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Behavioural response of bivalve molluscs to calcium hydroxide

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ABSTRACT

Blue mussels (*Mytilus edulis*) that are cultivated in the marine area around Prince Edward Island, Eastern Canada, are susceptible to the heavy biofouling of their shells by an invasive solitary tunicate, *Styela clava*, which rapidly proliferates. To mitigate this issue, mussel farmers periodically lift the longlines on which the mussels are suspended out of the water to spray a highly alkaline (~12.7 pH units) calcium hydroxide solution onto fouled individuals. Here, we tested the hypothesis that calcium hydroxide exerts behavioural stress on mussels and other bivalves. Field surveys revealed that the alkalinity of the seawater in the vicinity of longlines increased (9.3–11.7 pH units) immediately after treated mussel sleeves were returned into the water column. Thereafter, pH values declined rapidly, and met federal water quality guidelines (7.0–8.7 pH units) within 3.1 ± 0.5 min (range 0.3–10.5 min, $n = 31$ sleeves). Cultivated mussels challenged to both emersion and calcium hydroxide closed their valves for 14.0 ± 3.3 min ($n = 18$) compared to 6.5 ± 1.6 min ($n = 17$) by control mussels (emersion only). We subsequently assessed how three benthic bivalve species (*M. edulis*, *Crassostrea virginica* (eastern oyster) and *Argopecten irradians* (bay scallop)) respond to weak (pH ≤ 9.2) but sustained (3-h daily for 3 days) alkalinity conditions. All three species consistently responded by completely or partially closing their valves. However, all behavioural responses were short-lived (0.2–4.7 h), and were generally confined to the treatment period. In conclusion, spraying calcium hydroxide onto cultivated mussels has limited impact on seawater alkalinity and the behaviour of nearby bivalves.

1. Introduction

Limestone, or calcium carbonate ($CaCO_3$), is a naturally-occurring sedimentary rock that consists of calcium, magnesium carbonate and/or dolomite, in addition to small amounts of other minerals. When limestone is heated to temperatures >1000 °C it loses carbon dioxide (CO_2), and is converted to calcium oxide (CaO). The process of burning limestone to produce granulated calcium oxide has been practiced for centuries for use in agricultural purposes, such as reducing soil acidity. Among mariculturists, there is also a long history of using calcium oxide to control microorganisms in fish/shrimp/prawn ponds (Boyd and Fast, 1992; Gräslund and Bengtsson, 2001; Shahidul Islam et al., 2004), predatory starfish in oyster beds (Wood, 1908) and sea urchins in kelp beds (Bernstein and Welsford, 1982).

Calcium hydroxide ($Ca(OH)_2$) is known as hydrated lime, flaked lime or builders lime, and is prepared by hydrating calcium oxide in a process that creates a more stable form of lime for storage purposes. Calcium hydroxide has been used for decades to remove the predatory sea star (*Asterias vulgaris*) from oyster (*Crassostrea virginica*) and mussel (*Mytilus edulis*) seed collection systems (Barkhouse et al., 2007; MacKinnon et al., 1993). It is also commonly applied to bivalve stocks prior to their transfer from one embayment to another. This process is aimed at controlling the spread of various marine pests, such as barnacles. However, over the past decade, calcium hydroxide has also been increasingly used to control invasive fouling tunicates, particularly the solitary clubbed tunicate *Styela clava*. There are several reasons for controlling solitary tunicate populations in bivalve farms. For instance, solitary tunicates are efficient filter-feeders that compete with bivalves for limited particulate food resources; consequently, reducing the capacity for coastal systems to produce bivalves (Comeau et al., 2015). In addition, the rapid proliferation of these tunicates results in massive crop loss, because their weight breaks the byssal thread attachment of mussels, causing them to fall to the bottom where they lose their commercial value (Davidson et al., 2016). High-pressure water is usually used to eradicate soft-bodied tunicates, such as *Ciona intestinalis* (Ramsay, 2014). However, *S. clava* has a leather-like tunic that cannot be perforated easily using this method. To date, the most efficient strategy to control the on-site infestation of *S. clava* involves the lethal application of calcium hydroxide (Ramsay et al., 2014).

In the mussel industry, growers usually apply calcium hydroxide in late summer/early autumn after *S. clava* has settled onto the mussel sleeves and has reached a length of 20–25 mm. At this point, calcium hydroxide is dissolved in large onboard tanks to create a saturated

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(~4%) and highly alkaline (~12.7 pH units) lime solution that contains approximately 40 g of calcium hydroxide per 960 ml of seawater (Ramsay et al., 2014). The solution is manually sprayed onto tunicate-infested mussel sleeves as they are lifted from the water using a hydraulic crane (Fig. 1b, c). Following a short period of air exposure (45–90 s) that induces the mortality of *S. clava*, the treated sleeves are slowly re-immersed into the water. In recent years, some growers have developed automated low-pressure spray systems to speed up the treatment process (Fig. 1d). The main components of these automated systems are a mixing tank, a booth with multiple low-pressure nozzles (that hangs over the side of the boat) and a recovery system (to collect unused lime solution). Boats equipped with an automated sprayer system treat a 180 m longline containing 400 sleeves in approximately 30 min, compared to 1 h for manual application practices.

Because calcium hydroxide is a chemical that increases the alkalinity of water, it is important to quantify how it affects the environment and non-targeted organisms. To date, the alkalinity footprint associated to calcium hydroxide application in shellfish farms has not been determined in relation to its duration and spatial boundary. There is also a paucity of published literature on the biological effects of calcium hydroxide, with existing studies primarily focusing on determining lethal exposure levels for the bacterium *Vibrio fischeri*, three-spine stickleback *Gasterosteus aculeatus*, sand shrimps *Crangon septemspinosa* and American lobster *Homarus americanus* (Burridge et al., 2010; Locke et al., 2009). To our knowledge, there have been no studies on the sublethal effects caused by applying calcium hydroxide to mussels in farms. Yet, bivalves warrant attention because they are extremely sensitive to their environment (Jørgensen, 1990). Bivalves filter large amounts of ambient water, resulting in their exposing their unprotected soft-tissues to deleterious substances. Furthermore, mussels respond behaviourally and physiologically to changes in the toxicity of substances in the water (Levinton et al., 2002; Nilin et al., 2012; Tran et al., 2003, 2010).

The present study focused on the use of calcium hydroxide ($Ca(OH)_2$) by mussel farms in the embayments around Prince Edward Island (PEI), Canada, which have been subject to *S. clava* (see Fig. 1a for affected farms). We tested the hypothesis that calcium hydroxide exerts behavioural stress on bivalves, including cultured mussels, when applied to control tunicate fouling in longline mussel farms. Our approach was based on measuring the "pH footprint" in mussel farms, and subsequently challenging bivalves in a controlled environment. The response behaviour was evaluated using high frequency valvometry, which involved closely monitoring the extent of valve opening. Valve activity recording devices have been available since the early 20th century (Hopkins, 1931; Nelson, 1921), and are now being increasingly integrated into evaluations of environmental changes or stressors (Basti et al., 2009; Comeau, 2014; Liao et al., 2009; Tran et al., 2010). The opening of values signals the activation of a complex nervous mechanism involving the heart and adductor muscles (Taylor, 1976). This action results in bivalves exposing themselves to the environment, and exercising metabolically demanding processes; namely, the collection and assimilation of food particles. Thus, atypical valve activity, such as a tendency towards valves closure, was assumed to indicate physiological stress. This study is expected to provide important information on current practices in the mussel industry; specifically, how to mitigate *S. clava* with the minimum environmental damage.

2. Methods

2.1. Field surveys of alkalinity

pH measurements were conducted in the Malpeque Bay area to characterize the calcium hydroxide footprint associated to tunicate-treatment practices. Measurements were conducted opportunistically in 2013–2015, when growers were applying calcium hydroxide to mussels fouled with *S. clava*. During these field surveys, water temperature ranged between 7 and 19 °C and water current velocity ranged between 7.1 and 17.1 cm s^{-1}. The water current was measured by deploying an acoustic Doppler current profiler (ADCP, Workhorse Sentinel 1200 kHz ADCP, Teledyne RD Instruments, Poway, CA, USA) on the seabed; the device pointed towards the surface, and measured the water current at sleeve depth (1.0 to 3.0 m below the surface).

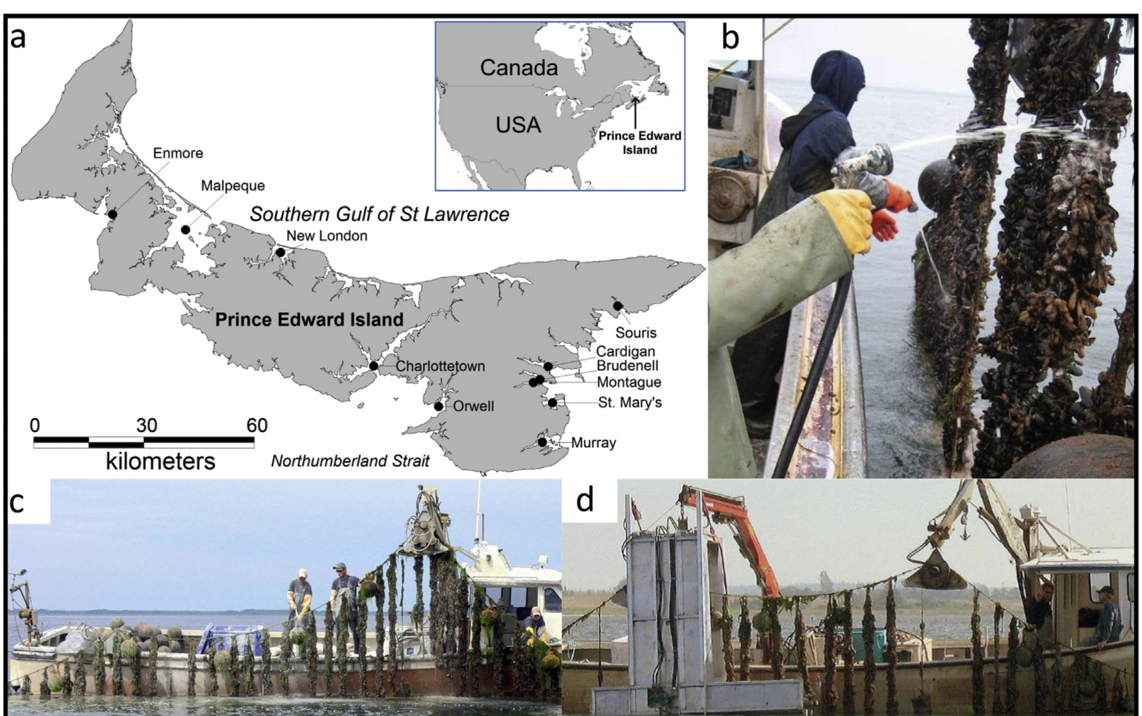

Fig. 1. (a) Distribution of the invasive solitary tunicate, *Styela clava*, in the waters around Prince Edward Island (PEI). (b, c) Mussel sleeves being manually sprayed with a saturated calcium hydroxide solution. (d) Automated/low pressure sprayer system designed to reduce labour costs and calcium hydroxide use.