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Survival and toxin accumulation in *Crassostrea virginica* larvae following exposure to various *Dinophysis* species

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ABSTRACT

This study investigates the effects of *Dinophysis* cells and their toxins on the viability of oyster larvae, a critical stage for the aquaculture industry. Specifically, the effects of toxigenic *Dinophysis* species (*D. acuminata*, *D. caudata*, *D. fortii*, and *D. ovum*) on 7-day-old *Crassostrea virginica* larvae were investigated through exposures to living cells, cell lysates (dead cells), dialysates (living cells separated by a membrane), and purified toxins in concentrations equivalent to 10 cells mL^{-1}. After 48 h, mortality and toxin uptake were assessed. Larvae experienced the highest mortality rates when exposed to living *D. caudata* (28 ± 4 %) and *D. fortii* (25 ± 6 %) cells, coinciding with elevated intra- and extracellular levels of pectenotoxin-2 (PTX2) and hydroxyPTX2. In contrast, no significant mortality was observed with lysates or dialysates from these species. *In addition, D. acuminata* (a low PTX2 producer) and *D. ovum* (producing only okadaic acid; OA) did not cause mortality under any condition. Exposure to purified OA had no detectable effect on mortality, however, exposure to pure PTX2 at 1.5 nM significantly increased larval mortality (17 ± 2 %), albeit to a lesser extent than exposure to living cells of *D. caudata* and *D. fortii*. Furthermore, dissolved PTX2 was adsorbed onto *Pavlova pinguis* cells, the larval food, potentially increasing toxin bioavailability for larvae. These results indicate that living *Dinophysis* cells producing high concentrations of PTXs (but not OA), along with other bioactive compounds, are responsible for the higher mortality in oyster larvae. Such exposure, along with other multi-stressor environmental factors such as ocean acidification, temperature and salinity fluctuations, hypoxia, poses risks to oyster recruitment, with potential socio-economic and ecological consequences for aquaculture.

1. Introduction

The United States (US) currently holds the third position in global bivalve consumption but is a relatively minor participant in the global aquaculture sector (presently ranking 18th in production), with 70–85 % of its seafood imported (National Marine Fisheries Service, 2022). The significant reliance on imports has resulted in a substantial deficit in the national seafood trade, surpassing $17 billion in 2020 (National Marine Fisheries Service, 2022). Government agencies at both the federal and state levels as well as private foundations and citizens groups have made a substantial commitment to research and programs focused on the restoration of mollusk populations, as fisheries face challenges from overharvesting, habitat loss and degradation, invasive competitors, and pathogens (Barton et al., 2015; Ekstrom et al., 2015; Leffler and Hayes, 2004). In recent years, the importance of hatchery production in generating larvae and seed for commercially, recreationally, and ecologically significant bivalve mollusks, particularly oysters, has significantly expanded in the US (Helm et al., 2004; Hollenbeck and Johnston, 2018). Despite numerous efforts to improve production rates and efficiency in bivalve hatcheries, the industry continues to face a frequent problem with unexplained high mortality rates during the larval phase (Barton et al., 2015; Gray et al., 2022). Traditionally,

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biological factors such as altered microbiome or ciliate contamination, poor water quality characterized by acidification of hatchery water, dramatic salinity and temperature fluctuations, and the presence of various natural or anthropogenic chemical contaminants, including toxic metals and organic and inorganic compounds, have been considered the primary threats to oyster larvae (Ekstrom et al., 2015). In recent decades, however, coastal eutrophication has increased, leading to an increase in harmful algal blooms (HABs), thus presenting a further challenge to the survival and restoration of oyster populations (Anderson et al., 2021; Glibert, 2020; Jones et al., 2006).

Over the last two decades, blooms of toxigenic dinoflagellate species of *Dinophysis*, responsible for diarrheic shellfish poisoning (DSP), have been well-documented in significant oyster cultivation regions across the United States. These include the Northwest Pacific, Gulf of Mexico, and Northeast and mid-Atlantic coasts, leading to the recurrent closure of shellfish harvesting activities in the protection of human health (Anderson et al., 2021; Ayache et al., 2023; Bates, 1998; Campbell et al., 2010; Lloyd et al., 2013; Trainer et al., 2013; Wolny et al., 2020).

Dinophysis species exhibit a wide range of annual peak cell abundances across these various geographic regions (Ayache et al., 2023). These blooms often occur between spring and summer, aligning with oyster hatchery production season, potentially leading to the coexistence of *Dinophysis* cells and their toxins with bivalve gametes and larvae in the water column (Hattenrath-Lehmann et al., 2018; Marshall, 1980; Pease et al., 2021; Sanderson et al., 2022; Swanson et al., 2010). Apart from its ecological and health-related significance, blooms of *Dinophysis* have the potential to negatively affect the rearing of larvae in hatcheries that depend on sourcing water from adjacent coastal areas (Gaillard et al., 2020; Pease et al., 2022; Rolton et al., 2022; Shumway, 1990). This is because mechanical seawater filtration systems can disrupt the cells, releasing toxins into the treated hatchery water used for algal feed preparation and larval development (Sanderson et al., 2022).

Most of the research on *Dinophysis* toxins, including diarrheic shellfish toxins (DSTs) such as okadaic acid (OA) and its derivatives (dinophysistoxins or DTXs), as well as non-DST compounds like pectenotoxins (PTXs), has primarily focused on their cytotoxic properties in economically valuable larval stages and adult bivalves. For instance, McCarthy et al. (2014) found that exposure of two bivalve species of global economic importance, the blue mussel, *Mytilus edulis*, and the Pacific oyster, *Crassostrea gigas*, to different concentrations of OA (1.2, 2.5 and 50 nM), increased DNA fragmentation significantly in both hemocytes and hepatopancreas cells, but they did not observe a dose-response relationship. Additionally, Nielsen et al. (2020) found sublethal effects in adult mussels (*M. edulis*) exposed to a DTXs- and PTX2-containing strain of *D. acuta*. The authors observed a severe negative impact on the clearance rate of *M. edulis*, with active feeding ceasing within a few hours of exposure to *D. acuta* cells. Furthermore, Basti et al. (2015) observed behavioral changes, pathologies in the digestive glands, and an increase in mortality rates in Japanese scallops (*Mizuhopecten yessoensis*) and noble scallops (*Mimachlamys nobilis*) when they were fed a monoclonal culture of PTX2-producing *D. caudata*. In contrast, Leite et al. (2021) showed that exposure to *Prorocentrum lima*, a species solely producing DSTs, did not affect the feeding behavior, shell closure, or clearance rates of the clam species *Anomalocardia flexuosa*.

Knowledge of *Dinophysis* species' adverse effects on juvenile life cycle stages is more limited. Gaillard et al. (2020) showed that the presence of PTX2 and PTX-producing *Dinophysis* species impaired oyster fertilization. Pease et al. (2022) reported increased mortality among oyster larvae exposed to PTX-producing *Dinophysis* spp. The current study was designed to refine conclusions from these prior studies, specifically aiming to determine which *Dinophysis* species and toxins cause oyster larval mortality. Specifically, this study investigated the effects of four *Dinophysis* species (*D. fortii, D. caudata*, *D. acuminata* and *D. ovum*) annually occurring in different coastal regions of the US, on the survival of Eastern oyster larvae (*Crassostrea virginica*). Each *Dinophysis* species is characterized by a distinct toxin profile; *D. fortii* produced both DSTs and PTX2, *D. caudata* exclusively produced hydroxyPTX2, *D. acuminata* produced both DSTs and PTX2 but at lower levels compared to *D. fortii*, and *D. ovum* produced only OA. These exposures were conducted at cell densities reflecting the typical abundance of these *Dinophysis* species during natural blooms in the Chesapeake Bay, Gulf of Mexico, and Pacific Northwest US coasts (10 cells mL^{-1}) where these species were isolated. Four distinct treatments were tested including (1) exposure of oyster larvae to living *Dinophysis* cells, (2) exposure to culture lysate, (3) indirect exposure to living *Dinophysis* cells separated by a dialysis membrane, and (4) exposure to purified OA and PTX2 toxins at concentrations equivalent to what is produced by 10 cells mL^{-1} of *D. ovum*, *D. acuminata*, and *D. fortii*, respectively.

2. Materials and methods

2.1. Culture maintenance

Four *Dinophysis* strains, each of a distinct species, were collected from diverse coastal regions within the US. Strain identification was determined using morphometric and DNA-based analyses, as described in Ayache et al. (2023). *D. acuminata* (DAVA01) was isolated from Nassawadox Creek, Chesapeake Bay, VA, in 2017; *D. ovum* (DOSS 2206) and *D. caudata* (DCSS 3191) originated from Surfside Beach, Gulf of Mexico, TX, in 2020 and 2019, respectively; and *D. fortii* (NWFSC 804) was collected from Budd Inlet in Olympia, WA, in 2019. These mixotrophic *Dinophysis* species were maintained in 0.22-μm filter-sterilized seawater (FSW) with a salinity of 25, pH 8.00 ± 0.02, and were fed every 3 days with the prey organism *Mesodinium rubrum* at a ratio of 1:5 predator: prey (Park et al., 2006). The ciliate *M. rubrum* (strain JAMR) was fed once a week with the cryptophyte *Teleaulax amphioxeia* (strain JATA). Both of these organisms were isolated in 2007 from samples collected from Inokushi Bay in the Oita Prefecture, Japan (Nishitani et al., 2008), and were grown with f/6 and f/2 culture media, respectively, prepared using 0.22-μm filter-sterilized seawater at a salinity of 25. All cultures were kept in a temperature-controlled incubator at 20 ± 1 °C and provided ~50 μmol photons m^2 s^{-1} photosynthetically active radiation (PAR) on a 14 h light:10 h dark cycle. Irradiance was delivered by Osram Cool White T8 fluorescent tubes (OSRAM GmbH, Munich, Germany). All cultures were monoclonal but non-axenic. The non-toxic marine microalga *Pavlova pinguis* (CCMP strain 459; Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, USA), used as a food source for oyster larvae, was cultivated in a 200 L aerated Kalwall tank with 0.2-μm FSW and f/2 nutrient media, at 20 °C with a salinity of 22.

2.2. Experimental setup

This study aimed to investigate the acute impacts of various *Dinophysis* species and their associated toxins on larval oysters through a series of 48-h laboratory experiments. Oyster larvae were exposed to four *Dinophysis* species, including three producers of DSTs: *D. fortii*, *D. acuminata*, and *D. ovum* strains, and three producers of PTXs *D. caudata, D. fortii, D. acuminata* strains. The intracellular toxin content and unique toxin profiles of these strains is shown in Table 1. Oyster larvae were inoculated at a density of 5 larvae mL^{-1} and were given a non-toxic food *Pavlova pinguis* which was added at a concentration of 30,000 cells mL^{-1}. Subsequently, the larvae were subjected to four treatments over the 48-h experimental period (Table S1): (A) living *Dinophysis* cells at 10 cells mL^{-1}; (B) *Dinophysis* cell lysates at a concentration equivalent to 10 cells mL^{-1}; (C); indirect exposure to living *Dinophysis* cells at 10 cells mL^{-1} whereby larvae were physically separated from cells by a dialysis membrane; and (D) purified OA and PTX2 toxins at concentrations, of 0.2, 0.3, and 1.5 nM, equivalent to the amount of intracellular toxin in *D. ovum*, *D. acuminata*, or *D. fortii* at 10 cells mL^{-1}, respectively.

Diploid oyster larvae, *Crassostrea virginica*, were collected at the early umbo planktonic veliger stage, seven days after fertilization, with an

Table 1
Cell length and intracellular toxin content of the four *Dinophysis* species. Okadaic acid (OA), dinophysistoxin-1 (DTX1), pectenotoxin-2 (PTX2), and hydroxyPTX2. Data are mean ± SD, $n = 3$.

			Intracellular toxin content (pg cell^{-1})			
Species	Strain ID	Cell length (μm)	OA	DTX1	PTX2	H_2PTX2
D. ovum	DOSS 2206	44 ± 4	15.1 ± 2.4	NA	NA	NA
D. acuminata	DAVA01	45 ± 2	NA	0.3 ± 0.1	23.8 ± 1.6	NA
D. fortii	NWFSC 804	73 ± 2	NA	4.9 ± 0.6	124.6 ± 18.4	NA
D. caudata	DCSS 3191	73 ± 5	NA	NA	NA	74.3 ± 3.9

average shell length of 130 ± 20 μm. The oysters were sourced from the Aquaculture Genetics and Breeding Technology Center (ABC) at the Virginia Institute of Marine Science (VIMS).

Dinophysis cells for treatments A to C were harvested from cultures in the exponential growth phase. All experiments were conducted in triplicate in 1-L glass jars containing 500 mL of filtered hatchery seawater (1 μm), at a salinity of 25 and pH of 8. The hatchery water was tested for dissolved DSTs and PTXs to confirm it was toxin-free, with control treatment analyses further verifying the absence of these toxins. Water exchanges were not conducted over the experimental period, as preliminary trials determined pH and dissolved oxygen held constant over 48 h at the experimental levels of light, *Dinophysis*, *Pavlova*, and larval cell densities.

Treatment A: Bioassays were performed with living *Dinophysis* cells (each species administered individually) to evaluate the impact of their interaction with oyster larvae, as well as the potential effects of the continuous production of toxins or bioactive extracellular compounds (BECs). Prior to the beginning of the experiment, *Dinophysis* spp. cultures were filtered through a 10-μm nylon mesh and rinsed with FSW to remove any cryptophyte and ciliate cells, as well as extracellular toxins and BECs. Following this cleansing step, *Dinophysis* cells were then resuspended into jars containing 500 mL of FSW at a density of 10 cells mL^{-1}, while oyster larvae were added at a concentration of 5 cells mL^{-1} creating a 2:1 *Dinophysis*: oyster larvae ratio. All treatments received a single inoculation of *Pavlova pinguis* at 30,000 cells mL^{-1} at the start of the experiment. A control was also included, with oyster larvae fed exclusively with *P. pinguis*.

Treatment B: Lysate bioassays were used to examine the effect of intracellular toxins associated with these HAB species. Therefore, clean, sieved *Dinophysis* cultures of each strain, with a cell concentration equivalent to those used in the living-cell treatment (10 cells mL^{-1}), were centrifuged for 10 min at 3000 ×*g* at 4 °C (5804R, Eppendorf, Hauppauge, New York, NY, USA) and the pellet was resuspended in 1 mL of 100 % methanol and bath-sonicated for 15 min at 25 kHz to lyse cells and centrifuged again as described above. The cell lysate was then added to the jars containing the FSW, oyster larvae, and *Pavlova pinguis*. A vehicle control was included to test for any effects due to the small amount of methanol added with each lysate: methanol was added to jars containing FSW, larvae, and *Pavlova pinguis* at the same final percentage as the treatment, 0.2 %.

Treatment C: Living *Dinophysis* cells were physically isolated from the oyster larvae using a permeable dialysis membrane tubing (Spectra/Por® 7, 10,000 Da) in these bioassays. Dialysis membranes were rinsed with FSW and then filled with 10 mL of each *Dinophysis* strain at a concentration of 10 cells mL^{-1} using cultures that had been previously sieved, washed, and resuspended in FSW. The sealed dialysis membranes were then immersed and suspended in jars containing 500 mL of FSW, larvae, and *Pavlova pinguis*. This approach was used to prevent direct larvae-to-cell contact between oyster larvae and *Dinophysis* species but allow for production of BECs and toxins by living *Dinophysis* strains, and exchange of chemicals across the dialysis membrane throughout the experiment. As a control for any effect of the membrane on larval survival, larvae and *Pavlova pinguis* were exposed to dialysis membranes filled with 10 mL of FSW, without *Dinophysis* spp.

Treatment D: The impact of purified OA and PTX2 was assessed in isolation from cellular interactions and other BECs associated with these HAB species. Purified OA and PTX2 used were purchased commercially, and one concentration of OA was administered to simulate the intracellular toxin content of 10 cells mL^{-1} of *D. ovum* (equivalent to 0.2 nM of OA). Two concentrations of PTX2 were administered to simulate the toxin levels produced by either 10 cells mL^{-1} of *D. acuminata* or *D. fortii* (equivalent to 0.3 nM and 1.5 nM of PTX2, respectively). Oyster larvae were initially supplemented with *P. pinguis* in FSW, and the pure toxin administered to the jars. A control was also included, with oyster larvae fed exclusively with *P. pinguis*, and methanol was added to reach a final concentration of 0.03 %. The effects of hydroxyPTX2 (H_2PTX2) were not tested due to lack of purified material.

After the 48-h exposure period in the various treatments, oyster larvae and treatment water were collected to evaluate larval mortality, toxin accumulation within the larvae, and extracellular toxin concentrations.

To better understand the bioavailability of PTXs in the above treatments, two additional tests were conducted without oyster larvae. These tests aimed to (1) investigate both the enzymatic conversion of PTX2 to PTX2-seco acid (PTX2-sa) in experimental water (Miles et al., 2004), (2) the potential adsorption of dissolved PTX2 to *P. pinguis* cells, the food used in larval treatments A-D. The presence of PTX2-sa was examined based on the hypothesis that this analog may result from the breakdown of PTX2 after its release into the water column by *Dinophysis* cells, as suggested by Takahashi et al. (2007). Three solutions of 10 mL each were prepared by spiking FSW, methanol, and Milli-Q (MQ) water with PTX2 at a concentration of 5 ng/mL. These solutions were left for 48 h to monitor the conversion of PTX2 to PTX2-sa (Fig. 4), and the water was collected and stored at −20 °C for later toxin analysis. In a second test, a 10-mL culture of *P. pinguis* at 30,000 cells mL^{-1} was spiked with PTX2 at a concentration of 15 ng/mL and incubated for 48 h to evaluate the adsorption of PTX2 to the cells (Table 2). After the incubation period, the samples were collected and centrifuged for 10 min at 3000 ×*g* at 4 °C, and the cell pellets were separated from the supernatants. The cell pellets were then resuspended in 1 mL of 100 % methanol, and all the samples were stored at −20 °C for later toxin analysis. These additional tests were conducted under the same conditions of temperature, salinity, and light intensity as previously described or oyster experiments.

2.3. Microscopic analysis- mortality assessment

For the assessment of larval mortality, jars were sieved using a 60-μm mesh to collect oyster larvae, which were then rinsed and suspended in 20 mL of FSW (0.22 μm-filtered). Subsequently, 2 mL subsamples were transferred to a well plate, where they were subjected to microscopic analysis for oyster larva enumeration using light microscopy at 40 × magnification (Olympus CKX53 or IX50 inverted microscopes, Olympus Corp., Shinjuku, Tokyo, Japan). To minimize stress and accurately assess larval mortality, the well plate was left undisturbed under the microscope for 5 min before observations and counting started. Oyster larvae

Table 2
Intracellular and extracellular PTX2 toxin concentration in *P. pinguis* culture spiked with 15 ng/mL PTX2 after 48-h of exposure.

	P. pinguis culture spiked with 15 ng/mL of PTX2	
	Intracellular PTX2 (ng/mL)	**Extracellular PTX2 (ng/mL)**
PTX2 attachment to larval food *P. pinguis*	13 ± 1.3 79 % ± 8 %	3.4 ± 0.4 21 % ± 2 %

were closely monitored for an additional 3 min and were counted as dead if they exhibited no ciliary movement or had an intact but empty shell. After the counting of dead oyster larvae, 10 μL of Lugol's iodine solution was added to each well plate causing larval mortality and allowing for a total count of larvae in each well. The average percentage of dead larvae for each treatment across three replicates was then calculated.

2.4. Toxin analyses of Dinophysis culture, treatment water, and oyster larvae

Toxin extraction and quantification were carried out to quantify (1) the intracellular toxin content in the stock culture of each *Dinophysis* species and *P. pinguis* cells after exposure (testing the bioavailability of PTX2 via potential adsorption onto *P. pinguis* cells), (2) the extracellular toxin concentrations in the treatment water, MQ and methanol solutions, and (3) the toxins accumulated in the oyster larvae after 48-h of exposure within treatments A – D. To extract intracellular toxins, a 10-mL sample was collected from of the stock culture of each *Dinophysis* strain during their exponential growth phase and from *P. pinguis* cells after 48-h of exposure. Samples were centrifuged at 3000 *g* at 4 °C for 15 min (5804R, Eppendorf, Hauppauge, New York, USA) to separate the culture into cell pellet and supernatant. Cell pellets (intracellular toxins) were extracted with 1 mL 100 % methanol, sonicated at 25 kHz for 15 min, centrifuged, and then syringe-filtered through a 0.22-μm, 13-mm filter (Millex PVDF, Durapore), and stored at −20 °C for later toxin analysis.

Any impact of the centrifugation speed and duration on *Dinophysis* cells, employed in this study, was independently assessed, revealing minimal leakage of dissolved toxins from the cells into the surrounding media. Specifically, only 15 % of the intracellular toxins were released due to the centrifugation process as described above (Table 3). This indicates that the centrifugation parameters used were effective in maintaining the integrity of the cells and minimizing toxin release.

To extract the extracellular toxins from the treatment water, 40-mL samples were collected from treatments A, B, and C at the end of the exposure period. Additional 7-mL samples were collected from treatment C, from within the dialysis membrane, to quantify the concentration of extracellular toxins inside the membrane. The samples were filtered through a 60-μm mesh to remove the larvae, followed by centrifugation at 3000 *g* at 4 °C for 15 min, specifically aimed to remove living *Dinophysis* cells from treatment A and C. Furthermore, 10-mL samples were collected from the tests described above, to assess the potential conversion of PTX2 to PTX2-sa in FSW, MilliQ water, and 100 % methanol solutions. The samples were then subjected to extraction, concentration, and desalination using a 60-mg Oasis HLB solid-phase extraction (SPE) cartridge (Waters, Milford, MA, USA). The cartridge was pre-conditioned with 3 mL of 100 % methanol followed by 3 mL of molecular-grade water, and the supernatants (40 and 7 mL) were then loaded to the cartridge, washed with 3 mL of molecular-grade water, and subsequently aspirated for 1 min to remove excess water. Toxins were eluted with 1 mL of 100 % methanol, filtered using a PVDF syringe filter (0.2 μm, 13 mm), and the eluate was kept at −20 °C for later toxin analysis.

Oyster larvae were subjected to a meticulous preparation process to ensure accurate toxin quantification. The larvae were initially sieved and washed twice with FSW upon collection to minimize toxin adsorbed on their shells; this effort, therefore, allows for the reporting of the toxins accumulated within the larvae throughout the 48-h exposures. To extract intracellular toxins from oyster larvae, 100 % methanol was added to the larvae, and extraction was aided by probe sonification: sonification on ice for 3 min, with 30-s pulses at 40 % amplitude using a Branson Digital Sonifier-450. The resulting methanolic extract was centrifuged for 5 min at 3000 *g* and 4 °C and the supernatant was filtered through a 0.22-μm syringe filter (13-mm, Millex PVDF, Durapore) and stored at −20 °C for later toxin analysis.

Toxin analyses were performed using a tandem quadrupole Xevo TQD ultra-performance liquid chromatography system (Waters) coupled to an electrospray ionization (ESI) source with a trapping dimension and at-column dilution (UPLC– MS/MS with trap/ACD) following Onofrio et al. (2020). Certified calibration solutions of PTX2, OA, and dinophysistoxin-1 (DTX1) were obtained from the National Research Council Canada (NRCC, Halifax, NS, Canada). HydroxyPTX2 was identified based on retention time, *m/z* ratio, and the detection of specific product ion transitions under positive ionization mode. Quantification was performed using a PTX2 standard curve, following the methodology outlined by Miles et al. (2004, 2006) (Table S2). The limits of detection (LOD) were 0.04 nM for OA and 0.02 nM for PTX2.

A modified version of the method described in McCarron et al. (2011) was applied to evaluate the conversion of PTX2 to PTX2sa in treatment water and oyster larvae. The analysis was performed on an Agilent 1260 LC coupled to an Applied Biosystems API4000 QTrap equipped with a turbospray ionization source. A binary mobile phase was used, with A (water) and B (95 % aqueous acetonitrile) each containing 5-mM ammonium acetate (pH 6.4). A Phenomenex Synergi MaxRP C12 column (50 × 2 mm i.d., 2.5 μm) was eluted at 300 μL/min with a linear gradient of 25 %–100 % B over 5 min, held for 1 min before a flow rate increase to 650 μL/min for 3 min. The flow was then decreased to 300 μL/min and B reduced to 25 % over 2 min before equilibrated until the next run. Selective reaction monitoring (SRM) transitions in positive ionization mode transitions were PTX2 876.5 > 823.5 and 876.5 > 213.1, PTX2sa 894.5 > 823.5, and 894.5 > 213. SRM transitions combined with retention times were used to identify PTXs present in samples. Five-point calibrations were acquired using a mixed standard prepared from certified calibrants of PTX2 and an in-house reference material of PTX2sa. Estimated LODs for PTX2 and PTX2sa were 0.36 and 0.31 ng/mL respectively.

2.5. Statistical analysis

All bioassays were carried out in triplicate, and the data were presented as mean values ± standard deviations (SD). Statistical analyses were conducted to assess potential variations in oyster larval mortality and toxin content across distinct treatments and among different *Dinophysis* species along with their respective toxins. To ensure the validity of the analysis, assumptions of independence (Durbin-Watson test), homoscedasticity (Bartlett test), and normality (Shapiro-Wilk test) of the residuals were assessed. When the previous assumptions were not met, data were log-transformed, alternatively, Mann-Whitney U or Kruskal-Wallis tests were used, followed by a Conover test. A two-way ANOVA was conducted, with the treatments and the *Dinophysis* species as factors, followed by a Tukey post hoc test to determine the sources of significant differences. Statistical significance was determined based on *p*-value <0.05, for a significance level of $\alpha = 0.05$, and were carried out using RStudio version 1.2.5033 (R Development Core Team, 2021).

3. Results

3.1. Effect of Dinophysis spp. and purified toxins on oyster larvae mortality

The percent mortality of larvae exposed to living cells, lysates and

Table 3
Impact of centrifugation speed and duration on PTX2 leakage from cells into media.

	D. acuminata culture	
Extraction protocol validation	**Intracellular PTX2 (ng/mL)**	**Extracellular PTX2 (ng/mL)**
	15.86 ± 0.72	2.88 ± 0.21
	85 % ± 4 %	15 % ± 1 %

dialysates of 4 strains of *Dinophysis,* as well as purified OA and PTX2 toxins, is shown in Fig. 1. The oyster larvae exhibited statistically significant increases in percent mortality when subjected to living cells of *D. caudata* and *D. fortii* species, 28 ± 4 % and 25 ± 6 %, respectively, compared to the control group, which displayed a baseline percent mortality of 9 % (Fig. 1A).

In contrast, exposure to living cells of *D. acuminata* or *D. ovum* did not result in significant mortalities in oyster larvae, with only 7 % observed in both species (Fig. 1). Furthermore, exposure to the lysate of any *Dinophysis* strain or exposure to membrane-separated, living *Dinophysis* cells also failed to elicit a significant effect (Fig. 1). The mortality percentages in the lysate treatments ranged from 8 % to 9 % and 11 % to 13 % in dialysate treatments (Fig. 1).

Similarly, exposure to purified OA and the lower concentration of purified PTX2 did not have a notable impact on oyster mortality, producing 12 % and 13 % mortality, respectively, compared to the control group (10 %) (Fig. 1). The high PTX2 treatment (1.5 nM), however, produced 17 ± 2 % mortality (Fig. 1).

3.2. Toxin accumulation in oyster larvae

Following 48 h of exposure, toxin analysis revealed that both PTX2 and hydroxyPTX2 accumulated in the oyster larvae in treatments A – D (Fig. 2), however, OA and PTX2-sa were not detected in oyster larvae in any of the treatments. Notably, significantly higher levels of PTXs were detected in treatment A involving larvae exposed to living *Dinophysis* cells $p < 0.05$. The highest concentrations of PTXs coincided with the highest percent of larval mortality, particularly in oyster larvae exposed to living *D. caudata* (8.36 pg larva^{-1} of hydroxyPTX2) and *D. fortii* cells (5.7 pg PTX2 larva^{-1}) in treatment A (Figs. 1 and 2). Oyster larvae exposed to living *D. acuminata* cells also exhibited significant accumulation of PTX2 within their cells (0.62 pg larva^{-1}, $p < 0.05$), albeit approximately one order of magnitude lower than those detected in treatment A involving *D. caudata* and *D. fortii* species (Fig. 2).

Toxins (PTXs) accumulated in oyster larvae in the lysate and dialysate treatments (B and C) but were substantially less than in the living cell treatments (A). *D. acuminata* lysates transferred about 11 % as much toxin as living cells and dialysates about 3 % as much. *D. caudata* lysates and dialysates transferred 3 % and 2 %, respectively, and *D. fortii* lysates and dialysates 9 % and 6 % (Fig. 2).

Similarly, when oyster larvae were subjected to purified OA or treatments involving *D. ovum* species (living cells, lysate, or dialysate), no detectable amounts of this toxin were found within their cells. In contrast, when oyster larvae were exposed to purified PTX2 at concentrations of 0.3 and 1.5 nM, a minimal accumulation of PTX2 within the oyster larvae was observed (0.03 and 0.16 pg larva^{-1} respectively; Fig. 2, right panel), and was 1- to 2-orders of magnitude lower in comparison to the levels found in the living *D. acuminata* and *D. fortii* bioassays under treatment A (0.6 and 5.7 pg cell^{-1}, respectively; Fig. 2, left panel).

3.3. Toxins remaining in the treatment water after the 48-h exposure experiments

Following 48 h of exposure, only PTX2 and hydroxyPTX2 were found in the treatment water (treatments A – C). The extracellular toxin concentration in treatment D was not measured, as a known concentration was introduced at the start of the exposure experiments for this treatment. The living treatment (A) resulted in the highest PTX concentrations exposed to oyster larvae in comparison with the other treatments (for *D. fortii* and *D. caudata* 0.54 and 0.18 nM, respectively; Fig. 3). Significantly lower extracellular PTX concentrations were measured in the lysate treatment (B) and were 0.23, 0.12 and 0.02 nM for *D. fortii*, *D. caudata* and *D. acuminata*, respectively ($p < 0.05$). For the dialysate treatment (C), toxin concentrations both inside and outside of the dialysis membrane were examined. Significantly higher concentrations of PTXs were found inside the dialysis membrane, where *Dinophysis* cells were present, for *D. fortii* and *D. caudata* species, showing unequal

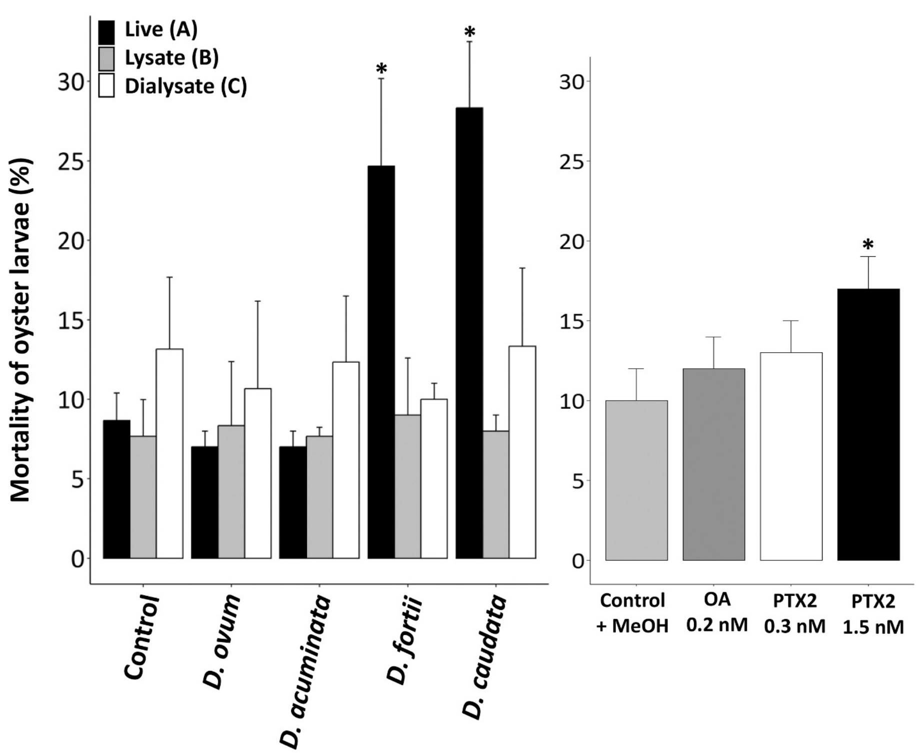

Fig. 1. Percent mortality of oyster larvae *C. virginica* following 48 h of exposure to various treatments: in the left panel: live *Dinophysis* cells (A), lysate (B), and dialysate (C); and in the right panel: exposure to 0.2 nM of purified OA, or 0.3 or 1.5 nM of PTX2 (treatment D). Data are mean ± SD, $n = 3$. Asterisks indicate significant differences compared to the control group.

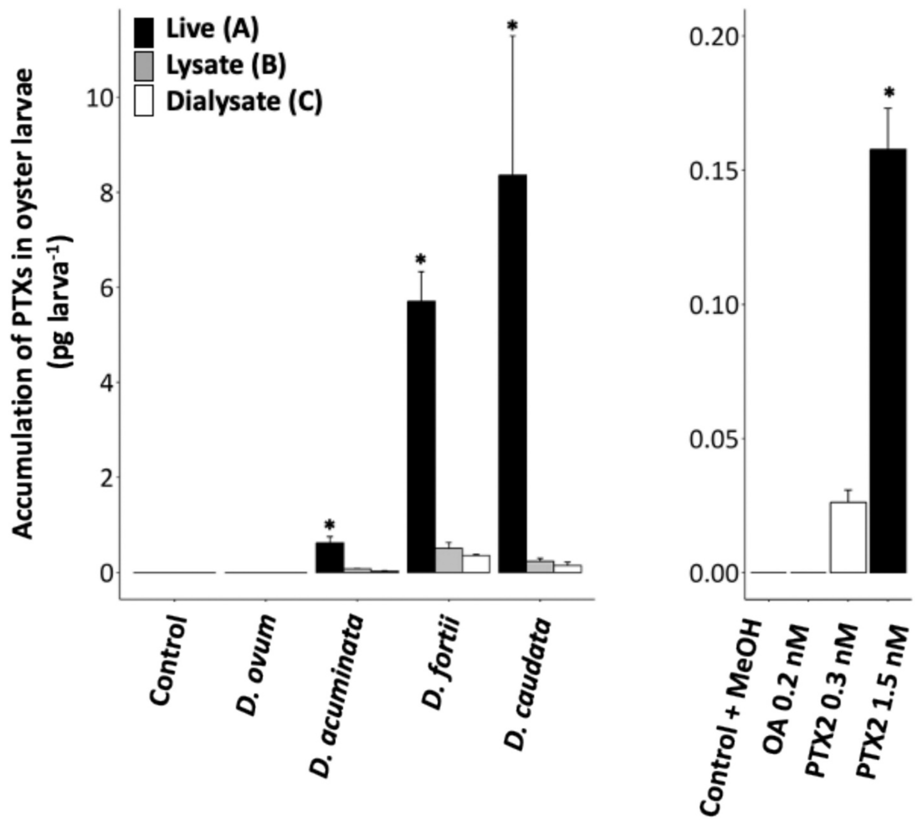

Fig. 2. Accumulated PTXs (PTX2 + H_2PTX2) in oyster larvae (expressed in pg larva^{-1}) after 48 h of exposure to various treatments: in the left panel: live *Dinophysis* cells (A), lysate (B), and dialysate (C). In the right panel: exposure to purified OA at 0.2 nM and PTX2 at 0.3 or 1.5 nM (treatment D). Data are mean ± SD, n = 3. Note the difference in scales between the left and right panels. Asterisks indicate significant differences compared to the control group.

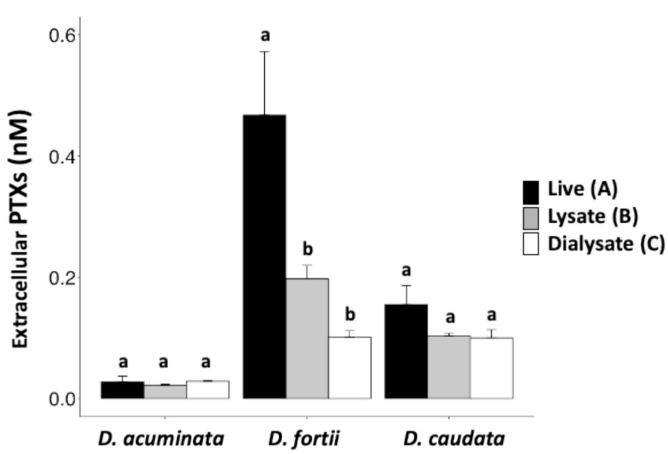

Fig. 3. PTXs concentration (nM) measured in the extracellular media after 48 h of exposure in the live (A), lysate (B) and the dialysate (C) treatments for the different species that produce PTXs. Data are presented as mean ± SD, n = 3. Treatments with superscript letters were significantly different within each species.

partitioning between the inside and outside of the membrane ($p < 0.05$), with the highest concentrations detected inside the membrane of *D. fortii* and *D. caudata* bioassays (0.40 and 0.41 nM). Only 30 % of the extracellular PTXs were found outside the dialysis membrane, with 0.12 and 0.11 nM for *D. fortii* and *D. caudata*, respectively (Fig. 3). Conversely, there was no significant difference in PTX2 concentrations between the inside and outside of the dialysis membrane for the *D. acuminata* species (0.02 nM and 0.03 M, respectively). Furthermore, in the tests assessing the bioavailability of PTX2 in treatment water, no evidence was found of PTX2 conversion to PTX2sa in FSW, MilliQ, or 100 % methanol after 48 h, in the absence of oyster larvae (Fig. 4). Moreover, in the second test, significantly higher concentrations of PTX2 (13 ng/mL), accounting for 79 % of the total detected toxin, were adsorbed onto *P. pinguis* cells, while only 21 % (3.4 ng/mL) remained in the dissolved form in the treatment water (Table 2).

4. Discussion

The aim of this study was to explore the impact of four species of the dinoflagellate *Dinophysis* spp., and their associated toxins on the larvae of the economically and ecologically important oyster species, *Crassostrea virginica*. The findings revealed significant mortality when larvae were exposed to living *D. caudata* and *D. fortii* species (treatment A), which contained the highest intracellular content and extracellular concentrations of pectenotoxins (H_2PTX2 and PTX2, respectively) across all strains and treatments. Moreover, exposure to pure PTX2 at a concentration of 1.5 nM, equivalent to that contained within the same number of *D. fortii* cells, led to a decrease in larval viability, though to a lesser extent compared to exposure to living *D. fortii* cells. In addition, when comparing the amount of accumulated toxins within oyster larvae across all treatments, only PTXs were detected in the larvae, with their load (toxin per larvae) being significantly higher in the living treatment (A), particularly when *D. caudata* and *D. fortii* were present. Conversely, exposure to okadaic acid (OA) and a strain of *D. ovum* that only produces OA, demonstrated no measurable effect on the survival of oyster larvae (treatments A – D).

These findings align with the only prior studies available that have explored the impact of exposing early life stages of oysters, including gametes and larvae, to *Dinophysis* species and their toxins (Gaillard et al.,

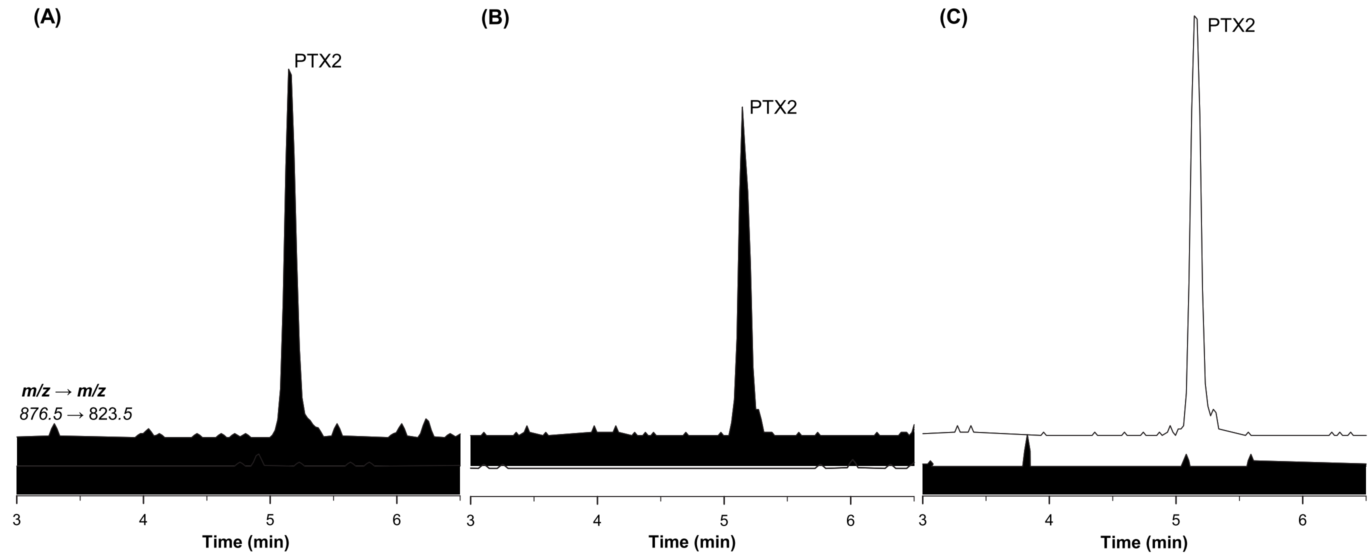

Fig. 4. A) Filtered seawater and B) MilliQ water C) methanol all spiked with 5 ng/mL PTX2. The selected reaction monitoring (SRM) method included transitions for PTX2 (*m/z* 876.5 > 823.5) and PTX2 seco acid (*m/z* 894.5 > 823.5).

2020; Pease et al., 2022). Consistent with our study, these authors did not observe any significant negative effect of OA and its producer on oyster gametes and larvae, despite examining toxin concentrations that were one to two orders of magnitude higher than those examined in the present study (Gaillard et al., 2020; Pease et al., 2022). Instead, the authors suggested that PTX2 and PTX2-producing *Dinophysis* species were the primary contributors to the negative effects observed in the early life stages of oysters. In particular, Pease et al. (2022) observed higher inactivity and increased mortality in larvae of the oyster species *C. virginica* after a 96-h exposure to living cells of *D. acuminata*, producers of OA and PTX2, (at a concentration of 1000 cells mL^{-1}), culture lysate (equivalent to 10,000 cells mL^{-1}), and purified PTX2 (208 nM, equivalent to 10,000 cells mL^{-1}). Furthermore, the exposure to purified PTX2 resulted in 50 % larval mortality within 48 h, and induced complete immobilization of larval oysters (Pease et al., 2022). In line with Pease et al. (2022) findings, exposing oyster larvae to a reduced cell concentration of 10 cells mL^{-1} in living cell exposure experiments, using the same *D. acuminata* species albeit with a different strain, did not have any negative impact on the viability of eastern oyster larvae.

In addition, Gaillard et al. (2020) reported that a 2-h exposure of Pacific oyster *C. gigas* gametes to *D. sacculus* cultures (producers of OA and PTX2), at concentrations ranging from 0.5 to 500 cells mL^{-1}, resulted in increased oocyte mortality with a detrimental impact on fertilization success. Moreover, exposure to purified PTX2 (at concentrations ranging from 5 to 50 nM) induced elevated reactive oxygen species (ROS) production and a reduction in fertilization success (Gaillard et al., 2020). The authors suggested that PTX2 could impact the polymerization of actin filaments, potentially affecting the cytoskeleton of oocytes and spermatozoa and, consequently, their viability to produce gametes. Indeed, PTXs have been shown to interfere with actin assembly/disassembly, thereby affecting cell cytoskeletal functions and leading to cell death, at concentrations ranging from nM to μM in mammalian and finfish cell lines (e.g. human, rat, rabbit, salmon; (Allingham et al., 2007; Ares et al., 2005; Dominguez et al., 2010; Hori et al., 2018).

Building upon these previous investigations, the present study investigated the harmful effects of unstudied species, *D. fortii* and *D. caudata*, along with their related toxins, PTXs, on the survival of oyster larvae. The *D. caudata* strain used in this study was previously analyzed for its toxin profile using High-Resolution Mass Spectrometry (HRMS) (Ayache et al., 2023), and it was shown to exclusively produce a newly identified PTX analog, hydroxyPTX2. This study investigated the impact of this PTX analog on *C. virginica* larvae, revealing its higher toxicity compared to PTX2 when administered as an intracellular toxin within *Dinophysis* species (treatment A). Despite *D. fortii*'s intracellular PTX2 content being nearly twice as high (125 pg cell^{-1}), its effect on larval mortality was similar to that of *D. caudata* (producer of H_2PTX2 at 74 pg cell^{-1}), resulting in 25 % and 28 % mortality, respectively. This finding suggests that H_2PTX2 is more potent than PTX2, however, investigations using purified H_2PTX2 material, which is currently unavailable, are needed to quantify its toxic effects relative to PTX2 and other bioactive extracellular compounds produced by *D. caudata* that may have contributed to observed larval mortality.

The effects of PTXs are not limited to larval animals. Basti et al. (2015) conducted the first feeding experiments with a PTX2-producing *D. caudata* strain (isolated from Japan) exposed to adult Japanese scallops, *Mizuhopecten yessoensis*, and Noble scallops, *Mimachlamys nobilis*. Both scallop species displayed altered behavior, including mucus hypersecretion, reduced escape response, paralysis, and death (73 % and 40 %, respectively) after a week of being fed a daily ration of *D. caudata* at a concentration of 10 cells mL^{-1}. The authors suggested that the observed effects were not solely attributed to PTX2 but might involve other unidentified bioactive extracellular compounds (BECs) from *D. caudata*. This hypothesis was supported by the lack of a correlation between scallop mortality and the increase of PTX2 content in *D. caudata* (Basti et al., 2015), and by the ability of adult scallops to oxidize PTX2 to PTX6, an analog reported to be non-toxic to adult Japanese scallops (Suzuki et al., 2005).

In response to marine HAB events, adult bivalves employ strategies including cessation of feeding, closure of their valves, or rejecting toxic cells to minimize toxin exposure (Lassudrie et al., 2020; Bricelj and Shumway, 1998; May et al., 2010). However, in the current study, the observed toxic effects on oyster larvae could not be attributed to the consumption of *Dinophysis* cells. The 7-day-old larvae used in the current study (~ 130 μm in length) have not developed the ability to ingest cells of this size (ranging from 44 to 73 μm in length; (Carriker, 2001). Instead, the effects are likely due to dissolved toxins and/or BECs present in the surrounding water. Alternatively, the filtration of toxins adsorbed to small live *Pavlova pinguis* cells by larvae could have facilitated their exposure to toxins or BECs. A similar mechanism of accumulation of dissolved toxins has been observed by Jauffrais et al. (2013). The authors showed that the bioavailability of dissolved toxins (AZA1 and 2) to adult blue mussels increased when cell debris from lysed *Azadinium spinosum* cells and non-toxic food from *Isochrysis affinis* *galbana* were present. The toxins were subsequently accumulated and distributed across different tissues depending on the exposure route; either in the digestive glands through feeding activity or in the gills via respiratory/ filtration processes (Jauffrais et al., 2013).

Exposure of oyster larvae to purified PTX2 at a concentration of 0.3 nM did not yield any significant impact on their survival, unlike the higher concentration of 1.5 nM (Fig. 1). Consequently, it seems that this lower PTX2 concentration was below the effective concentration or toxic threshold capable of influencing their survival. This implies that the threshold concentration of PTX2, where a minimal yet significant effect on larval survival is detected, falls between 1.5 and 0.3 nM, highlighting the necessity for further investigations to characterize the lower toxicity threshold.

The treatments involving cell lysate and dialysate also did not exhibit any significant impact on the survival of oyster larvae across the tested species. However, larval exposure to living *Dinophysis* cells that can produce PTXs was necessary to cause significant mortalities. Besides the exposure of oyster larvae to higher extracellular PTXs, the increased mortality during living cell exposure (treatment A) may be due to (1) the production of BECs facilitated through contact with larvae, or (2) interference with larval feeding, ventilation, or swimming due to physical contact with large *Dinophysis* cells (i.e., *D. fortii* and *D. caudata*, which are approximately half the size of the oyster larvae). Findings from the study by Tang and Gobler (2012) support the latter hypothesis. The authors noted that the spirolide-producing dinoflagellate *Scrippsiella trochoidea* caused 100 % mortality in *C. virginica* larvae during 3-day exposures through a physicochemical rather than strictly chemical mechanism, such as clogging of larval feeding apparatuses by materials produced by *S. trochoidea* (e.g., lipids, and extracellular polysaccharides).

Furthermore, previous research on the *Alexandrium* genus highlighted its allelopathic effects, impacting the growth of various phytoplankton and zooplankton species, and inducing pathologies in marine organisms, including bivalves (Satake et al., 2019; Tillmann et al., 2016; Tillmann and John, 2002). These effects were interpreted as defensive reactions triggered by *Alexandrium* cells and their BECs, with the exclusion of paralytic shellfish toxins (PST). For example, Castrec et al. (2018) identified distinct cytotoxic effects in adult oysters (*C. gigas*) exposed to a non-PST, BEC-producing strain of *A. minutum*. These effects included altered valve activity and gill tissue, as well as reduced feeding activity compared to a PST-producing strain. Similarly, Borcier et al. (2017) found that juvenile scallops (*Pecten maximus*) exposed to BECs produced by *A. minutum* displayed cytotoxic effects, resulting in cessation of growth due to tissue alterations and a decline in filtration rate. In this study, the observed effects on oyster larval mortality may be partly attributed to BECs in addition to PTXs. This hypothesis is supported by the significant reduction in larval mortality observed in treatment (D) with 1.5 nM purified PTX2, as well as when the oyster larvae were physically isolated from *Dinophysis* cells using a dialysis membrane. Worth noting, in the dialysate treatment (C), the concentration of extracellular PTXs measured both inside and outside the membrane equaled or exceeded those found in the living treatment (A) for *D. fortii* and *D. caudata* bioassays, respectively. However, since the majority of the extracellular toxin was retained in the membrane, it remains unclear if BECs or cell contact were the cause of additional mortality in living cell treatments. The cutoff of the dialysis membrane (10,000 Da) used in this study was sufficiently large to allow the transfer of PTXs, DSTs, and BECs across the two compartments. However, it would also retain larger molecules, such as mucilage and other organic matter produced by *Dinophysis* within the membrane. These retained macromolecules could have played a significant role in adsorbing the toxins and other BECs, potentially contributing to their retention within the membrane. This hypothesis is supported by Pizarro et al. (2013), who found that in plankton net-hauls from turbulent, detritus-rich water columns with undetectable levels of *Dinophysis* cells, most detected toxins were adsorbed by organic aggregates.

During the examination of PTXs concentrations in the water post lysate treatment (B), it was noted that only a small fraction persisted: 0.02, 0.23, and 0.12 nM, corresponding to 7 %, 16 %, and 14 %, for *D. acuminata*, *D. fortii*, and *D. caudata*, respectively, compared to the initially estimated PTXs levels (Fig. 3 and Table 2). While some PTXs accumulated in the oyster larvae, a significant portion of these toxins were likely adsorbed onto the *P. pinguis* cells. This was demonstrated in an additional test designed to investigate the fate of dissolved PTX2 toxins in the media (Table 2). The test results indicated that there was no conversion of PTX2 into PTX2-sa in the water (Fig. 4). Furthermore, the majority of the dissolved PTX2 (79 %), was found to be adsorbed onto the *P. pinguis* cells (Table 2). This adsorption process likely made the toxin more bioavailable for the oyster larvae to assimilate, suggesting that the larval food could play a significant role in the dynamics of PTX2 availability in the surrounding water.

Additionally, the lack of effect in the lysate treatment could be attributed to the absence of introducing BECs into the treatment water. Given that the lysate treatment utilized only the extracted cell pellets and excluded supernatant water containing extracellular released BECs, their potential impact on the treatment was omitted.

Oyster larvae accumulated PTXs in all tested treatments, even without significant mortalities. It is crucial to underscore that although acute exposure may not result in immediate mortality, the stress induced by factors like PTX accumulation has the potential to impact critical physiological aspects and ultimately impact the survival or settlement of these vulnerable early life stages of oysters. This was demonstrated in the studies conducted by Pease et al. (2022) and Gaillard et al. (2020), where exposure to *Dinophysis* cells and toxin content in certain treatments did not lead to immediate mortality of the larvae or the exposed gametes. Instead, Pease et al. (2022) observed a state of inactivity in the larvae after a 48-h exposure, while Gaillard et al. (2020), observed a significant reduction in fertilization success following the exposure of gametes. In addition, several studies have recently documented the impact of the accumulation of other HAB toxins on the development and survival of oyster larvae (Castrec et al., 2019, 2020; Rolton et al., 2016). For instance, in Castrec et al. (2020), the authors found that Pacific oyster larvae (*C. gigas*) fed with toxic *Alexandrium minutum* accumulated paralytic shellfish toxins (PST). The exposure did not significantly impact their survival (5 % mortality); however, sublethal effects were noted, including a reduction in larval growth, a delay in development, alterations in swimming behavior (sinking), and a significant decrease in their settlement rate (9 % settlement yield compared to 17 % in the control treatment). Moreover, in Rolton et al. (2016) and Castrec et al. (2019), HAB toxins (brevetoxin and paralytic shellfish toxins, respectively) were accumulated within the oocytes and spermatozoa of *C. virginica* and *C. gigas*, respectively. While there was no notable impact on the percentage of fertilization success, the larvae resulting from this neonatal exposure showed significantly smaller sizes and higher mortality rates during the initial post-fertilization period. Besides the acute lethal effects, the uptake and elimination of HAB toxins seems to be an energy-intensive process, leading to higher maintenance costs and a reduction in energy allocated for other critical physiological functions like growth and feeding, as well as disease resistance. Therefore, these developmental delays and decreased overall fitness may have severe consequences for hatchery production and the oyster population as a whole, including a delayed rate of larval settlement and an increase of their susceptibility for predation.

In this study, we used exposures of 10 cells mL^{-1} which is a commonly observed density during spring and summer *Dinophysis* blooms, but which is much lower than peak densities that have been observed occasionally in recent years in the Northeast Atlantic or Northwest Pacific coasts of the US where major oyster production sites are located (Ayache et al., 2023; Gobler et al., 2017; Trainer et al., 2013; Wolny et al., 2020). These exceptional blooms, particularly for *D. acuminata*, have been reported with concentrations exceeding 200 cells mL^{-1} to $>$1000 cells mL^{-1} in some regions (Ayache et al., 2023).

Considering that natural and hatchery spawning, as well as early larval development of oysters, occurs primarily between mid-March and mid-September in the Chesapeake Bay for example, there is a high likelihood of temporal overlap between *Dinophysis* cells and their associated toxins with the planktonic larvae. Therefore, the potential for exposure during these unusual events suggests that early oyster life stages may face PTX concentrations 60 to 200 times higher than those tested in this study. A similar scenario was demonstrated in Pease et al. (2022), where over 50 % of the larvae died after 24 h of exposure to high *D. acuminata* cell concentration (1000 cells mL^{-1}) and all larvae stopped swimming after exposure to pure PTX2 toxin (equivalent to 208 nM, 10,000 cells mL^{-1}). In addition, in this current study, exposure experiments lasted only two days, thus, it is possible that moderate bloom densities, and short time exposure of *Dinophysis* blooms may have little to no impact on oyster larval survival, particularly in the case of *D. acuminata*. However, prolonged blooms and toxin exposures that last weeks could potentially lead to a decline in larval survival and development, as well as impede their growth into settled, healthy juveniles. Hence, continuing these experiments until the eyed and setting juvenile stages would offer valuable insights into the longer-term effects of toxin exposure. Additionally, exploring any potential carryover effects that could influence altered resilience to additional stressors, such as disease susceptibility, or the ability to tolerate temperature or salinity fluctuations, would be of great interest. In recent studies by Onofrio et al. (2021); and Sanderson et al. (2022), the authors reported the persistent presence of dissolved PTX2 toxin year-round in the water column across various sites in the lower Chesapeake Bay and coastal bays of Virginia even in the absence of detectable cells. In addition, in the Sanderson et al. (2022) study, low concentrations of PTX2 were found in hatchery treated water used for oyster larval production at five different hatcheries during spawning and early larval development of *C. virginica*. The authors noted that the surveyed hatcheries did not experience larval mortality events or production issues, however, the measured PTX2 concentrations were 2 to 3 orders of magnitude lower than those examined in this study. The authors also highlighted the effectiveness of sand and activated charcoal filtration in one of the studied hatcheries, resulting in an 81 % reduction in PTX2 concentrations from the source water. However, these toxin levels were detected during non-bloom conditions and in the absence of *Dinophysis* cells in the external hatchery water. Therefore, it is crucial to assess the efficacy of filtration steps in removing higher toxin concentrations expected during typical or exceptional bloom events and to evaluate the impact of the exposure of any remaining toxin on oyster larvae.

5. Conclusion

The implications of these results are considerable, given the economic value of *C. virginica* for both oyster farmers and coastal communities in the US. This oyster species has experienced a drastic decline in both harvest and abundance over the past 130 years (Mace et al., 2021; Rothschild et al., 1994) and any further impact on the development and survival of this organism in its early life stages will pose additional challenges to the restoration efforts of the oyster populations in the US. While mortality resulting from infectious diseases remains the primary concern for this sector, new studies have shown links to marine HABs in outbreaks of bivalve mortalities, specifically by influencing the immune system and defense mechanisms of bivalves (Gray et al., 2022; Lassudrie et al., 2020; Trainer and King, 2023). Hence, the exposure to *Dinophysis* cells and their harmful compounds (PTXs and BECs), which have lethal and sublethal effects during early life stages (present study), coupled with increased vulnerability to diverse environmental disturbances, could potentially threaten oyster recruitment, yields, and aquaculture production and lead to significant socio-economic and ecological consequences (Shumway, 1990).

CRediT authorship contribution statement

Nour Ayache: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marta Sanderson:** Writing – review & editing, Visualization, Methodology, Conceptualization. **Jessica Small:** Writing – review & editing, Validation, Resources, Methodology, Investigation. **Amanda B. Chesler-Poole:** Writing – review & editing, Methodology. **Sabrina D. Giddings:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation. **Lisa Campbell:** Writing – review & editing, Validation, Methodology, Investigation. **Vera L. Trainer:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Michael L. Brosnahan:** Writing – review & editing, Validation, Methodology, Investigation. **Juliette L. Smith:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2025.742294.

Data availability

Data will be made available on request.

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Corrigendum to "Survival and toxin accumulation in *Crassostrea virginica* larvae following exposure to various *Dinophysis* species" [Aquaculture, Volume 602, 30 May 2025, 742294]

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The authors regret an error in the abbreviation of a toxin analog in the text. "HydroxyPTX2" was incorrectly cited as "H_2PTX2" instead of "HOPTX2" which could mislead readers, as "H_2PTX2" refers to another analog produced by a different *Dinophysis* species. Furthermore, this error occurs seven times throughout the text.

The authors would like to apologise for any inconvenience caused.

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