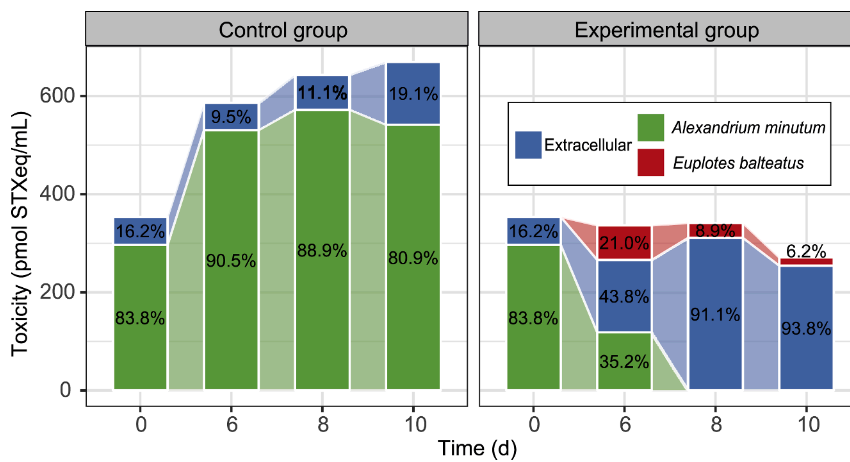


**Fig. 3.** Changes in toxicity of intracellular (*A. minutum* and *E. balteatus*) and extracellular PSTs within different treatment groups.

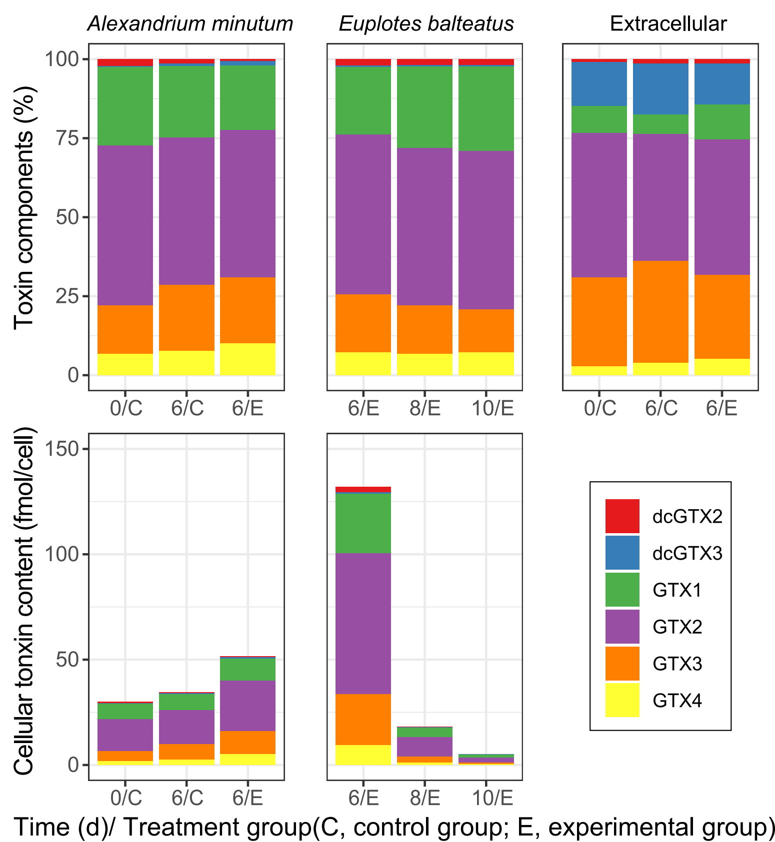


**Fig. 4.** The percentage composition of PSTs and the cellular toxin contents.

growth plateaued, with minimal changes in cell density, reaching 16,367 ± 777 cells/mL by 15 d

In the experimental group where *A. minutum* was exposed to *E. balteatus*, the initial density of *A. minutum* was 10,167 ± 638 cells/mL. During the 0–3 d period, the cell density increased slowly, reaching 11,122 ± 643 cells/mL by the third day. From 4 d to 7 d, there was a rapid decrease in cell density, dropping from 10,911 ± 370 cells/mL to 356 ± 45 cells/mL. During days 8 to 15, the density of *A. minutum* remained at a lower level, reaching 68 ± 25 cells/mL by 15 d

In co-cultivation with *A. minutum*, the initial density of *E. balteatus* was 18 ± 3 cells/mL. During the 0–10 d period, its density maintained an increasing trend, with a faster rate from 5 d to 10 d, rising from 197 ± 15 cells/mL to 3319 ± 270 cells/mL. From 10 d to 15 d, the density of *E. balteatus* gradually decreased, reaching 1009 ± 74 cells/mL by 15 d As illustrated in Fig. 2, the specific growth rate of *E. balteatus* showed a declining trend during the initial 0–2 days of the experiment, while during this period, the density of *A. minutum* displayed a gradual increase. This observation suggests that in the early stages of co-cultivation, most *E. balteatus* individuals were unable to feed on *A. minutum* cells effectively. However, after a 2-day adaptation period, *E. balteatus* exhibited significant consumption of algal cells, accompanied by a corresponding enhancement in its growth rate. By day 4, the ingestion rate of *E. balteatus* on *A. minutum* reached 20.7 cells/*E.b*/d. By day 5, *E. balteatus* achieved its peak growth rate, approximately 1.0 /d. As the density of *A. minutum* decreased to lower levels within the experimental group, the ingestion rate of *E. balteatus* on algal cells dropped to near-zero levels. Consequently, *E. balteatus* exhibited a negative growth rate between days 10–15.

### 2.2. Toxicity of intracellular and extracellular PSTs within different treatment groups

The toxicity of extracellular PSTs (dissolved in the water column) and intracellular PSTs (contained by *A. minutum* and *E. balteatus* cells) at key experimental phases are illustrated in Fig. 3. In the control group, intracellular PSTs accounted for over 80 % of the total toxicity and exhibited a trend of increasing followed by stabilization. This trend showed a notable positive correlation with changes in algal density. The toxicity of extracellular PSTs showed an increasing trend, but the magnitude of change was smaller compared to intracellular toxins: at 0 d, it was 57 ± 5 pmol STXeq/mL, and at 10 d, it was 128 ± 5 pmol STXeq/mL. The contribution of extracellular PSTs to total toxicity decreased from 16.2 % to 9.5 % during 0–6 d, then increased to 19.1 % during 6–10 d The toxicity of the entire water column in the control group significantly increased from 354 ± 25 pmol STXeq/mL at 0 d to 670 ± 39 pmol STXeq/mL at 10 d

In the experimental group, the toxicity of the entire water column remained around 350 pmol STXeq/mL during 0–8 days and decreased to 271 ± 11 pmol STXeq/mL by day 10. Comparatively, at day 10, the total toxicity in the experimental group was 40.4 % of that in the control group. Meanwhile, there were significant changes in the composition of intracellular and extracellular toxins in the experimental group. By day 6, the proportion of extracellular toxins in total water column toxicity increased to 43.8 %, further increasing to 93.8 % by day 10. The proportion of intracellular toxins decreased to 56.2 % by day 6 (with *A. minutum* accounting for 35.2 % and *E. balteatus* accounting for 21 %); by days 8 and 10, the contribution of PSTs from *A. minutum* cells had decreased to negligible levels, with intracellular toxins mainly originating from *E. balteatus*, albeit at very low proportions of 8.9 % and 6.2 %, respectively.

### 2.3. PSTs components of *E. balteatus, A. minutum* and the water column

The composition of intracellular and extracellular PSTs in different treatment groups, as well as the cellular toxin content of *A. minutum* and *E. balteatus* across key experimental phases, are depicted in Fig. 4. There were no notable differences in the composition of extracellular and intracellular toxins between the experimental and control groups. However, extracellular PSTs contained additional dcGTX3 compared to intracellular PSTs, indicating a possible process of de-carbamoylation either during the excretion of PSTs by *A. mimutum* or after release into the water column. The PSTs components of *E. balteatus* and *A. minutum* were almost identical to each other and remained constant over time, suggesting no obvious chemical structure changes within PSTs ingested by *E. balteatus*.

The cellular PSTs content varied between treatment groups and over time. At day 6, the cellular PSTs content of *A. minutum* in the experimental group was notably higher than that of the control group at the same time, possibly due to the grazing pressure induced by *E. balteatus*. The cellular PSTs content of *E. balteatus* could be several times higher than that of *A. minutum*. However,after the cell density of *A. minutum* in the water column was reduced, the accumulated PSTs in *E. balteatus* rapidly decreased, with minimal change in their percentage composition. Combining the trend of changes in extracellular PSTs in the experimental group (Fig. 3), it can be inferred that most PSTs produced by *A. minutum* were transferred to the water in soluble form through the grazing and digestion process of *E. balteatus*.

## 3. Discussion

The concept of biotic control or mitigation of HABs through grazers has a long history but is often considered impractical due to the substantial resources required to maintain a large population of grazers (Anderson et al., 2012; Shirota, 1989). However, more efficient selection of grazers can alleviate this issue to some extent. Compared to the reported grazers of *A. minutum*, the removal efficacy and reproductive capacity of *E. balteatus* are superior. The density of *A. minutum* decreased from 10,911 ± 370 cells/mL to 356 ± 45 cells/mL in 3 days (from day 4 to day 7) (Fig. 1); meanwhile, the density of *E. balteatus* increased from 104 ± 13 cells/mL to 1351 ± 53 cells/mL. Assuming exponential growth of *E. balteatus* during this period and disregarding the intrinsic growth of *A. minutum*, the average daily ingestion rate of *E. balteatus* on *A. minutum* was calculated as 7.2 cells/*E.b*/d, indicating that each *E. balteatus* individual grazed 7.2 cells of *A. minutum* daily. Throughout the experiment, the maximum daily ingestion rate of *E. balteatus* on *A. minutum* reached 20.7 cells/*E.b*/d at day 4, while *E. balteatus* maintained robust growth (the maximum specific growth rate equals 1.0 /d), indicating that *A. minutum* is a nutrient-adequate food source for *E. balteatus* to survive and reproduce (Fig. 2).

Copepods are extensively studied grazers of microplankton. The daily ingestion rate of *Acartia tonsa* on *A. catenella* can reach 3000 cells/ind./d due to its larger size and higher clearance rate compared to ciliates. However, their low density renders them ineffective in substantially eliminating algal cells, with laboratory experiments demonstrating no more than a 3 % removal per day (Abdulhussain et al., 2020). Shipboard grazing experiments conducted in the Gulf of Maine and on Georges Bank suggested that mesozooplankton grazers (including copepods, rotifers, cladocerans, and meroplankton) may be capable of retarding *A. fundyense* bloom development at low concentrations typical of the early stages of a bloom but not at higher concentrations once a bloom becomes established (Turner, 2010). In comparison, although the daily ingestion rate of *E. balteatus* is much lower compared to copepods, its strong reproductive capacity and high eventual density result in *E. balteatus* removing >90 % of the *A. minutum* cells from the water column within 3 days (Fig. 1), bringing the concept of bio-mitigation of HABs into a promising and practical realm. It is important to note that this experiment was conducted under controlled conditions with a single species, and the efficacy of grazers in natural conditions would be significantly influenced by environmental changes and species diversity, potentially yielding different results.

Further study indicated that the *A. minutum* used in this study produces various PSTs, including GTX1, GTX4, GTX2, GTX3, dcGTX2, and dcGTX3 (Fig. 4). *E. balteatus* ingests *A. minutum* cells and consequently accumulates PSTs within its cells. The observed cellular toxin content of *E. balteatus* can reach up to five times that of *A. minutum*, while *E. balteatus* maintains growth and reproduction (Fig. 1, Fig. 4). As the density of *A. minutum* decreases, the daily ingestion rate of *E. balteatus* on algal cells also decreases, while *E. balteatus* continues to proliferate through binary fission. These processes may decrease the toxin content in individual *E. balteatus* cells but will not decrease the total toxin content in the *E. balteatus* population or increase the dissolved PSTs in the water. However, results showed that (1) the cellular toxin content of *E. balteatus* decreased rapidly, reaching only about half of that of a single *A. minutum* cell by day 8, with a reduction of over 80 % compared to day 6; (2) the overall toxicity of the *E. balteatus* population also decreased, while the extracellular toxin content increased notably; (3) the PSTs components in *E. balteatus, A. minutum*, and the water column were almost identical (Fig. 3, Fig. 4). These observations suggest that the feeding process of *E. balteatus* on *A. minutum* does not alter the chemical structure of PSTs. *E. balteatus* acts like a nutritional sieve, retaining matter beneficial for its growth and excreting PSTs through unclear mechanisms. Similarly, *Notiluca scintillans* has been reported to ingest toxic *A. minutum* without accumulating any toxins, suggesting rapid detoxification/excretion after feeding (Frangopulos et al., 2011). The ciliate *Favella taraikaensis* can also actively feed on *A. tamarense* and grow rapidly, with PSTs scarcely accumulating within the ciliate (Kamiyama and Suzuki, 2006). It can be inferred that unicellular grazers might not be sustained vectors for PSTs but rather transport PSTs from algal cells into the water column in a dissolved form.

Cellular PSTs content in *A. minutum* induced by *E. balteatus* was notably high compared with the control (Fig. 4). Similar phenomena

have been extensively reported, leading to a basic consensus that grazing pressure can induce changes in toxin production of toxigenic algae, although the magnitude of these changes varies in different studies owing to different species and experimental conditions (Selander et al., 2015, 2006). However, due to the greater reduction in algal cell density, the total PSTs toxicity of the algal population exhibited a rapid decrease during the experiment (Fig. 3).

Overall, under the grazing effect of *E. balteatus, A. minutum* in the experimental group were eliminated within several days, with most of the toxins temporarily accumulated in *E. balteatus* before being released into the water column. By the end of the experiment, the combined extracellular and intracellular toxicity in the experimental group was lower than at the start of the experiment, and approximately half of that in the control group at the same time point. These results suggest that the grazing process of *E. balteatus* on *A. minutum* substantially decrease intracellular PSTs in the water column, however by converting most of them into extracellular portions. Theoretically, this process can alleviate the biomagnification of PSTs through food chains, thereby mitigating their impact on higher trophic levels. The degradation of extracellular PSTs could be driven by chemical conditions and/or microbial activity, necessitating extended temporal examinations with additional analytical methodologies. Further investigation and discourse are also warranted to evaluate the potential ecological ramifications of intracellular PSTs vis-à-vis those dissolved in the water column.

## 4. Materials and methods

### 4.1. Biotic materials and culture conditions

*E. balteatus*, sourced from coastal waters of Fujian, China, were isolated and maintained by the Protozoan Germplasm Repository of Minjiang University. Seawater was filtered through a 5 μm nylon fiber membrane, and rice grains were added as a substrate for bacteria, which in turn served as food for the *E. balteatus. A. minutum* (GY-H46), originally isolated from Daya Bay, Guangdong, China, was purchased from Shanghai Guangyu Biotechnology Co., Ltd., China. Algal culture media was made by filtering seawater through a 0.22 μm mixed fiber membrane and heated at 121 °C for 30 min, and L1 medium (Guillard and Hargraves, 1993) without silicate was then added for algal culture.

The glassware used in the biotic culture was pre-rinsed with 5 % v/v HCl for 24 h to remove contaminants, thoroughly washed with Milli-Q water, and heated at 121 °C for 30 min. The culture was performed on shelves with light control in an air-conditioned room. The room temperature was 22 ± 1 °C, the light intensity above the culture containers was approximately 90 μmol photons/($m^2$·s), and the light:dark cycle was 12 h:12 h.

### 4.2. Experimental procedure

*E. balteatus* cultures were filtered through a 100 μm nylon mesh 48 h prior to the experiment to eliminate most residual food particles, and the density of *E. balteatus* was determined using a microscope right before the experiment. *A. minutum* was cultivated to a density of approximately 10,000 cells/mL prior to the experiment, during its exponential growth phase. Three liters of algal suspension were transferred to each of two conical flasks. Using the method described below, *E. balteatus* were added to one of the conical flasks to achieve a final density of approximately 20 cells/mL; the other conical flask was left untreated as a control group. The volume of *E. balteatus* culture required was calculated based on its density. A specific volume of starved *E. balteatus* culture was gravity-drip filtered using an 11 μm nylon membrane (Merck Millipore Ltd., Tullagreen, Carrigtwohill, Ireland) and washed several times using sterile seawater to remove most symbiotic bacteria. Subsequently, the nylon membrane was immersed in the algal culture and shaken several times to detach most of the filtered *E. balteatus*. The algal culture mixed with *E. balteatus* was then sampled and counted to determine the actual initial densities of *A. minutum* and *E. balteatus*.

Three counting samples were collected from each treatment daily for 15 days. During sampling, 4 mL of the culture was pipetted into a centrifuge and fixed using 20 μL of Lugol's solution. Each sample was counted three times to determine the densities of *A. minutum* and *E. balteatus*. To determine the densities of *E. balteatus* and algal cells in the samples, cell numbers were counted using either a Sedgwick–Rafter counting chamber or a cell culture plate, depending on the different densities and appropriate counting volumes, under a microscope (Olympus CKX53, Tokyo, Japan).

The sampling times for PSTs were determined based on count results to ensure that data from key experimental phases were captured: the start of the experiment, when *E. balteatus* significantly feed on algal cells, when algal cells are mostly cleared, and after complete clearance. During each sampling, three 200 mL portions of culture medium were extracted from two conical flasks. Gravity filtration was conducted using 30-micron nylon membranes (Haining Yibo Filtration Equipment Factory, Zhejiang, China), and the membranes were then transferred to 2 mL cell disruption tubes (Lysing Matirx E, MP biomedicals, Ohio, US). Subsequently, the filtered liquid from the nylon membrane underwent low-pressure filtration (< 0.04 MPa) using glass fiber filters with 0.7 μm pores (GF/F, Whatman™, Buckinghamshire, UK), and the GF/F membranes were transferred to 2 mL cell disruption tubes. The filtrates after GF/F membrane filtration were then transferred to polyethylene sampling bottles and stored at −20 °C before further procedure. Given the significant volume increase of *E. balteatus* after ingesting algal cells, with most exceeding 30 μm, the portion retained by the nylon membrane represented the intracellular PSTs component of *E. balteatus*, while that retained by the GF/F membrane after passing through the nylon membrane represented the intracellular PSTs component of *A. minutum*. The toxin presented in the remaining filtrate represented the extracellular PSTs component.

### 4.3. PSTs samples preparation and quantification

For the extraction of intracellular PSTs, the membranes were freeze-dried (FD-250,101 GXB, Hangzhou Furuijie Technology Co., LTD, China) in the cell disruption tubes until there was no loss in mass within 6 h. Subsequently, the membranes were cut into small pieces, and 1 mL of 0.1 mol/L acetic acid solution was added to the tubes. Cell disruption was carried out using the FastPrep-24 5 G Sample Preparation System (MP Biomedicals, Ohio, USA). Following the disruption process, centrifugation was performed at 10,000 rpm for 10 min (Centrifuge 5424, Eppendorf AG, Hamburg, Germany). The resulting supernatant was aspirated using a syringe and then filtered through a 0.2 μm pore size Syringe Filter (Pall Corporation, Puerto Rico, USA) into sample bottles. These prepared samples were stored at −20 °C before analysis using LC-MS.

The quantification of saxitoxin (STX) and its 11 variants (GTX1, GTX2, GTX3, GTX4, GTX5, dcGTX2, dcGTX3, C1, C2, neoSTX, and dcSTX) was conducted by the Key Laboratory for Marine Bioactive Substances and Modern Analytical Technology, The First Institute of Oceanography, Ministry of Natural Resources (Qingdao, China). PSTs certified calibration solutions were obtained from the National Research Council Canda (Halifax, Nova Scotia, Canada). The LC-MS procedure followed the method established by Pan et al. (2023), using a 1290 Infinity II UHPLC combined with a G6470A triple quadrupole MS system (Agilent, Santa Clara, CA, USA) equipped with an Agilent jet stream electrospray ionization source. Intracellular PSTs extracted using acetic acid were directly analyzed using LC-MS. Extracellular toxin samples were centrifuged at 10,000 rpm for 10 min and then analyzed using the on-line SPE-LC-MS/MS method with a Merck ZIC—HILIC column (2.1 mm × 20 mm, 5.0 μm; Darmstadt, Germany) as the on-line SPE column and a Poroshell 120 HILIC-Z column (2.1 mm × 50 mm, 1.9 μm; Agilent, America) as the chromatographic separation column. Detailed gradient programs of loading and eluting process were performed as described by

Pan et al. (2023). Limit of detection (LOD) and limit of quantification (LOQ) were defined as concentrations when the signal-to-noise ratio is 3 and 10 for the quantitative ion, respectively (Table S1).

### 4.4. Data analysis

Conversion factors were utilized to express the toxicity of the sum of the PSTs variants as saxitoxin toxicity equivalent (*STXeq*) due to the differing toxicity of individual PSTs variants. The *STXeq* concentration in the sample was calculated as follows:

$$STXeq = \sum_{i=1}^{n} T_i \cdot R_i$$

where $T_i$ is the concentration of individual PSTs variants, and $R_i$ is the toxicity factor (Oshima, 1995) of them, respectively.

The specific growth rate of *E. balteatus* was calculated based on the method of Frost (1972), and using the following equation:

$$k_i = \ln\left(\frac{CE_{i+1}}{CE_i}\right)$$

where $k_i$ is the specific growth rate of *E. balteatus* on day i, $CE_i$ and $CE_{i+1}$ are the cell concentration of *E. balteatus* on day $i$ and $i+1$, respectively.

The daily ingestion rate of *E. balteatus* on *A. minutum* was estimated as follows, where the growth of the algae was not considered:

$$I_i = \frac{CA_i - CA_{i+1}}{\overline{CE}_{i:i+1}}$$

where $I_i$ is the daily ingestion rate on day *i; $CA_i$* and $CA_{i+1}$ are the cell concentrations of *A. minutum* on day $i$ and $i+1$, respectively; $\overline{CE}_{i:i+1}$ was calculated according to Heinbokel (1978) to estimate the average *E. balteatus* number from day $i$ to day $i+1$ in an exponentially growing culture (see also Jakobsen and Hansen (1997)).

$$\overline{CE}_{i:i+1} = \frac{CE_{i+1} - CE_i}{\ln(CE_{i+1}/CE_i)}$$

where $CE_i$ and $CE_{i+1}$ are the cell concentrations of *E. balteatus* on day $i$ and $i+1$, respectively.

The cell density and PSTs values were averaged from triplicate samples to determine the mean density/values ± one standard deviation. The statistical calculations were performed using SigmaPlot 14.0 (Systat Software, San Jose, CA).

## CRediT authorship contribution statement

**Jing Li:** Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Jinrong Wang:** Funding acquisition, Conceptualization. **Xiuping He:** Software, Methodology, Investigation. **Haifeng Gu:** Writing – review & editing, Conceptualization. **Xin Xu:** Validation, Funding acquisition, Formal analysis, Data curation. **Chen Liang:** Resources. **Yongchao Wang:** Validation, Investigation. **Xiao Xu:** Validation, Investigation. **Linxuan Jia:** Validation, Investigation. **Junhui Chen:** Supervision, Methodology. **Miaohua Jiang:** Resources. **Jianming Chen:** Supervision, Resources, 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.

### Data Availability

Data will be made available on request.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.wroa.2024.100229.

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