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Assessment of chemical treatments for controlling *Didemnum vexillum*, other biofouling, and predatory sea stars in Pacific oyster aquaculture

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ABSTRACT

Aquatic invasive species in biofouling communities continue to cause ecological and economic impacts globally. *Didemnum vexillum*, an aggressive invasive tunicate, can have detrimental impacts on the shellfish aquaculture industry and a reliable method of control is required. In this study, cultured Pacific oysters (*Crassostrea gigas*) fouled with *D. vexillum* were treated with various concentrations of brine, freshwater, hydrated lime, and acetic acid at four exposure times (0.5, 1, 5, and 10 min). Results demonstrated that only lime and acetic acid significantly reduced total biofouling and *D. vexillum* coverage. Lime concentrations of 1 and 2% at 5 and 10 min exposures and 4% at 5 min exposure successfully removed up to 92.3% of *D. vexillum* fouling while maintaining high (≥80%) oyster survival. This led to the testing of 1, 2, 3, and 4% lime at 1 and 5 min exposures on *C. gigas* in a laboratory setting to determine potential impacts of these treatments on oyster survival, growth, and condition. The same treatments were also repeated on the mottled sea star, *Evasterias troschellii*, a major predator of Pacific oysters, to ascertain effects on sea star mortality. Combined, the results of field and laboratory experiments demonstrated that exposure to 3 and 4% hydrated lime for 5 min was effective in removing total biofouling, *D. vexillum* fouling, and predatory sea stars without causing significant adverse effects on oyster survival, growth, or condition. This study provides insights to assist the shellfish aquaculture industry with possible solutions to control invasive tunicates and predatory sea stars.

1. Introduction

In the shellfish aquaculture industry it is common for culture equipment and product to become overgrown with other marine organisms, a phenomenon known as biofouling. Biofouling increases labor costs for farmers and often reduces shellfish value (Adams et al., 2011; Watson et al., 2009). In extreme cases, biofouling can cause shellfish mortality through predation, competition, and/or restriction of water flow (Daniel and Therriault, 2007; Lambert and Lambert, 1998).

Invasive species, generally opportunistic and fast-growing organisms, often become dominant components of biofouling communities. A growing number of invasive tunicates have recently been observed in coastal marine environments and their presence is becoming a growing concern for the shellfish aquaculture industry (Lambert and Lambert, 2003; LeBlanc et al., 2007; McKindsey et al., 2007; Switzer et al., 2011). In southern Brazil, Rocha et al. (2009) reported the presence of 16 non-indigenous tunicate species at shellfish aquaculture sites. In San Quinton Bay (Mexico), the majority of fouling species on Pacific oysters (*Crassostrea gigas*) were non-indigenous tunicates (Rodriguez and Ibarra-Obando, 2008). Over the past decade, the blue mussel (*Mytilus edulis*) farming industry on the east coast of Canada has been challenged by a number of invasive tunicates including solitary species (*Styela clava* and *Ciona intestinalis*) and colonial ones (*Botrylloides violaceus* and *Botryllus schlosseri*) (LeBlanc et al., 2007; Locke et al., 2009; Ramsay et al., 2008). On the west coast of Canada, the presence of at least four non-indigenous tunicate species – *B. violaceus*, *B. schlosseri*, *S. clava*, and *Didemnum vexillum* – has been documented (Carver et al., 2006; Daniel and Therriault, 2007). Although it appears that these species have not impacted shellfish aquaculture on the west coast of Canada to the same extent as on the east coast, they are of great concern based on ecological and economic impacts noted elsewhere, especially *D. vexillum*.

Didemnum vexillum has spread from Japan to Europe, New Zealand, and both coasts of North America (Bullard et al., 2007; Lambert, 2009). This tunicate can grow quickly on stable substrates (natural or artificial), smothering and outcompeting native wild species and cultured shellfish, thereby posing a significant risk to the marine environment in general and shellfish aquaculture specifically (Daniel and Therriault, 2007; Valentine et al., 2007, 2009). This invasive tunicate has been reported in eelgrass (*Zostera marina*) beds, an important habitat for many larval/juvenile organisms, in New England, USA (Carman and Grunden, 2010) and Morris et al.

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(2009) have demonstrated that *D. vexillum* coverage may affect larval recruitment of the bay scallop (*Argopecten irradians irradians*). *Didemnum vexillum* covers hundreds of square kilometers of pebble gravel habitat on the Georges Bank fishing grounds off the northeastern coast of the USA, where it may have severe impacts on larval fish, shellfish, and benthic communities (Lengyel et al., 2009). In northwestern North America, *D. vexillum* has been discovered in British Columbia (BC), Canada (Daniel and Therriault, 2007) and, more recently, in Oregon, Washington, and Alaska, USA (S. Chan, Oregon Sea Grant, pers. comm.).

Despite the growing concern about invasive tunicates in shellfish aquaculture, no obvious solutions have been demonstrated to fully mitigate this problem. Several potential control options have been proposed – including various biological, mechanical, and chemical treatments – but results have been highly variable. Carman et al. (2009) found that the common periwinkle (*Littorina littorea*) consumed decaying *D. vexillum*, but was not effective at controlling it in aquaculture settings, leading the authors to suggest that non-biological methods for controlling this invasive tunicate were required. Sea urchins have been shown to reduce biofouling (as a result of their grazing activity) on cultured bivalves and cultivation gear in pearl oyster (*Pinctada imbricata*) culture in Venezuela (Lodeiros and Garcia, 2004) and scallop (*Pecten maximus*) culture in the Irish Sea (Ross et al., 2004), however, those studies did not examine effects on invasive tunicates specifically. Epelbaum et al. (2009) suggested that green sea urchins, *Strongylocentrotus droebachiensis*, efficient grazers of several invasive tunicate species tested in laboratory experiments, might prove useful for reducing tunicate fouling in shellfish aquaculture. Subsequent field trials, however, showed that, while *S. droebachiensis* did reduce *D. vexillum* fouling on cultured Pacific oysters over time, the level of fouling at the end of the study was still relatively high compared to oysters treated with a 4% hydrated lime solution, again suggesting that chemical treatments may be preferred (Switzer et al., 2011). Mechanical methods, such as pressure washing and manual removal, were effective for immediate reduction of tunicate coverage in bivalve aquaculture (Enright, 1993; Switzer et al., 2011), however, these methods are labor-intensive and may lead to the spread of colonial species as the fragments are capable of recolonization (Paetzold and Davidson, 2010).

Several studies have been conducted to assess the potential of chemical treatments to combat invasive tunicate fouling. Antifouling treatments of acetic acid (Forrest et al., 2007; LeBlanc et al., 2007; Piola et al., 2010) and hydrated lime [$Ca(OH)_2$] (Locke et al., 2009; Piola et al., 2010) have been tested on some invasive tunicate species, but the potential effects of these treatments on shellfish survival, non-target organisms, and the environment remain unclear (Locke et al., 2009). Other treatments tested, on the east coast of Canada, with limited applicability (due to unsatisfactory tunicate mortality and/or low shellfish survival) included sodium hydroxide, citric acid, formalin, detergents, UV light, steam, hot water, and electricity (LeBlanc et al., 2007). Denny (2008) tested a variety of chemicals on *D. vexillum* colonies in New Zealand and found that acetic acid, sodium hydroxide (lye or caustic soda), and sodium hypochlorite (bleach) were effective in reducing tunicate fouling on seed mussels (*Perna canaliculus*), but only the 0.5% bleach solution resulted in 100% tunicate mortality with limited impact on the seed mussels. Piola et al. (2010) tested acetic acid, hydrated lime, and sodium hypochlorite on *D. vexillum* in New Zealand and suggested acetic acid was preferable to hydrated lime, at least when applied as a spray. More recently, Switzer et al. (2011) showed that a 4% hydrated lime dip was very successful at removing *D. vexillum* from cultured Pacific oysters, but suggested dip concentrations and/or durations could be manipulated to maximize tunicate mortality and minimize shellfish loss. In addition, potential "eco-friendly" solutions such as freshwater or brine treatments might prove useful, but to the best of our knowledge, these treatments have not yet been evaluated rigorously for their potential to control tunicate fouling in oyster culture.

Another issue for the shellfish aquaculture industry is the control of predatory organisms, in particular, sea stars. A number of studies have shown that quicklime (calcium oxide, CaO) has a devastating effect on various species of sea stars and other echinoderms while remaining rather harmless to most other marine fauna, including a variety of shellfish species in general and oysters in particular (Bernstein and Welsford, 1982; Galtsoff and Loosanoff, 1939; Loosanoff and Engle, 1942; MacKenzie, 1977; Needler, 1940; Shumway et al., 1988; Wood, 1908). In BC (and other areas around the globe) the majority of shellfish production occurs in suspension (*i.e.* off bottom) and most sea stars arriving at these shellfish aquaculture sites are predicted to result from planktonic settlement, not from movement of juvenile or adult individuals. Thus, if a treatment is applied after a settlement event, mortality due to sea star predation could be reduced (*i.e.* little risk of re-invasion post-treatment). In BC, the mottled sea star (*Evasterias troschellii*) is a particularly problematic predatory species for shellfish farmers, but the effects of quicklime or hydrated lime on this particular sea star species have not been examined.

The main objective of the present study was to examine the effectiveness of four chemical treatments (acetic acid, brine, freshwater, and hydrated lime) – applied at various concentrations and for various exposure times – for the removal of overall biofouling, and *D. vexillum* fouling in particular, on cultured Pacific oysters (*C. gigas*). Once successful treatments were identified in the field, we further examined the effects of these treatments on oyster survival, growth, and condition in a laboratory setting. In addition, we tested the effects of successful treatments on mortality rates of the mottled sea star (*E. troschellii*), a common predator in oyster aquaculture in BC.

2. Materials and methods

2.1. Field experiment

The study was conducted at a shellfish aquaculture site in Lemmens Inlet (near Tofino, BC, Canada; 49° 12.591′N, 125° 51.778′W) from September 3 to October 9, 2009 (five weeks). This area was selected due to the increasing abundance of *D. vexillum* observed by local shellfish growers (M. Woods, Equinox Oysters, pers. comm.). The study site consisted of a steel net pen platform (L × W: 6.3 × 6.3 m) adjacent to an oyster farm (suspended long lines) in an area dominated by rocky substrate. Seawater temperature ranged from 13.7 to 16.9 °C during the study period, with an average of 15.0 °C. Salinity was 29.5 ppt, as measured at the beginning of the experiment. Temperature and salinity readings were taken at a depth of 2.5 m.

Experimental treatments used in this study were selected based on promising results from previous chemical-treatment studies on invasive tunicates (*e.g.* Denny, 2008; Piola et al., 2010), existing knowledge gaps, and a requirement for treatments to be environmentally friendly, affordable, readily available, and easy to use in an aquaculture setting. Chemicals selected were brine, freshwater, hydrated lime, and acetic acid. Concentrations of brine (40, 50, 70 ppt), freshwater (0, 5, 20 ppt), hydrated lime (1, 2, 4%), and acetic acid (0.25, 1.25, 5%) were prepared in 8-L volumes in a laboratory using ambient freshwater before being transported to the field site. Brine and freshwater solutions were prepared using Instant Ocean® Sea Salt. Acetic acid solutions were prepared from regular-strength (5%) household vinegar, as we speculated that it would be more readily available to shellfish growers than glacial acetic acid, and likely less expensive. All treatments were tested at exposure times of 0.5, 1, 5, and 10 min. All three factors were fully crossed, resulting in 48 treatment combinations (*i.e.* four chemical types × three concentrations × four exposure times) with each treatment having five replicates. Fouled Pacific oysters (30–36 months old) were collected from suspended longline clusters at the farm and separated by hand. Oysters that were particularly heavily