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Experimental exposure of the blue mussel (*Mytilus edulis*, L.) to the toxic dinoflagellate *Alexandrium fundyense*: Histopathology, immune responses, and recovery

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Abstract

Mussels (*Mytilus edulis*) were exposed to cultures of the toxic dinoflagellate *Alexandrium fundyense* or the non-toxic alga *Rhodomonas* sp. to evaluate the effects of the harmful alga on the mussels and to study recovery after discontinuation of the *A. fundyense* exposure. Mussels were exposed for 9 days to the different algae and then all were fed *Rhodomonas* sp. for 6 more days. Samples of hemolymph for hemocyte analyses and tissues for histology were collected before the exposure and periodically during exposure and recovery periods.

Mussels filtered and ingested both microalgal cultures, producing fecal pellets containing degraded, partially degraded, and intact cells of both algae. Mussels exposed to *A. fundyense* had an inflammatory response consisting of degranulation and diapedesis of hemocytes into the alimentary canal and, as the exposure continued, hemocyte migration into the connective tissue between the gonadal follicles. Evidence of lipid peroxidation, similar to the detoxification pathway described for various xenobiotics, was found; insoluble lipofuchsin granules formed (ceroidosis), and hemocytes carried the granules to the alimentary canal, thus eliminating putative dinoflagellate toxins in feces. As the number of circulating hemocytes in *A. fundyense*-exposed mussels became depleted, mussels were immunocompromised, and pathological changes followed, i.e., increased prevalences of ceroidosis and trematodes after 9 days of exposure. Moreover, the total number of pathological changes increased from the beginning of the exposure until the last day (day 9). After 6 days of the exposure, mussels in one of the three tanks exposed to *A. fundyense* mass spawned; these mussels showed more severe effects of the toxic algae than non-spawning mussels exposed to *A. fundyense*.

No significant differences were found between the two treatments during the recovery period, indicating rapid homeostatic processes in tissues and circulating hemocytes.

Keywords: *Alexandrium fundyense*; Harmful algal blooms; Hemocyte; Histopathology; Immunology; *Mytilus edulis*

1. Introduction

Toxic or otherwise inimical microalgal proliferations in aquatic ecosystems, referred to as harmful algal blooms (HABs), were first recorded by ancient civilizations and have been observed ever since (Fogg, 2002; Landsberg, 2002). These HABs, caused by a wide variety of microalgal species, can affect many sympatric marine organisms (Shumway, 1990; Fogg, 2002; Landsberg, 2002). HABs are increasingly impacting seafood production, especially aquaculture. As bivalve mollusks are filter-feeding animals that can bio-accumulate toxins and other compounds, toxins produced by HAB species can accumulate in bivalve tissues and affect their predators, including top predators in food webs such as humans (Azanza and Taylor, 2001; Landsberg, 2002). To protect public health, monitoring and management programs for bivalve toxins have been implemented (Shumway et al., 1990; Rehnstam-Holm and Hernroth, 2005); HAB-related closures to shellfish harvest can result in great economic losses to the aquaculture industry (Shumway, 1990; Whyte, 1997; EUROHAB, 1998). Moreover,

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of all cultured bivalves, mussels accumulate Paralytic Shellfish Poisoning (PSP) toxins most rapidly and to a greater degree compared to other harvested shellfish (Shumway et al., 1990; Gosling, 2003).

In addition to accumulating and passing toxins to higher trophic levels, bivalves themselves can be affected by HAB events. For example, it has been shown that toxic algae can produce histopathological lesions and impair immune responses in commercially harvested bivalve species exposed to them (Wikfors and Smolowitz, 1995; Wikfors et al., 2000; Hégaret and Wikfors, 2005a,b; Pearce et al., 2005; Hégaret et al., 2007a,b; da Silva et al., 2008; Galimany et al., 2008a,b).

The dinoflagellate *Alexandrium fundyense* is a HAB species that produces a group of neurotoxins called saxitoxins (Anderson et al., 1990). These toxins affect many marine organisms and, through dietary consumption, can cause a syndrome known as PSP which impairs sodium-channel signal transmission between neurons (Daranas et al., 2001; Landsberg, 2002; Bricelj et al., 2005). Blooms of this dinoflagellate and trophic transfer of toxins can lead to effects upon finfish, including erratic swimming and mortality (Samson, 2002; Martin et al., 2006; Sephton et al., 2007) and on shellfish, reducing clearance rate, growth rate of soft tissues, and condition index (Bricelj et al., 1993; Landsberg, 2002). Nevertheless, when no other type of alga is available, *A. fundyense* can be used as a food source by mussels despite its toxicity (Bricelj et al., 1993).

This study investigated the effects of *A. fundyense* exposure upon blue mussels, *Mytilus edulis*, under experimental conditions and the recovery of the mussels after the exposure was terminated, with a focus on histopathology and hemocyte immune functions of exposed mussels.

2. Materials and methods

2.1. Experimental animals

Mussels, *Mytilus edulis* (44.1–77.2 mm shell length), were collected from Westcott Cove, Stamford, Connecticut, USA from an intertidal beach on the north shore of Long Island Sound in May of 2007. Mussels were cleaned of fouling organisms and acclimated for 4 days before the experiment, the first 3 days in the experimental tanks with filtered sea water and the fourth day fed with *Rhodomonas* sp. (RHODO, see below) at a concentration of 1×10^4 cells ml^{-1}.

2.2. Algal cultures

The harmful algal species tested in this study was the BF2 strain of the dinoflagellate *A. fundyense* (Balech), obtained from the Milford Microalgal Culture Collection (isolated from the Gulf of Maine, U.S.A.). In addition, a non-toxic cryptophyte, the RHODO strain of *Rhodomonas* sp., was used as a non-toxic, control alga.

The microalgae were cultured in 20-L glass carboy assemblies using aseptic technique (Ukeles, 1973). Cultures were harvested semi-continuously to maintain consistency in culture quality over the course of the study and were harvested in late-log or early-stationary phase. *A. fundyense* was grown in F/2-enriched (Guillard and Ryther, 1962; Guillard, 1975), filtered seawater from Milford Harbor, and *Rhodomonas sp.* was cultured in E-medium (Ukeles, 1973). Both cultures were maintained at 20 °C with 24 h light. Algal-cell densities were determined by hemocytometer counts with the light microscope.

To determine PSP toxicity in mussels, the mouse bioassay was performed according to APHA (1970), using mussels exposed for 8 and 9 days to *A. fundyense*. Briefly, 100 g of mussel meat was collected per replicate sample and homogenized, and an equal volume of 0.18N HCl was added to the homogenate. Sample pH was adjusted to 3, and the mixture was brought to boil at 100 °C for 5 min. Then the extract was centrifuged for 5 min at 3000 rpm and decanted. Mice (ICR females, 17–23 g) were inoculated with 1 ml of this extract intraperitoneally and observed for symptoms and mortality for 60 min. A standard PSP toxin reference solution (diluted to 0.33 μg/ml H_2O pH 3) served as a positive control. The conversion factor (CF) was determined by injecting 5 mice IP with a dilution of the reference solution that produces a median death time of 5–7 min. The death time was converted to mouse units (MU) using Sommer's tables. Mice that survived the 60 min time period were given a MU value of <0.875. Calculated MU values were converted to corrected mouse unit, CMU, with a weight-correction factor, and median value MCMU was calculated to represent the true median death time of the group. Concentration of the toxin was determined by the following formula: μg toxin/100 g meat = MCMU × CF × dilution factor × 200.

Toxins within *A. fundyense* cells harvested from cultures used for the exposure experiment were analyzed by HPLC. An HPLC-UV-FL (Alliance 2695, Waters) was used to determine the toxic PSP compounds in *A. fundyense* using Lawrence's methodology (Lawrence et al., 1995).

2.3. Experimental design

Six hundred (600) mussels were distributed randomly into six 20-L glass aquaria, i.e. 100 animals per aquarium. Triplicates of each of two treatments were done in this experiment: mussels were fed either *A. fundyense* or *Rhodomonas* sp., each at 4×10^3 cells ml^{-1} during the first 9 days of the experiment, and all mussels were fed *Rhodomonas* sp. during the last 6 days to test recovery. All mussels were given a regime of 16 feedings per day, one every 90 min, using computer-automated valves attached to the cover of a rearing-chamber system (Smith and Wikfors, 1998).

Samples of mussels were collected on day 0 (before exposures), after 3, 7 and 9 days of exposure to the experimental microalgal treatments, and on days 12 and 15 to study the recovery process. At each sampling time, 60 mussels were removed from the aquaria (10 from each, individual aquarium) and analyzed for stomach contents, histopathology, and hemocyte parameters. Feces and pseudofeces also were examined microscopically throughout the experiment.