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Effects of the toxic dinoflagellate *Alexandrium catenella* on histopathogical and escape responses of the Northern scallop *Argopecten purpuratus*

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ABSTRACT

Juvenile Northern scallops *Argopecten purpuratus* were exposed to cultures of the paralytic shellfish toxin (PST) producing dinoflagellate, *Alexandrium catenella*, or a non-toxic microalga as a control, T-iso. After 3 and 6 days of exposure to either *A. catenella* or T-iso, scallops were stimulated to elicit an escape response by exposing them to the predatory sea star *Meyenaster gelatinosus*. We monitored the escape response of the scallops in terms of reaction time after first contact with the sea star, number of claps (burst of rapid valve closures) until exhaustion, clapping time, clapping rate, the time scallops spent closed when exhausted, and recovery from the initial number of claps, clapping time and clapping rate. Additionally, histopathological and stress responses (through heat-shock protein [hsp70] induction), as well as accumulation of Paralytic Shellfish Poisoning (PSP) toxins, were monitored on scallops after 3 and 6 days of exposure to *A. catenella*. After 6 days of exposure, scallops exposed to *A. catenella* accumulated PSTs and reacted more rapidly with a higher clapping rate, however the duration of their escape response was shorter than controls, when exposed to *M. gelatinosus*. Additionally, scallops exposed to *A. catenella* showed histopathological features, especially after 6 days of exposure, including increased melanization of the tissues and myopathy, with high levels of degeneration of the muscle fibers. A six-day exposure to *A. catenella* also caused an increase in prevalence of rickettsiales-like organisms within scallop tissues. This study suggests that PST accumulation can affect the interaction between the Northern scallop and both pathogens and predators, potentially increasing their susceptibility to either of them.

1. Introduction

For the last few decades, harmful algal blooms (HABs) have been recurring on the Chilean coasts, progressively extending from the south and the austral region northwards (Molinet et al., 2003). The genus *Alexandrium* can produce saxitoxin (STX) and derivatives, collectively known as "Paralytic Shellfish Toxins" (PSTs), which are responsible for "Paralytic Shellfish Poisoning" (PSP) of human consumers of contaminated seafood. From 1991 to the present, several "red tides" – blooms of *Alexandrium catenella* – occurred in southern Chile. The largest *A. catenella* red tide was documented in the summer-fall of 2002. Cell densities in this bloom were as high as 7.8×10^5 cells L^{-1} (Clement et al., 2002, Molinet et al., 2003), and toxicity values were above 22,000 μg STX-eq. 100 g^{-1} in tissue of clams. This event caused intoxication of more than 100 persons, among which eight died (Molinet et al., 2003). PSTs attributable to *A. catenella* blooms have resulted in closures of fisheries over extended areas and periods of time in Chile. Little information is available on the impact of PSTs or *A. catenella* on bivalve physiological and behavioral responses to these toxins, alone or combined with other challenges.

Northern scallops, *Argopecten purpuratus* Lamarck 1819, are distributed throughout the Eastern Pacific coast of South America from Sechura, Perú (6°S), to Tongoy Bay, Chile (31°S) (von Brand et al., 2006). This region's scallop fishery was originally based upon natural beds but overfishing nearly extirpated scallops. Scallop cultivation then started to be developed, mainly in Tongoy, Guanaqueros, and Caldera Bays (von Brand et al., 2006). Several trials have also been conducted to extend northern scallop aquaculture southward (Gonzalez et al., 1999). Northern scallop

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aquaculture represents a very important economic activity in northern Chile, with an annual production of 18,781 tons (von Brand et al., 2006), and any environmental stress, predator, or disease outbreak could jeopardize this industry.

One of the main, natural predators of northern scallops is the sea star *Meyenaster gelatinosus* (Ortiz et al., 2003), which elicits a strong escape response in scallops (Brokordt et al., 2006). The latter authors demonstrated that *A. purpuratus* shows a highly stereotyped and consistent escape response when stimulated by *M. gelatinosus*, starting with a series of valve claps (alternating adduction and abduction of the valves). If the stimulus is maintained until exhaustion, most scallops close their valves firmly and remain closed for a certain period, after which the valves slowly reopen (Brokordt et al., 2006). Exhaustion or "fatigue" is defined here when scallops cannot respond to further stimulations and remain closed (Chih and Ellington, 1983; Brokordt et al., 2006). Indeed, the permanent closure of the valves is supported by the catch contraction of the tonic muscle, which requires almost no energy (Tsutsui et al., 2007). Predation would be more likely to occur when a resting (recovering from previous exercise, that did not induced exhaustion) or exhausted scallop is captured by *M. gelatinosus.*

STX present in *Alexandrium* spp. belongs to a class of neurotoxic alkaloids that selectively blocks voltage-gated Na^+ channels in excitable cells, thereby affecting neural impulse generation in animals (Catterall, 1980). Recent studies demonstrated that *A. fundyense* and *Gymnodinium catenatum*, can cause paralysis of eastern oysters *Crassostrea virginica*, blue mussels *Mytilus edulis* and giant lions-paw scallops *Nodipecten subnodosus* (Hégaret et al., 2007a; Galimany et al., 2008; Estrada et al., 2010). Several other studies highlighted the ability of harmful algae from the genus *Alexandrium* to impact the behavioral response of oysters (*C. virginica* and *C. gigas*), quahogs (*Mercenaria mercenaria*) and mussels (*Perna viridis*) (Tran et al., 2010; May et al., 2010). An exposure to the toxic dinoflagellate *Alexandrium* causes decrease in shell valve gape (in oysters, mussels and clams) and increase in valve microclosures in oysters *C. gigas*, which are represented by sporadic spikes of voltage induced by variation of distance between electromagnetic electrodes glued onto both shells and indicating shell closure of a short duration during a continuous recording of the valve movements of oysters. Other studies demonstrated that *M. edulis* exposed to the toxic dinoflagellate *Protogonyaulax tamarense* (now *A. tamarense*), showed erratic closure of the valves (Gainey and Shumway, 1988). Moreover, histological observations of tissues of mussels exposed to *A. fundyense* indicated the presence of hemocytes in the gills, as well as degeneration of muscle tissue, including digestive gland, gonads, and the adductor muscle (Galimany et al., 2008). Similar observations were made by Haberkorn et al. (2010) on adductor muscles of *C. gigas* exposed to *A. minutum*, wherein several stages of muscle degeneration could be observed from myopathy to total hyaline degeneration of the tissues. These observations suggest that *Alexandrium* sp. blooms impair muscle structure and function and consequently contractile capacity. This would then affect the control and strength of the adductor muscle during shell opening and especially during closing, thereby impairing defense against predators.

The aim of our study was to investigate the impact of *A. catenella*, the common cause of harmful algal blooms on the Chilean coasts, on the escape response of the northern scallop *A. purpuratus* when exposed to the predatory sea star *M. gelatinosus*. Feeding behavior, escape responses, histopathological and compensatory defense responses through stress protein induction, as well as accumulation of PSP toxins in the digestive gland, were measured on juvenile scallops exposed to *A. catenella* for 3 and 6 days.

2. Materials and methods

2.1. Experimental animals

Ninety-six juvenile *A. purpuratus* (30–35 mm) were obtained from the Universidad Católica del Norte (UCN) aquaculture center, from Tongoy Bay (30°16′S; 71°35′W), and taken to the UCN laboratory at Coquimbo. There, scallops were kept for 1 wk in 25-L tanks supplied with filtered, running seawater (∼1.8 L min^{-1}, ∼16 °C), aerated, and fed with a diet of 50% *Isochrysis galbana* and 50% *Chaetoceros calcitrans*, before the beginning of the experiment. The size of scallops averaged 33.48 mm in length, 32.90 mm in width and 11.95 mm in height.

Sea stars, *M. gelatinosus*, were obtained from La Herradura Bay (29°59′S, 71°22′W) and maintained in a 500-L tank for the time of the experiment in running seawater (16 °C) in the wet laboratories of UCN, Coquimbo. Each sea star was used only 3 times as experimental predators to assess scallop escape response.

2.2. Algal cultures

The *A. catenella* strain used, ACCH-01 was isolated from a cyst collected in Puerto Santo Domingo in August 2004 (Varela et al., 2004) and obtained from the Centro I-MAR, from the Universidad de Los Lagos in Puerto Montt, Chile. This strain was selected from several *A. catenella* strains available in culture for high PSP content (62 pg STX-eq. $cell^{-1}$; Varela et al., 2004). Cultures of *A. catenella* were grown in L1-medium made with autoclaved, 1-μm-filtered seawater (Guillard and Hargraves, 1993). The microalgae were cultured in 2-L glass bottles at 15 °C with 24-h light. Cells were harvested in early stationary phase, usually approaching a cell density of 5000 cells mL^{-1} and sent to the Universidad Católica del Norte (UCN) wet laboratories at Coquimbo.

The *Isochrysis* sp. (Tahitian strain) T-iso culture, a common aquaculture feed, was used as the control and complementary diet for the experiment. T-iso was cultured in 1000-L, open tanks in the Central Culture Laboratory of UCN, Coquimbo and harvested after 3–6 days of growth, usually at a cell density approaching 10^6 cells mL^{-1}. Algal cell densities were determined by hemocytometer counts under a light microscope.

2.3. Experimental design

Ninety-six scallops were distributed randomly into eight 20-L tanks (i.e. 12 scallops per tank). Scallops were maintained in a continuous flow (5 mL min^{-1}) of 16 °C, filtered seawater from La Herradura Bay, with constant aeration and to which algal feeding of either T-iso, as a control or a mixed diet of T-iso and *A. catenella* had been added. Four replicates of two different treatments were run in this experiment:

(1) A control group of scallops was fed a unialgal culture of T-iso, at a constant cell density of 10^5 cells mL^{-1}.
(2) A Harmful Algal Bloom (HAB) exposed group of scallops was given T-iso at the same density (10^5 cells mL^{-1}) with *A. catenella* added at 500 cells mL^{-1}.

The biodeposits were removed and examined every day for presence of intact cells.

After 3 and 6 days of exposure to either T-iso or the HAB treatment, five scallops from each tank were tested for escape response and sampled for production of heat shock proteins (HSPs) and examined histopathologically. At the end of the 6 days, the two scallops left in each tank were sampled for quantification of PSP toxins in the soft-tissue.