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Impact of the Toxic Dinoflagellate *Alexandrium catenella* on the Valve Movement of *Mytilus edulis*: A Comparison between Two Populations with Contrasting Histories Exposure

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Abstract: Shellfish aquaculture farms, due to their coastal position, face the threat of exposure to harmful algal blooms. Such blooms can release, among others, paralytic shellfish toxins (PST) produced by the dinoflagellate *Alexandrium catenella* and are known to cause the restriction of bivalve harvesting sites. Shellfish can accumulate PSTs in levels that are poisonous for humans, therefore making them unfit for consumption. Thus, the ability to detect PSTs before they reach the critical threshold is crucial for minimizing losses in the industry. Previous studies have demonstrated that toxic algae detection is possible with the use of an early warning system based on the valve-gaping behaviour of blue mussel *Mytilus edulis*. However, some studies observed the presence of toxin resistance in other species of bivalves when they are regularly exposed to PSTs. If no resistance is observed whatever the past history of the populations would be with regard to PST exposure, this species could be appropriate as a sentinel candidate. In this study, we compare the valve-gaping behaviour of two blue mussel populations with contrasting long-term histories of PSTs events (i.e., regularly vs. not previously exposed to the PSTs producer) were compared using experimental exposure of *A. catenella* to *M. edulis*. It was found that mussels from both populations exhibited similar gaping behaviour patterns when exposed to *A. catenella*. For both populations, the number of valve closures and closure duration tended to increase in the presence of *A. catenella*, which suggested an avoidance response to the toxic dinoflagellate. In conclusion, our results support the use of *M. edulis* without origin discrimination

Keywords: biotoxins; early warning system; harmful algal blooms (HABs); paralytic shellfish poisoning (PSP); PSP resistance; valvometry

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1. Introduction

Paralytic shellfish toxins (PST) are toxins produced by some dinoflagellate species, such as *Alexandrium catenella* [1]. This species is typically found along the eastern coast of North America during the summer [2,3], thriving in conditions characterized by a salinity range of 9 and 19 °C [4–6]. PSTs can lead to large economic losses for the shellfish aquaculture industry, as filter feeding bivalves consume toxic algae out of the water column, which can lead to toxin accumulation within their tissues [7,8]. While PSTs are not toxic to the bivalves themselves, they do pose a severe health risk to humans when they are consumed [9], triggering symptoms such as headache, nausea, facial paraesthesia and muscle paralysis leading respiratory problems [10]. During PST outbreaks, shellfish harvesting is

prohibited until toxicity levels fall below a regulatory threshold (80 µg STX eq/100 g; [11], which often requires several weeks or months.

Deploying bivalve sentinel species equipped with valvometry sensors may provide an early warning of harmful algal blooms (HAB) [12–14]. Such a warning system could minimize the economic losses by harvesting some of the cultured stock prior to a PST outbreak. Blue mussels (*Mytilus edulis*) may have such sentinel attributes since (1) their valve-gaping behaviours are known to be sensitive to harmful algae [14], (2) they can be easily transferred between areas, and (3) they continue pumping water under challenging conditions, including in contaminated environments [15,16]. In more detail, when *M. edulis* was exposed to natural seawater containing *A. catenella* its valve gaping behaviours was altered, resulting in longer valve openings (yawning) of longer durations, which in turn suggested a partial and temporary muscle paralysis [14]. This behavioural change was detected at low *A. catenella* concentrations, up to a week before the toxin accumulation in mussels triggered a harvest prohibition. However, studies on soft-shell clams, *Mya arenaria*, revealed the development of PSTs resistance [17–19], which conceivably reduces their usefulness in early warning systems based on valve-gaping. In keeping with this information, one intriguing question is whether mussels develop this kind of resistance, which would be problematic for the sensitivity of the system over time. Such resistance implies that different populations of mussels would respond differently to the presence of *A. catenella* and may not equally serve as early warning systems.

To validate the potential use of *M. edulis* valvometry as an early warning tool in the Gulf of St. Lawrence, we compared the gaping behaviour of mussels originating from two different areas: one area that is not exposed to *A. catenella* and one area exposed yearly to high *A. catenella* blooms. The objective of this study was to determine whether the valve gaping response of *M. edulis* varied among mussel sources with different *A. catenella* exposure histories, ultimately testing for PSTs of adaptive behaviours related to PST in the blue mussel.

2. Materials and Methods

2.1. Animal Collection

To compare the gaping behaviour of mussels, two populations of mussels were collected and held under controlled laboratory conditions. The first source was collected from a lease in St. Peter's Bay, Prince Edward Island, Canada (46° 26′ 30.7″ N, 62° 44′ 51.3″ W) in October 2021 (63.45 ± 2.79 mm SE; Standard Error). Over the last 20 years, *A. catenella* has never been documented in this area [20]. Contrarily, mussels (61.27 ± 3.60 mm SE) that were regularly exposed to *A. catenella* were collected in October 2021 from the St Lawrence Estuary at Métis bay (48° 40′ 49.10″ N, 68° 1 ‘58.20″ W). These two stations are far enough to consider that the mussels come from two distinct populations [21]. These mussels are exposed annually to *A. catenella* with a high risk to have more of 1000 cells L^{-1} in the summer between June to September [6,22]. Once at the laboratory, mussels were placed in 300 L maintenance tanks filled with 1-µm continuously aerated filtered seawater, a salinity of ≈28, and a natural photoperiod, where they were acclimated for four weeks. Mussels were fed daily with live Tisochrysis lutea CCMP 1324 (CCMP: strain reference of the company National Center for Marine Algae and Microbiota), Chaetoceros muelleri CCMP 1316, and Pavlova lutheri CCMP 1325 (1:1:1) at a rate of 60,000 cells L^{-1} mussel-1. Algae were batch-cultured in f/2 medium (with Si for the diatom *C. muelleri*; Guillard, 1975) at 18 °C, under continuous illumination, in 20 L tanks supplied continuously with CO_2 to maintain a pH of ≈8 and a light intensity of µmol m^{-2} s^{-1}. Cell counts were determined with a Multisizer 4e Beckman Coulter counter with a 50-µm pore orifice.

This experiment used the "AT6" strain of *A. catenella* that was isolated from the St. Lawrence Estuary at the Maurice Lamontagne Institute (Department of Fisheries and Oceans; DFO) during a red tide event that occurred in 2008. The toxin concentration of this strain was measured and showed values around 3 pg STXeq cell–1. The alga *A. catenella* was cultured in f/2 medium without Si under continuous illumination with a photosynthetic

active radiation of 100 µmol quanta m^{-2} s^{-1}, at a temperature of 18 °C with constant CO_2 addition to maintain a pH of 8.

2.2. Experimental Design

Following the acclimation period, 36 mussels were connected to the valvometry monitoring system [13,23] prior to initiating the experiment. Wired mussels were distributed into 6 tanks, each containing 6 mussels (3 from each source), which were filled with 75 L of 1 µm filtered seawater (Figure 1). The experiment was maintained at 18 °C, a temperature selected between the optima range of both species (i.e., 20 °C for mussels [24] and 14 °C for *A. catenella* [6]. The first 24 h (T0 period) served to establish a baseline level for gaping behaviours. After this initial 24 h period, *A. catenella* was introduced at a concentration of 10,000 cells L^{-1} for 42 h (T1 period). This *A. catenella* concentration aligns with the maximum concentration used by Durier et al. (2022) [14] and ensured the regulatory limit for bivalve harvest. Following the T1 period, the water in each of the tanks was completely replaced, and mussels were exposed to *Tetraselmis suecica*, a non-toxic alga, at a concentration of 5000 cell mL^{-1} for the next 24 h (T2 period). The gaping behaviours were continuously monitored from the T0 to the T2 period to investigate how they changed with these different exposures.

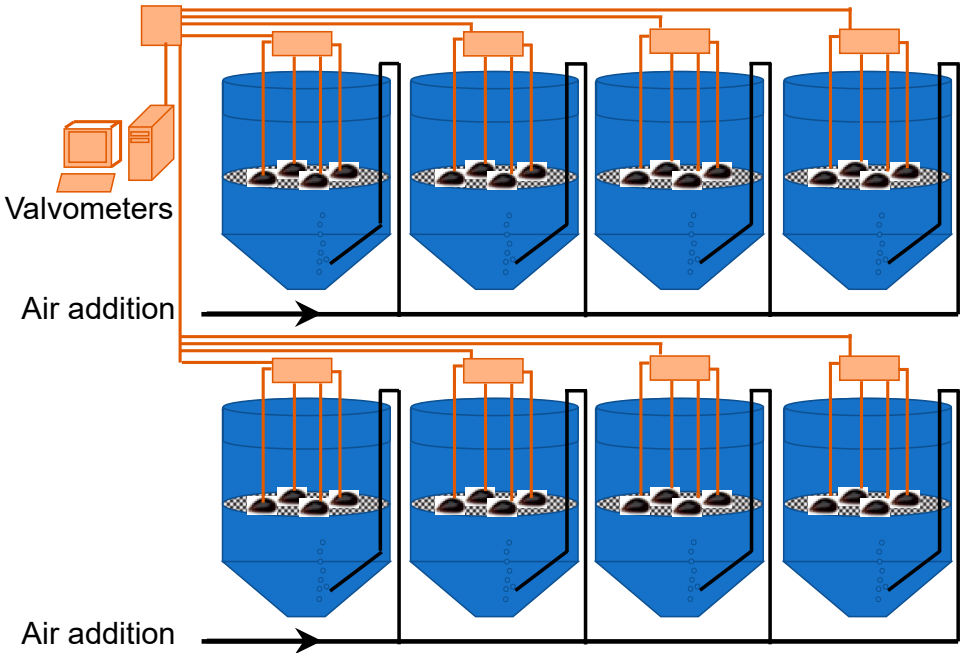

Figure 1. Experimental design used during the experiment with 8 tanks for mussels mixed origins (St. Lawrence estuary and Prince Edward Island).

2.3. Valvometry

To record the mussel gaping behaviour, a Hall element sensor (HW-300a, Asahi Kasei, Tokyo, Japan; 0.5 g) was attached to one valve and a magnet was attached to the opposing valve using Solarez® UV epoxy resin (Wahoo International, Vista, CA, USA). The sensors were installed on all experimental animals and connected to a 4-channel dynamic strain recorder (DC-204 R, Tokyo Sokki Kenkyujo Co., Tokyo, Japan) equipped with a memory card. The magnetic flux between the sensor and the magnet was converted into voltage values and concomitantly recorded. The sensors and magnets were attached to the mussel valves with a starting target voltage of ≈−80,000 µV to ensure the system was similarly and proper placed on all individuals. We used 10 measures s^{-1} to obtain a high resolution

of gaping behaviours. Using the R version 1.4.1717 [25], voltages were converted from µV values to Valve Opening Amplitude (VOA), calculated as:

$$\text{VOA} = [(opening - min)/(max - min)] \times 100 \quad (1)$$

VOA is expressed as a percentage of the valve opening amplitude at a given time, where max and min values correspond to the maximum and the minimum opening in µV measured during the experiment. Five behavioural indicators were subsequently calculated from the VOA: (1) number of closures, (2) total closure duration, (3) average VOA, (4) average closure duration, and (5) number of micro-closures [13]. Mussels were considered closed when the VOA was <10% of the maximum value, while micro-closures were defined by a 3% reduction of VOA within 0.1 s. All five indicators were compared between mussels in both populations for each experimental period (T0, T1 and T2).

2.4. Statistical Analysis

Due to the non-normal distribution of the data, univariate two-way permutation analyses of variance (PERMANOVAs) were conducted using the vegan package in R [26]. If a significant effect was detected, a posteriori PERMANOVA pairwise test was used [27]. The effect of *A. catenella* (i.e., period T0, T1, T2) and mussel population (i.e., St. Lawrence Estuary vs. Prince Edward Island) were examined for each of the 5 behavioural indicators tested.

3. Results

Variation within Behavioural Indicators

No discernible interaction was found between the different exposures (T0, T1 and T2) and mussel populations for any of the behavioural indicators observed (Table 1). The effect of mussel population was found to be statistically insignificant for all measured behavioural indicators the exception of the average VOA (Table 1), which displayed higher averages in mussels from the St. Lawrence Estuary than in mussels from Prince Edward Island. Additional distinctions were observed in the behaviour of mussels before, during, and after *A. catenella* exposure, specifically in terms of the number of closures and total closure duration (Figure 2). Both the number of closures and total closure duration showed similar trends between the populations where values tended to increase during T1 in response to *A. catenella* exposure. This suggested a decrease in the amount of time mussels were open during such events.

Table 1. Statistical results of PERMANOVAs examining five behavioural indicators of valve movements in mussels from Prince Edward Island and St. Lawrence Estuary during *Alexandrium catenella* exposure events. Values in bold denote *p*-value < 0.05.

Indicator	Factor	DF	Pseudo-F	*p*-Value
Number of Closure	Mussel population	1	3.366	0.07
	Period	**2**	**10.632**	**0.0001**
	Interaction	2	0.96	0.403
Total Closure Duration	Mussel population	1	0.225	0.637
	Period	**2**	**16.958**	**0.0001**
	Interaction	2	0.285	0.754
Average Valve Opening Amplitude	Mussel population	1	**19.49**	**0.0001**
	Period	2	**16.726**	**0.0001**
	Interaction	2	1.208	0.303

Table 1. *Cont.*

Indicator	Factor	DF	Pseudo-F	*p*-Value
Microclosure	Mussel population	1	0.041	0.949
	Period	2	0.969	0.457
	Interaction	2	0.464	0.808
Average Closure Duration	Mussel population	1	0.809	0.55
	Period	2	1.505	0.123
	Interaction	2	0.546	0.824

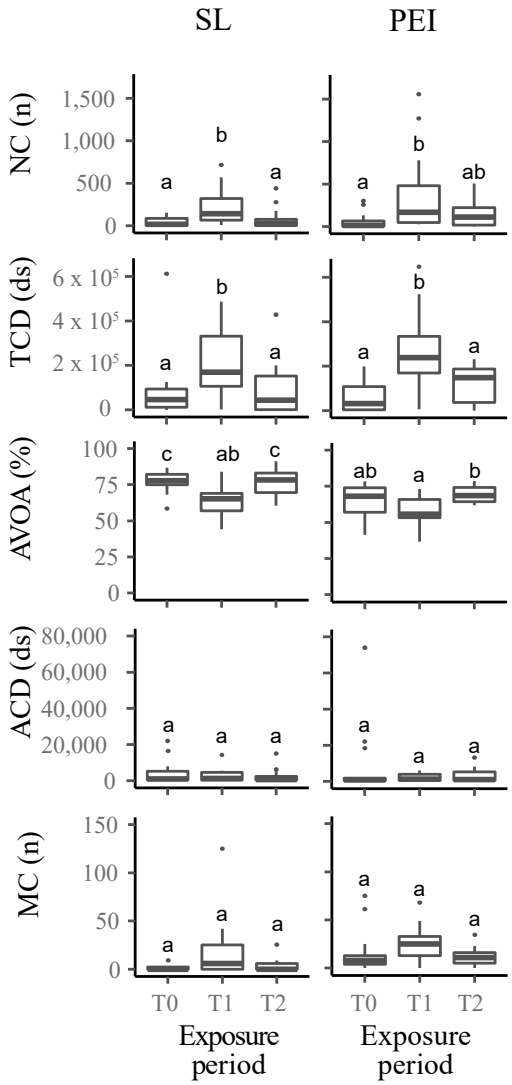

Figure 2. Mussel's behavioural indicators exposed to toxic algae for mussels from St. Lawrence estuary (SL) and Prince Edward Island (PEI). NC: Number of closures. TCD: Total Closure Duration. ACD: Average Closure Duration. AVOA: Average Valve Opening Amplitude. MC: Microclosure. T0: Acclimation and reference behaviour, T1: *A. catenella* exposition, T2: food addition. The letters a, b and c are significantly different.

4. Discussion

Mussels from both Prince Edward Island and the St. Lawrence Estuary showed similar changes in their valve-gaping behaviours when exposed to *A. catenella*, suggesting that this species of phytoplankton can impact the overall functioning of mussels and that resistance

has not developed over time with exposure history. When mussels were exposed to *A. catenella* in the current study, the most notable response was an avoidance behaviour with a recorded rise in the number of valve closures and a prolonged valve closure duration. It is noteworthy that Durier et al. (2022) [14] reported a contrasting behavioural response, since mussels in that study displayed a decrease in valve closures in the presence of toxic dinoflagellates. The contrasting behavioural responses between the two studies could perhaps be due to different concentrations of *A. catenella* which was higher in the present study than in Durier et al. (2022) [14].

No differences were detected for any of the behavioural indicators between the two mussel populations, except for average VOA. The absence of differences in behavioural indicators between the populations shows that the mussel gaping behaviour remains similar despite divergences in exposure histories. Generally, when mussels were exposed to toxic algae, both populations showed a decrease of their VOA. The differences in average VOA observed between the two populations prior to *A. catenella* exposure may be partially explained by differences in the shell morphology due to their habitat. Mussels from Prince Edward Island were cultivated under suspension culture, while those from the St. Lawrence Estuary were grown along the coastal shore. Tidal habitats such as the St Lawrence Estuary, subject mussels to emersion periods and higher hydrodynamic shear stress, prompting the development thicker shells [28], which may result in increases of the average VOA. Durier et al. (2022) [14] similarly demonstrated that change in the mussel gaping behaviours in the presence of *A. catenella* were mainly related to the number of closures, the total closure duration, and the average closure duration. Thus, the difference between the average VOA of the two populations shouldn't impact the early warning systems ability to detect toxins. Overall, this study highlights the absence of adaptive gaping behaviour in *M. edulis* during exposure of *M. edulis* to *A. catenella*. We know that the soft-shell clam, *M. arenaria*, regularly exposed to toxic algae showed adaptation to the presence of *A. catenella* toxins [17–19]. It would be interesting to monitor the gaping behaviour of soft-shell clams that are frequently exposed to *A. catenella* to to determine whether the reported PST resistance is also manifested in gaping behaviour. Some studies have also verified the usefulness of valvometry to detect changes in mussel behaviour by monitoring valve movements in the presence of a HAB. The absence of differences in gaping behaviours between mussels from the St. Lawrence Estuary and those from the Prince Edward Island suggest that mussels from different origins can be used to detect the presence of *A. catenella*, regardless of variations in their exposure histories. Together, these results create an optimistic outlook for the use *M. edulis* with valvometry as an early warning system to detect HAB events. This system would ideally assist in mitigating the economic losses for the shellfish aquaculture industry.

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