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Research article

Preliminary evaluation of effects of invasive tunicate management with acetic acid and calcium hydroxide on non-target marine organisms in Prince Edward Island, Canada

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Abstract

The proliferation of invasive tunicates in Prince Edward Island (PEI) estuaries has necessitated the development of approaches for managing tunicates that foul aquaculture structures, especially *Styela clava* and *Ciona intestinalis*. Spraying or immersion with a saturated solution of hydrated lime (calcium hydroxide) or 5% acetic acid are effective against these tunicates, but are also biocidal to a variety of non-target organisms as demonstrated by bioassays with the bacterium *Vibrio fischeri*, sand shrimp *Crangon septemspinosa* and threespine stickleback *Gasterosteus aculeatus*. Both chemicals have the potential to alter estuarine pH, which should remain within the limits 7.0-8.7 mandated by federal water quality guidelines. Acetic acid is no longer used as a commercial treatment in PEI and currently poses no risk to the environment. The pH of saturated hydrated lime solutions used for immersing mussel socks reached 12.6, but the "pH footprint" in the estuarine water column was limited to a radius of <1 m around the treatment site and rapidly returned to ambient pH. In studies elsewhere, heavy applications (>5 tonnes/ha) of quicklime (calcium oxide) pellets mainly converted to hydrated lime within minutes, but chemical conversion of hydrated lime to harmless calcium carbonate took up to 18 days depending on temperature, pellet size, and the amount of quicklime applied. Conversion of hydrated lime to calcium carbonate should be more rapid at the much lower daily application rates (<0.007 tonnes/ha) of hydrated lime in powdered form, which represented the maximum amount likely to be used in mussel aquaculture for tunicate management in PEI. In most PEI estuaries, dilution by tidal mixing alone is probably sufficient to return the pH to normal values within a tidal cycle at these relatively low inputs of hydrated lime, even without taking into account the chemical conversion which would be occurring simultaneously. Decisions about whether to use chemical treatments must balance economic and potential environmental costs of treatment against the known economic consequences of unmanaged tunicate biofouling. A recent shift to use of pressure washing will reduce the potential for impacts associated with chemical treatments, but may not be feasible in estuaries infested with *Styela clava*.

Key words: invasive tunicate, pest management, mussel aquaculture, acetic acid, hydrated lime, calcium hydroxide, toxicity, bioassay, biofouling

Introduction

The proliferation of invasive tunicates as fouling organisms in the marine environment has led to the development of a suite of approaches to manage infestations. Management goals include reducing tunicate density or biomass to levels that avoid adverse effects (e.g., reduced growth or survival of cultured bivalves from smothering or competition for food; handling and processing issues in aquaculture; handling issues for seasonal removal of floating docks), and preventing spread to uninfested localities either directly by removal of tunicates from vectors (e.g., from infested bivalves before processing or transfer, or from boat hulls), or by reducing the reservoir of propagules available for transport by vectors (e.g., Forrest et al. 2007; Locke et al. 2009).

The need for tunicate management has motivated a search for control methods that are environmentally benign, inexpensive and logistically feasible. The requirement that treatments that kill tunicates have no long-term environ-

mental consequences is particularly important on aquaculture sites, where the cultured species must remain safe for human consumption and the health and productivity of the environment must not be compromised. To this end, options that have been explored include: chemical treatments with sodium hydroxide, acetic acid, citric acid, formalin, detergents, chlorine bleach, and hydrated lime; and physical treatments using air drying, ultraviolet light, steam, hot water, electricity, smothering, pressure-washing, and puncturing (Carver et al. 2003; Coutts and Forrest 2007; LeBlanc et al. 2007).

Tunicate pests requiring management in Prince Edward Island (PEI) estuaries have included *Molgula* sp., *Styela clava* Herdman, 1881, *Botrylloides violaceus* Oka, 1927, *Ciona intestinalis* (Linnaeus, 1757), and to a lesser extent *Botryllus schlosseri* (Pallas, 1766). The latter four are non-indigenous species in PEI, as is at least one species of *Molgula* (*M. manhattensis* (De Kay, 1843)) (Locke unpub. data). The primary goal of tunicate management in PEI is the removal of large masses of tunicates from blue mussel (*Mytilus edulis* Linnaeus, 1758) aquaculture infrastructure. Also important is the prevention of dispersal of tunicates on harvested mussels and eastern oyster (*Crassostrea virginica* (Gmelin, 1791)), or on settlement devices used as collectors for larvae of these bivalves, which are then often transferred to different estuaries for culture. Pressure washing, calcium hydroxide (hydrated lime), or some combination of these treatments are generally applied to bivalve aquaculture gear on the aquaculture site. Until recently, dilute (5%) acetic acid (vinegar) was also in use as an experimental treatment. Tunicate treatments are commonly perceived by those involved in the industry to have negligible impacts on non-target biota and the environment, but potential impacts in PEI have not been rigorously studied.

Acetic acid is a weak organic acid, registered in Canada with the Pesticide Management Regulatory Agency for use as an organic herbicide in terrestrial applications (e.g., Registration Number 28163, http://pr-rp.pmra-arla.gc.ca/portal/page?_pageid=34,1&_dad=portal&_schema=PORTAL) but not, to our knowledge, registered for aquatic application. Immersion in 4-5% acetic acid is an effective means of control for a variety of marine biofoulers including the tunicates *Ciona intestinalis*, *Styela clava*, and *Didemnum vexillum* Kott, 2002 (Carver et al. 2003; Coutts and Forrest 2005; Sharp et al. 2006; Forrest et al. 2007; Pannell and Coutts 2007). Acetic acid is an attractive choice for pest control because it is a naturally occurring, low molecular weight organic compound (Spencer and Ksander 1999). Because 4-5% acetic acid is ordinary household vinegar, this treatment is often perceived as posing little or no risk to the environment. Acetic acid is, however, biocidal to a variety of organisms (e.g., Forrest et al. 2007).

In commercial applications in PEI, 5% acetic acid was found to be effective as a spray treatment (Fig. 1a) for colonial, but not solitary, tunicates (N. MacNair, PEI Department of Fisheries, Aquaculture and Rural Development, unpub. data). Immersion in 5% acetic acid for <1 minute is effective against solitary tunicates but is considered impractical for use in aquaculture because of dilution of the acid by water from immersed mussel socks (N. MacNair, PEI Department of Fisheries, Aquaculture and Rural Development, pers. comm.; LeBlanc et al. 2007). With both immersion and spray, there is mortality of mussels. If the mussels are shaken so they close their valves and the soft tissue is not exposed to acetic acid, mortality/loss of attachment is minimized in the range of 5-20% (LeBlanc et al. 2007; N. MacNair, pers. comm.).

Unlike acetic acid, “lime” has a long history of use in biological control for aquaculture or fisheries purposes (e.g., Wood 1908). At least two forms have been used: quicklime (calcium oxide, CaO), which is produced by heating limestone (calcium carbonate, $CaCO_3$) to drive off the carbon dioxide; and hydrated lime (calcium hydroxide, $Ca(OH)_2$) which is produced by adding water to quicklime. Neither form is registered as a pesticide in Canada. Quicklime, formerly listed as a hazardous substance by the U.S. Environmental Protection Agency, was removed from that list in 1979 (Shumway et al. 1988). Quicklime has been used in PEI and in Long Island Sound as a treatment to clear oyster beds of starfish (Needler 1940; MacKenzie 1977) and in California to eliminate sea urchins from kelp beds (Bernstein and Welsford 1982). Hydrated lime has been used for years to control predatory starfish on mussel seed (spat) collectors in PEI; the method, which consists of briefly immersing each collector in a trough filled with a saturated solution of hydrated lime in seawater, has been adapted for tunicate management on mussel socks and other aquaculture gear (Figure 1b). Alternatively, a low-volume hydrated lime sprayer may be used.

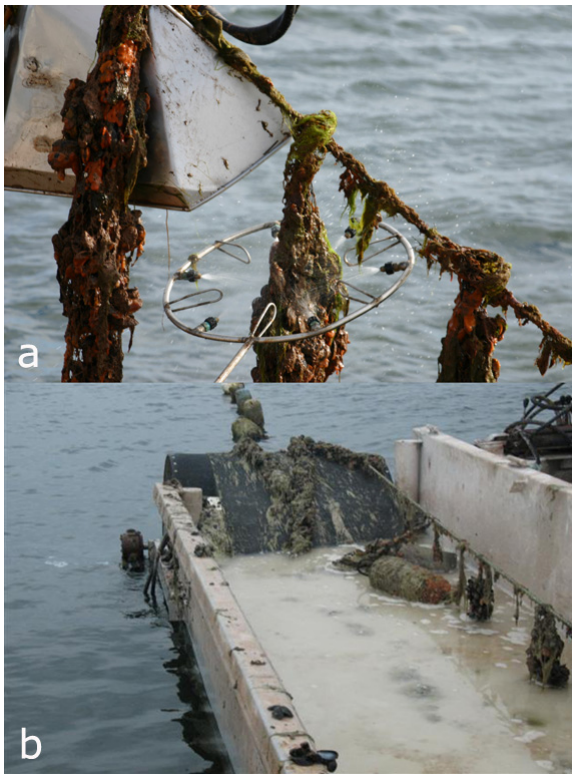

Figure 1. Typical methods for the application of acetic acid and hydrated lime to control tunicates on mussel aquaculture leases in Prince Edward Island (Photo credit: PEI Department of Fisheries, Aquaculture and Rural Development):
(a) This circular sprayer is moved by hand over the mussel sock to apply 5% acetic acid to all sides of the sock from nozzles located around the sprayer. Metal loops extending toward the centre of the sprayer help to position the sock equidistant from all nozzles
(b) A bag of hydrated lime is added to sea water in a trough attached to the side of the boat, then the mussel sock is dragged through the length of the trough.

MacNair and Smith (2000) found that immersing oyster spat collectors in a 4% hydrated lime solution for 1 min effectively killed *Molgula* sp. Mortality of mussels following immersion of a mussel sock in a trough is up to 10-15%; as with acetic acid, mortality increases if the valves of the mussels are not closed during treatment (N. MacNair, pers. comm.).

The goal of this study was to investigate the toxicity of acetic acid and hydrated lime in order to identify potential effects on non-target biota in coastal waters of PEI. We conducted bioassays of hydrated lime and acetic acid effects on bacteria, a crustacean, and a fish, representing different components of the ecosystem. We also conducted experiments to compare the toxicity of acetic acid versus citric and hydrochloric acids. We investigated the buffering capacity of representative estuaries by estimating the potential rates of application of hydrated lime, and the theoretical ability of the estuaries to absorb or mitigate hydrogen ion imbalance through physical dilution and mixing processes. Target pH levels were 8 (mid-point of the ambient pH we observed in PEI estuaries) and 9 (an approximation of the upper limit of the Canadian federal guideline for marine and estuarine pH, i.e., 8.7 pH units) (CCME 1999). In the field, we determined the "pH footprint" around limed treatment sites, and observed, *in situ*, the response of organisms to application of hydrated lime.

Methods

Toxicology tests - laboratory

Acute lethalities of hydrated lime and acetic acid were investigated using 96 h static bioassays for threespine stickleback *Gasterosteus aculeatus* Linnaeus, 1758 and sand shrimp *Crangon septemspinosa* Say, 1818. Chronic toxicities of hydrated lime and acetic acid were investigated by sand shrimp survival and growth in 14-day exposure. Sublethal toxicities of hydrated lime, acetic acid, citric acid, and hydrochloric acid were studied using the luminescent bacterium *Vibrio fischeri* (Beijerinck, 1889). Toxicology tests were conducted in the Environment Canada Toxicology Laboratory, Moncton, NB. Laboratory grade samples of glacial acetic acid (Fisher 99.7%), hydrochloric acid (Baker Lot C46039 36.5 to 38%) and citric acid (Fisher Lot 070975), and agricultural grade hydrated lime (Havelock Lime Co., Havelock, NB) were tested. Threespine stickleback used in the bioassays were obtained from Lawrencetown, NS. Sand shrimp were collected from Kouchibouguac Bay, NB. The freeze dried bacteria were purchased from Osprey Scientific (Mississauga, ON).

The 96 h bioassays of threespine stickleback were conducted according to the standard method of Environment Canada (1990). Test solutions were prepared for the samples in natural seawater (salinity 28-30‰), aerated for 30 min, and initial water quality (temperature, dissolved oxygen, salinity and pH) was measured. Ten fish (mean wet biomass 0.33 g ± 0.12 SD, N=20) were randomly introduced into

20 L of each test concentration (control and five levels of each of hydrated lime and acetic acid from 32 to 3,200 mg/L). The tests were checked for mortalities frequently the first day, then once a day thereafter. Any dead fish were removed. Water quality was measured daily. The 96 h LC50s (concentration calculated to cause 50% mortality) were calculated following Stephan (1977). A monthly reference toxicant test conducted with phenol ensured that normal operating conditions were maintained, and that the population of fish used in the test was of normal sensitivity.

The 96 h toxicity tests of hydrated lime and acetic acid on sand shrimp were conducted by a standard method developed by the laboratory. The test solutions were prepared in natural seawater, and ten replicate 1 L mason jars were filled with each test concentration (control and five levels of each of hydrated lime and acetic acid from 5 to 50,000 mg/L) and acclimated to 15±1ºC. Initial water quality was then measured. One sand shrimp was introduced into each test vessel. The tests were checked for mortalities and water quality was measured daily. The 96 h LC50s were determined as for the fish experiment.

Chronic toxicities (survival and growth in 14 day exposure) were investigated using sand shrimp (mean wet weight 3.00±SD 1.307 mg, N=20). Test set up was the same as for the 96h test, except that in the chronic experiment twenty replicates were used per test concentration and solutions were control and five levels of hydrated lime from 3.2 to 320 mg/L. Testing was conducted as static renewal with 80% of the test solution replaced three times a week with fresh solution. The temperature was checked daily. Water quality was measured three times a week. The test tanks were checked for mortalities once a day. The test organisms were fed three times a week at time zero and after renewals with 100 mg of frozen brine shrimp with some live brine shrimp for the first week, and 200 mg of frozen brine shrimp in week two. After 14 days, each surviving sand shrimp was weighed (dry weight). The 14-day LC50 was calculated. Analysis of variance was used to examine the effect of treatments on sand shrimp weights (log-transformed to normalize) (JMP Version 4, SAS Institute 2000).

Sublethal toxicities of hydrated lime, acetic acid, citric acid, and hydrochloric acid were compared using the bacterial Microtox test. The three acids were tested to determine whether the efficacy of acetic acid as a biocide was due to factors other than pH. The Microtox test consists of 15-minute static bioassays on the luminescent bacterium *Vibrio fischeri* (Environment Canada 1992). If toxic materials are present they interfere with the cellular respiration of the organism, measured as a decrease in light output. A reference toxicant test was conducted with zinc sulphate for each batch of tests to ensure that the population used in the test was of normal sensitivity. The IC50, the concentration that causes inhibition of light by 50%, was calculated using Microtox Omni software (Azure Environmental 1999).

The No Effect Concentration for hydrated lime and acetic acid was approximated from the concentrations resulting in ≤ 10 % mortality in the fish and sand shrimp tests (this is considered an acceptable mortality in the controls), and light production within the range of the control values in the Microtox test.

Toxicity test – field

An experiment to compare the effects of 5% acetic acid (household vinegar) and citric acid adjusted to the same pH (2.6) on the survival of the vase tunicate, *Ciona intestinalis*, was conducted at an aquaculture site in Murray River (46º01'N, 62º35'W) from 29 August to 11 September 2007. Pieces were cut from a Styrofoam buoy fouled with vase tunicates, and trimmed so that each buoy piece supported 30 tunicates. If necessary, tunicates were carefully peeled from the buoy without damaging the remaining specimens to obtain the correct number of tunicates. Each buoy piece was placed in a tank of estuary water (21.2-24.5ºC, 27‰, pH 8.16) on the boat until treated. Holding time was < 1 hr. Citric acid was adjusted to pH 2.6 by dilution. Triplicates of tunicate-infested buoys were dipped into 1 L containers of either acetic or citric acid for 5 or 10 sec followed by 10 sec of air-drying, or were exposed only to 10 sec air-drying to act as controls. Each container was used for dipping until its pH diverged by more than 0.2 pH unit. Each buoy piece was placed in a labeled cage that was attached to a mussel line at ~0.5 m depth. After eight days, the cages were retrieved and the tunicates were counted and classified as “healthy” (still attached to the buoy, and siphoning within 1 min when placed into a bucket of estuary water) or “moribund” (detached from the buoy, attached but not siphoning, or obviously dead and decomposing).

To determine the longer-term survival of tunicates that were attached but not siphoning, the buoys were returned to the cages for five days, when the status of the tunicates was re-assessed and the experiment was terminated. Repeated-measures analysis of variance was used to compare survival rates among treatments (JMP version 4, SAS Institute 2000).

Field observations and pH at treatment sites

Opportunistic observations on treatment usage, "pH footprint" around treatment sites (measured with a YSI pH meter), and responses of non-target biota both in the treatment troughs and by a diver on and adjacent to treatment sites were made in PEI estuaries throughout the summer and autumn of 2007.

pH buffering capacity of estuaries

We investigated the buffering capacity of representative estuaries by estimating the potential maximum daily rates of application of hydrated lime, and the theoretical ability of the estuaries to neutralize the resultant hydrogen ion imbalance. We did not conduct this exercise for acetic acid, as its field use has been discontinued.

Four estuaries were chosen for analysis, including three where tunicate treatments were in use. Murray (including Murray River and Murray Harbour) and Cardigan (46º12'N, 62º30'W) (including Cardigan River, Cardigan Bay, Montague River, Brudenell River and St. Mary's Bay) are estuaries located in southeastern PEI, where invasive tunicates have been established since 1998 and were distributed throughout all mussel-growing areas of the estuaries in 2007. Hydrated lime is currently in use for tunicate control by some aquaculture growers in both estuaries, although other growers use pressure-washing or some combination of the two methods. In previous years several growers used 5% acetic acid. Malpeque (46º32'N, 63º50'W) (including Malpeque Bay, Bentick Cove, Bideford River, Chichester Cove and Marchwater) and Tracadie (46º24'N, 63º00'W) (including Tracadie Bay and Winter Bay) are located on the north shore of PEI. In 2007, hydrated lime was used in the portions of Malpeque Bay infested with tuni-cates; one grower had experimented with acetic acid previously. There were no solitary tunicates in Tracadie Bay in 2007 and growers did not use hydrated lime to control the two colonial species that were present, but this estuary was included in the study because it is one of the most heavily utilized in PEI for mussel aquaculture. If hydrated lime were being used in Tracadie Bay to control tunicates, this would most likely represent the "worst case" scenario for application rates. Mussel growers have on occasion used hydrated lime for starfish control on mussel seed collectors in the smaller, attached, Winter Bay.

Determining the potential volume of hydrated lime used in PEI estuaries

Aquaculture sites in PEI are leased from the Government of Canada. Growers may lease one or more sites in an estuary, but few growers own or have access to more than one set of equipment for treating tunicates. Therefore the number of growers, rather than the number of leases, most likely determines the quantity of lime that could potentially be used daily in an estuary. The number of growers (unique grower identification numbers) was obtained for each estuary from the Aquaculture Leasing Division (D. Mills, Fisheries and Oceans Canada, Charlottetown, PEI). This may overestimate the number of tunicate treatment units as some smaller growers may share equipment. Interviews with growers in each estuary indicated that in Murray, Tracadie and Cardigan, eight to ten longlines could be treated with lime per unit per day, so we used 10 as the maximum number. In Malpeque, six longlines would typically be treated daily. These differences reflect the kinds of gear that have been developed by growers in each estuary, as well as differences in the lengths of the lines. Growers generally add one or more 23-kg bags of hydrated lime to the trough before starting treatment, and add more at the end of each longline. Interviews with growers indicated two bags were used per line in Malpeque Bay where the lines were longer; growers in the other three systems added 1.5 bags per line (A. Morrison and N. MacNair, pers. comm.).

Lastly, we estimated the potential maximum daily use of hydrated lime in each estuary as the product of: number of growers, number of longlines that could be treated daily/grower, number of bags of hydrated lime used/longline, weight of bag of lime (22.7 kg). We consider this a worst-case scenario, representing the hypothetical amount of hydrated lime entering

the estuary on a day when every grower in the estuary was using hydrated lime to the maximum amount; and if all the lime slurry remaining in the troughs at the end of the day was dumped into the estuary. Currently, there is no code of practice specifically addressing the use of hydrated lime and this is a common means of disposal. The amount of hydrated lime entering the estuary if the slurry remaining in the trough at the end of the day was disposed on-shore was also estimated. The estimate was based on the potential maximum daily volume of dissolved lime, assuming all the hydrated lime dissolves and the mean solubility of hydrated lime in water is 1.65 g/L (solubility at 20ºC from National Lime Association 2007).

Calculating the ability of test estuaries to buffer hydrogen ion imbalances

Our field observations indicated that the pH of hydrated lime solutions used by growers was in the range of 12 to 12.6 pH units. The pH of a saturated calcium hydroxide solution varies with temperature; at 15 to 30ºC, the pH should range from 12.3 to 12.8 (National Lime Association 2007). These temperatures encompass the range of conditions when tunicates would typically be treated in PEI. The pH of sea water is generally in the range of 7.5-8.4 (Sverdrup et al. 1942; Pelejero et al. 2005), which is consistent with our observations in PEI estuaries. Our calculation was based on achieving a target pH of 8, the midpoint of the normal range, or 9, an approximation of the upper allowable pH limit (8.7) mandated in the Canadian guidelines for marine and estuarine pH (CCME 1999). For example, without taking into account the chemical buffering capacity of the estuary, and assuming complete mixing, a dilution factor of 10^4 could physically correct the elevated pH of a solution of hydrated lime, assumed to be 12 pH units, to seawater pH, assumed to be 8 pH units (i.e., a reduction of four orders of magnitude in hydrogen ion concentration). Similarly, a dilution factor of 10^3 could correct the pH from 12 to 9. We considered estimation of the chemical buffering capacity of the estuary to be a complicated question far beyond the scope of the present study.

To determine the potential for physical dilution, two ratios were calculated for each of the four estuaries: (1) tidal volume of the estuary (Gregory et al. 1993): maximum daily volume of dissolved hydrated lime for that estuary, and (2) total estuarine volume: maximum daily volume of dissolved hydrated lime. If either ratio was greater than 10^4:1, the "dilution factor" of a completely mixed estuary should be sufficient to obtain a mean pH of 8. A ratio of 10^3:1 indicated the volume should be sufficient to dilute the hydrated lime to pH 9.

The maximum application rate of hydrated lime per unit surface area (minimum estuarine surface area at chart datum (Gregory et al. 1993)) was estimated to enable comparisons with the application rates of quicklime (expressed in tonnes/ha) from the literature.

Results

Toxicology tests

Hydrated lime

The pH during the acute toxicity test of threespine stickleback with hydrated lime was 7.91-7.94 in the control, but reached 10.40-10.61 in the treatment with the most lime, 3200 mg/L (Table 1). There was no mortality of fish in the control, or in treatments up to 100 mg lime/L. The 96-hour LC50 was 457 mg/L (95% confidence limits of 262 - 785 mg/L). Based on the pH values measured at t=0 this LC50 was equivalent to 10.47 (10.26 - 10.52) pH units.

There was no mortality of sand shrimp in control (pH 6.60-8.03) to 50 mg lime/L (pH 8.17-9.12) treatments over 96 h, but 100% mortality in treatments with lime concentrations of 500 mg/L (pH 8.58-10.32) to 50,000 mg/L (pH 12.39-12.61) (Table 2). The 96 hour LC50 was 158 mg/L (50 - 500). The equivalent pH value would be 9.70 (9.12 - 10.3).

A chronic toxicity test could not be conducted for sand shrimp held at lime concentrations of 100 and 320 mg/L, as all sand shrimp were dead after 14 days (Table 3). Mortality of 5-15% occurred in the control and treatments with 3.2 to 32 mg/L of lime. The 14 day LC50 was 53.1 mg/L (48.3 - 58.4), or an equivalent pH of 9.20 (9.12 - 9.28). Exposure to lime did not affect the growth of sand shrimp (one-way ANOVA, $F=2.21$, $P=0.07$).

Light inhibition of bacteria in the Microtox test (IC50) occurred at lower concentration of hydrated lime than the lethal effects on fish or shrimp. The IC50 was 31.0 mg/L (18.8 - 51.4 mg/L), corresponding to a pH of ~ 9.0.

Table 1. Acute toxicity (96 hour) of hydrated lime to threespine stickleback.

Lime treatment	Control	32 mg/L	100 mg/L	320 mg/L	1,000 mg/L	3,200 mg/L
pH t=0 h	7.93	8.91	10.06	10.46	10.55	10.61
Mean pH	7.93	8.64	9.54	9.89	10.07	10.51
% mortality	0	0	0	50	70	100

Table 2. Acute toxicity (96 hour) of hydrated lime to sand shrimp.

Lime treatment	Control	5 mg/L	50 mg/L	500 mg/L	5,000 mg/L	50,000 mg/L
pH t=0 h	7.79	8.06	9.12	10.32	12.29	12.61
Mean pH	7.68	8.00	8.58	9.47	12.11	12.46
% mortality	0	0	0	100	100	100

Table 3. Chronic toxicity (14 day) of hydrated lime to sand shrimp.

Lime treatment	Control	3.2 mg/L	10 mg/L	32 mg/L	100 mg/L	320 mg/L
pH t=0 h	8.06	8.00	8.22	8.77	9.76	10.25
Mean pH	7.97	7.95	8.04	8.17	8.72	9.78
% mortality	5	10	15	5	100	100

Table 4. Acute toxicity (96 hour) of acetic acid to threespine stickleback.

Acetic acid treatment	Control	32 mg/L	100 mg/L	320 mg/L	1,000 mg/L	3,200 mg/L
pH t=0	8.10	7.48	6.00	4.20	3.61	3.16
Mean pH	7.95	7.70	7.01	4.22	3.64	3.17
% mortality	0	0	0	100	100	100

Table 5. Acute toxicity (96 hour) of acetic acid to sand shrimp.

Acetic acid treatment	Control	5 mg/L	50 mg/L	500 mg/L	5,000 mg/L	50,000 mg/L
pH t=0 h	7.82	7.85	7.52	3.95	3.02	2.29
Mean pH	7.96	7.94	7.64	3.93	2.95	2.26
% mortality	0	0	0	100	100	100

Table 6. Chronic toxicity (14 day) of acetic acid to sand shrimp.

Acetic acid treatment	Control	10 mg/L	32 mg/L	100 mg/L	320 mg/L	1,000 mg/L
pH t=0 h	8.07	7.82	7.43	6.06	6.11	3.64
Mean pH	7.99	7.95	7.86	7.56	5.50	3.64
% mortality	0	5	10	15	100	100

Acetic acid

There was no mortality of threespine sticklebacks in the control (pH 7.83-8.10) and with acetic acid up to 100 mg/L (pH 6.00-7.52), but 100% mortality at acetic acid concentrations at or above 320 mg/L (pH 4.20-4.23) (Table 4). The 96 h-LC50 was 178 mg/L (100 - 320 mg/L), equivalent to 5.02 (4.20 - 6.00) pH units.

Similarly, no sand shrimp died in the control or treatments up to 50 mg/L acetic acid (pH 7.17-8.03 in the three treatments), but all sand shrimp died in acetic concentrations at or above 500 mg/L (pH ≤ 3.93) (Table 5). The 96-h LC50 was 158 mg/L (50 - 500), equivalent to 5.45 (3.95 - 7.52) pH units.

All sand shrimp died during the 14 day chronic toxicity tests with acetic acid concentration ≥ 320 mg/L (mean pH 5.50) (Table 6). In treatments with ≤ 100 mg/L (mean pH of all treatments 7.56-7.99), ≤15% of the sand shrimp died. The 14 day-LC50 was 116 mg/L (85.9 - 157 mg/L), equivalent to 7.06 (6.95 – 7.17) pH units. There was no effect on growth of the sand shrimp when exposed to acetic acid (one-way ANOVA, F=1.97, P=0.11).

Sublethal effects on bacteria occurred at lower concentrations of acetic acid. The estimated IC50 of acetic acid in the Microtox test was 88.5 mg/L (32.2 - 244 mg/L), equivalent to 6.30 pH units.

Comparison of acids

Acetic, hydrochloric and citric acid elicited responses at different hydrogen ion concentrations in the Microtox test. The estimated IC50 of acetic acid was 88.5 mg/L (32.2 - 244 mg/L), equivalent to 6.30 pH units. The estimated IC50 of citric acid was 126 mg/L (no confidence limits could be calculated), corresponding to a pH of 5.75. The IC50 of hydrochloric acid occurred at ~195 mg/L, corresponding to a pH of ~4.60.

The field experiment with *Ciona intestinalis* also showed clearly the greater lethality of acetic compared to citric acid when used as an immersion treatment adjusted to similar pH (Figure 2). The treatments were significantly different (F=17.37, df=4,8, P=0.0005) by repeated-measures ANOVA. There was no significant change in survival from day 8 to 13 of the experiment (F=0.19, df=1,8, P=0.67) and no significant interaction between treatment and day (F=1.25, df=4,8, P=0.36). A Tukey's HSD test indicated that survival rates on day 8 in the two citric acid treatments did not differ from each other or from controls; but survival was significantly lower (P<0.05) in the two acetic acid treatments which did not differ from each other.

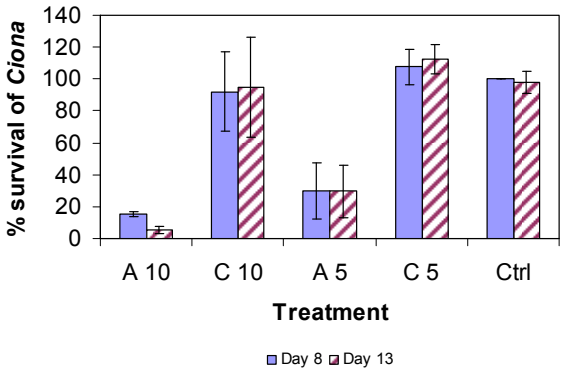

Figure 2. The results of an experiment comparing survival (mean and SD of 3 replicates) of *Ciona intestinalis* exposed for 5 and 10 sec to acetic (A5 and A10) and citric (C5 and C10) acids at pH 2.6-2.8, and control (Ctrl).

Effect levels

Hydrated lime resulted in mortalities exceeding ~10% (the acceptable level of control mortality) to threespine stickleback (96 h exposure) at 320 mg/L (pH 9.89), sand shrimp (96 h) at 500 mg/L (pH 9.47) and sand shrimp (14 d) at 100 mg/L (pH 8.72). Overall, the most sensitive endpoint for all the species tested was in the Microtox test with IC50 at 31 mg/L (pH 9.0) (Table 7).

Acetic acid resulted in comparable mortalities to threespine stickleback (96 h) at 320 mg/L (pH 4.22), and sand shrimp (96 h) at 500 mg/L (pH 3.93), but the 10-fold change in acetic concentration from the next nearest test level resulted in a large change of pH (from 7.01 and 7.64, respectively) so the true pH endpoints in each bioassay were more likely between 4.22-7.01 and 3.93-7.64. Sand shrimp (14 d) showed effects at 100 mg/L (pH 7.56). Overall, the most sensitive endpoint for all the species tested was in the Microtox test with 50% loss of function at 88.5 mg/L (pH 6.30) (Table 7).

Dilution factors for "no effect concentration" ranged from 500- to 1560-fold for lethal impacts of hydrated lime (Table 7). Similar dilution would be required for "no effect concentration" for lethal effects of acetic acid.

Table 7. Summary of results of acute (15 min or 96 h) and chronic (14 d) toxicity tests on threespine stickleback (*Gasterosteus aculeatus*), sand shrimp (*Crangon septemspinosa)* and bacterium (*Vibrio fischeri*) with hydrated lime, acetic acid, hydrochloric acid, and citric acid. Dilution factor is calculated for a 5% solution to reach Median Effective Concentration (LC50 or IC50) or No Effect Concentration. NC=not calculable; no replication in this test.

(a) Hydrated lime

	Median Effective Concentration			No Effect Concentration		
Species (time)	Concentration (mg/L)	pH	Dilution factor	Concentration (mg/L)	pH	Dilution factor
G. aculeatus (96 h)	457	10.47	109:1	100	9.54	500:1
C. septemspinosa (96 h)	158	9.70	316:1	50	8.58	1000:1
C. septemspinosa (14 d)	53.1	9.20	942:1	32	8.17	1560:1
V. fischeri (15 min)	31	9.0	1610:1	NC	NC	NC

(b) Acetic acid

	Median Effective Concentration			No Effect Concentration		
Species (time)	Concentration (mg/L)	pH	Dilution factor	Concentration (mg/L)	pH	Dilution factor
G. aculeatus (96 h)	178	5.02	281:1	100	7.01	500:1
C. septemspinosa (96 h)	158	5.45	316:1	50	7.64	1000:1
C. septemspinosa (14 d)	116	6.10	431:1	32	7.86	1560:1
V. fischeri (15 min)	88.5	6.30	565:1	NC	NC	NC

(c) Citric acid

	Median Effective Concentration			No Effect Concentration		
Species (time)	Concentration (mg/L)	pH	Dilution factor	Concentration (mg/L)	pH	Dilution factor
V. fischeri (15 min)	126	5.75	397:1	NC	NC	NC

(d) Hydrochloric acid

	Median Effective Concentration			No Effect Concentration		
Species (time)	Concentration (mg/L)	pH	Dilution factor	Concentration (mg/L)	pH	Dilution factor
V. fischeri (15 min)	195	4.60	256:1	NC	NC	NC

Table 8 (a). Mass balance calculation to determine capacity of selected Prince Edward Island estuaries to dilute the estimated hydrogen ion changes associated with maximum daily hypothetical application of hydrated lime to control tunicates on mussel aquaculture sites.

Estuary	Growers /estuary	Longlines limed /day/grower	Bags of lime used /line	Total lime used (kg)	Volume dissolved lime (m^3)	Tidal volume (m^3x10^6)	Estuary volume (m^3x10^6)	Ratio tidal: lime volume	Ratio estuary: lime volume
Tracadie	28	10	1.5	9534.0	5778.2	9.8	36	1696	6230
Murray	9	10	1.5	3064.5	1857.3	20.0	31	10768	16691
Cardigan	27	10	1.5	9193.5	5571.8	71.3	377	12797	67662
Malpeque	14	6	2	3813.6	2311.3	141.1	592	61049	256136

Table 8 (b). Conversion of maximum hypothetical daily hydrated lime applications to tonnes/ha for comparison with quicklime experiments from the literature.

Estuary	Hypothetical maximum daily hydrated lime (kg)	Estuary surface area (chart datum) (km^2)	Hypothetical daily rate of hydrated lime application (tonnes/ha)
Tracadie	9534.0	13.8	0.0069
Murray	3064.5	12.1	0.0025
Cardigan	9193.5	64.7	0.0014
Malpeque	3813.6	155.4	0.0002

Field observations and pH at treatment sites

In Murray River, only two growers used lime troughs to control tunicates on mussel socks in 2007. No growers were using acetic acid, although this treatment had been used as recently as 2005. Some growers who had formerly used acetic acid had switched to pressure-washing the mussels, and used hydrated lime sprayed through a nozzle only to treat buoys. This change had been made in large part because the replacement of *Styela clava* by *Ciona intestinalis* as the dominant fouling tunicate in Murray made pressure-washing a feasible method whereas it was ineffective against *Styela*. Growers had also found the acetic acid spray difficult to apply evenly, so its effect on tunicates was inconsistent and mussel mortalities were sometimes high (A. Morrison and N. MacNair, pers. comm.).

In Malpeque Bay, mussel socks were sprayed with hydrated lime rather than immersed in a trough. No growers used pressure-washing, as *Styela clava* was the fouling tunicate of concern in that estuary and large specimens are protected by a leathery tunic. Trials are planned for 2008 to determine the maximum size of *Styela* that can be controlled by pressure-washing (A. Morrison and N. MacNair, pers. comm.).

Growers in most PEI estuaries, even those lacking tunicate infestations, commonly utilize hydrated lime as an annual treatment for predators (starfish) on seed mussels. Since the arrival of fouling tunicates there are sometimes as many as three treatments annually on seed mussel lines, but the amount of lime used per treatment is usually lower than that used on larger mussels (which, if treated at all, are typically treated annually; or only at harvest).

Eastern oysters from infested PEI estuaries were treated in 2007 to prevent dispersal of tunicates via the oyster processing plant vector. This treatment consisted of a 30 second immersion in hydrated lime followed by 1 hour of air-drying. Treatment was done either on shore after harvest or at the plant before processing (N. MacNair, pers. comm.).

pH measured in hydrated lime troughs during immersion of mussel socks reached a maximum value of 12.6 pH units. A cloud of lime particles was visible in the water immediately below the area where the treated sock exits the lime trough, and the pH in this area was ~10, but readings rapidly (as fast as the pH meter could register the change) dropped to pH 8.3-9.0 approximately 0.7 m from the area of discharge, and were always <8.5 approximately 1 m from the area of discharge, at depths down to about 1 m (A. Morrison, pers. comm.). The cloud of lime dissipated before reaching the bottom. Observations by a diver during an experiment to determine behavioural responses to hydrated lime indicated that the cloud of particles, produced from releasing 45 kg of lime, settled to the bottom, but that organisms such as blue mussels, rock crabs (*Cancer irroratus* Say, 1817) and gastropods on the socks or nearby bottom continued their normal activities even when engulfed in the lime cloud (N. MacNair, pers. comm.).

Macroinvertebrates and fishes associated with the mussel socks were often found in the lime troughs after treatments. Fish (cunner *Tautogolabrus adspersus* (Walbaum, 1792) and rock gunnel *Pholis gunnelis* (Linnaeus, 1758)) were found dead in the trough at the end of the day. European green crab (*Carcinus maenas* (Linnaeus, 1758)) showed no obvious ill effects while in the trough, and specimens removed from the trough and held in estuary water appeared healthy and active more than a week later (A. Morrison, pers. comm.).

pH buffering capacity of estuaries

All four estuaries had sufficient tidal volumes or estuarine volume to dilute the hypothetical loading of dissolved hydrated lime to pH 9, assuming equal mixing of the hydrated lime throughout the volume of water (Table 8a). All but Tracadie Bay had sufficient tidal or estuary volume to maintain a pH of 8. Of the four estuaries examined, Malpeque had the greatest capacity to absorb pH changes at the current level of aquaculture.

Maximum hypothetical daily application rates of hydrated lime ranged from 0.0002 tonnes/ha (Malpeque Bay) to 0.0069 tonnes/ha (Tracadie Bay) (Table 8b).

Discussion

Hydrated lime

At the ecosystem level, addition of hydrated lime to PEI estuaries may have two positive consequences: countering the acidification of ocean waters, and improving water quality in eutrophic systems. Within the past two centuries, surface waters of the world have experienced a

pH reduction of about 0.1 pH units and further reductions of ~ 0.2-0.3 pH units are predicted by 2100 (OSPAR 2006), thus the addition of a chemical that increases pH, such as hydrated lime, may be locally beneficial. Hydrated lime may also improve water quality in estuaries that experience anaerobic events due to excess nutrient levels, which have become common in PEI over the past few decades (Raymond et al. 2002). Hydrated lime is sometimes added to anaerobic marine environments for odor control, to inhibit sulfate-reducing bacteria, and to precipitate algae, silt and phosphorous to the bottom (Nishimura and Seki 1983; Müezzinoğlu et al. 2000). These are short-term improvements which persist only as long as additions of lime are made.

Very little is published about the toxicity of hydrated lime. In odor control treatments of marine systems, 114 g/m^2 (1.14 tonnes/ha) of hydrated lime killed 99% of the sulfate-reducing bacteria and 66% of anaerobic bacteria; 198 g/m^2 (1.98 tonnes/ha) killed 99.8% of the anaerobic bacteria (Müezzinoğlu et al. 2000). At the application rates used for tunicate control in PEI, one minute of immersion in a saturated 4% solution (40,000 mg/L, pH ~12) of hydrated lime effectively killed the tunicate *Molgula* sp. but did not injure eastern oyster spat or the green alga *Codium fragile* ssp. *tomentosoides* (van Goor, 1923) (MacNair and Smith 2000). A typical mortality of blue mussels after immersion treatment of a mussel sock for tunicates is 10-15% (N. MacNair, pers. comm.). In our experiments, median effective concentrations that caused mortality were two orders of magnitude lower than a saturated solution but exposure time was considerably longer than we would expect to see associated with a single tunicate treatment: 96-h exposures of threespine stickleback at 457 mg/L (pH 10.5) and sand shrimp at 158 mg/L (pH 9.7), and 14-d exposure of sand shrimp at 53 mg/L (pH 9.2) (Table 7). Overall, the most sensitive endpoint was the sublethal response detected after 15 min in the Microtox bacterial test with IC50 at 31 mg/L of hydrated lime (Table 7). This would be equivalent to a pH observation of 9.0, which is likely to occur in the field only within about 1 m of treatment sites. In the field, European green crab *Carcinus maenas* survived at least a week with no obvious ill effects after exposure to saturated hydrated lime in a mussel sock treatment trough (pH ~12-12.6), although fishes (cunner *Tautogolabrus adspersus* and rock gunnel *Pholis gunnelis*) died by the end of the day (N. MacNair, pers. comm.).

Much of the published literature on "lime" effects in the marine environment relates to quicklime rather than hydrated lime and so a comparison of the chemistry and toxicity of these two chemicals is relevant. The mechanism of toxicity of both chemicals is closely associated with their strongly alkaline nature (pH ~ 12 in saturated solution). For example, similar lesions were caused to the aboral surface of starfish by quicklime settling through the water column, and to the oral surface by crawling over a deposited layer of hydrated lime (Loosanoff and Engle 1942). Hydrated lime is considerably less toxic than quicklime (usually only 15-20% as many mortalities when exposed for the same duration), but hydrated lime may have a longer exposure time (North and Shaefer 1963). Quicklime rapidly (<15 min) converts to hydrated lime after mixing with water, an exothermic reaction which liberates extreme heat (152.8 kcal/mole) and may burn the surface of susceptible organisms on contact (North and Shaefer 1963, Bernstein and Welsford 1982). The subsequent conversion of hydrated lime to calcium carbonate is slower, liberates less heat (18.8 kcal/mole) and requires carbon dioxide. The duration of this conversion is an important determinant of the overall effect of hydrated lime. The rate of conversion from hydrated lime to calcium carbonate depends on temperature, the particle size of the lime, and the availability of carbon dioxide (typically present at 0.009 kg/m^3 of sea water, and required at a rate of 0.59 kg of carbon dioxide to convert 1 kg of hydrated lime) as well as related ions such as bicarbonate which affect the equilibrium state (North and Shaefer 1963). Complete conversion of heavy applications (>5 tonnes/ha) of quicklime to calcium carbonate requires five to 18 days in sea water and most of this time is used in the conversion of hydrated lime to calcium carbonate (North and Shaefer 1963; McKenzie 1977; Bernstein and Welsford 1982). Using pH as an indicator of lime conversion, Loosanoff and Engle (1942) found that lighter applications of quicklime (~0.9 tonne/ha) increased pH by only 0.2-0.4 pH units to 8.2-8.5, and conversion to calcium carbonate occurred within two days even at 2˚C. Conversion of hydrated lime in PEI estuaries should occur even more rapidly given that the lime is in powdered form, water temperatures during application are usually >15˚C, and the maximum potential daily appli-

cation rates we calculated for tunicate control (<0.007 tonnes/ha) are two orders of magnitude lower than the "light" quicklime applications used by Loosanoff and Engle (1942). Indeed, according to our calculations, there is ~22 times as much carbon dioxide present as would be required for the conversion of 0.007 tonnes of hydrated lime to calcium carbonate in a 1-ha surface layer of thickness 1 m. The field observation that the "pH footprint" of hydrated lime in the water column around treatment sites was limited to a radius <1 m is consistent with rapid conversion of the hydrated lime to calcium carbonate.

Marine organisms that are unaffected by quicklime should not be affected by hydrated lime, as it is a degradation product of quicklime in sea water. Macroalgae (*Macrocystis pyrifera* (Linnaeus, 1771), *Gigartina canaliculata* Harvey, 1841, *G. leptorhynchos* J. Agardh, 1885, and *Egregia laevigata* Setchell, 1896); adults and larvae of blue mussel, eastern oyster, quahog *Mercenaria mercenaria* (Linnaeus, 1758), soft-shell clam *Mya arenaria* Linnaeus, 1758; adult and juvenile (carapace length 37 mm) American lobster *Homarus americanus* H. Milne Edwards, 1837; adult spiny lobster *Panulirus interruptus* (J.W. Randall, 1840); shrimps; some polychaetes; gastropods with opercula; encrusting corals; anemones; some sponges; and most fish species survive heavy applications (5-50 tonnes/ha) of quicklime (Galtsoff and Loosanoff 1939; Needler 1940; Loosanoff and Engle 1942; North and Shaefer 1963; McKenzie 1977; Shumway et al. 1988). Some of these organisms survived exposure to quicklime in tanks for >1 yr. Histological examination after six months of exposure revealed no damage to American lobster gills, mussel gills or worm parapodia, and gross examination showed no damage to any tested species other than starfish (Shumway et al. 1988).

Similarly, organisms affected by quicklime may be susceptible to hydrated lime. At heavy application rates, quicklime was lethal to all echinoderms (starfish, urchins, sea cucumbers); it also harmed jellyfish, some sponges, mature gastropods lacking opercula (e.g., abalone *Haliotis* sp.), larval gastropods, some polychaetes, bryozoans, larval American lobsters, some larval fishes, and adult flatfishes (Galtsoff and Loosanoff 1939; Loosanoff and Engle 1942; Loosanoff 1962; Bernstein and Welsford 1982; Shumway et al. 1988). Sublethal effects on the filtering and growth of eastern oysters and soft-shelled clams were observed in laboratory experiments (Loosanoff and Engle 1942; Turner 1970). Phytoplankton abundance was reduced for 24 h following application of quicklime (0.56 tonne/ha) in a PEI estuary, but subsequently recovered (Needler 1940).

While the toxicity of hydrated lime to larval fishes and lobsters has not been studied, these organisms were evidently affected by particles of quicklime although not necessarily by filtered solutions of quicklime. Eggs and fry of winter flounder, *Pseudopleuronectes americanus* (Walbaum, 1792), survived in strong solutions of quicklime, filtered to remove particles, but not in solutions where they contacted solid particles (Loosanoff and Engle 1942). Similarly, Loosanoff and Engle (1942) found that direct contact with particles of quicklime was lethal to stage 1, 2 and 3 American lobster larvae. Stage 1 larvae, the most resistant, survived only 40 min in solutions with quicklime particles. In filtered 9% solution, larvae survived 4 h (Loosanoff and Engle 1942). As a result of this series of experiments, Loosanoff and Engle (1942) and Shumway et al. (1988) recommended that quicklime not be used when larvae of lobsters and flatfish were present in the water.

Acetic acid

Like hydrated lime, the addition of excessive acetic acid has the potential to alter pH, but the effect would be adverse with respect to the local acceleration of the acidification of sea water. Acetic acid and acetate, which are produced by the anaerobic decomposition of organic matter and as photosynthetic products, are always present in equilibrium in the water column and sediments (Wu et al. 1997; Spencer and Ksander 1995; Vichkovitten and Holmer 2005). At high concentrations, acetate inhibits microbial sulfate reduction, is toxic to the roots of vascular plants including eelgrass (*Zostera marina* Linnaeus, 1753, the major vascular plant and a keystone species of PEI estuaries), and can contribute to eutrophication (Koschorreck et al. 2004). Acetic acid causes leakiness of cell membranes and loss of ions from roots, and occlusions of cell membranes, resulting in reduced permeability to oxygen, accompanied by reduced uptake of water and nutrients. Inhibition of sprouting and growth is well known for freshwater vascular plants, as well as the saltmarsh common reed *Phragmites australis* (Cavanilles, 1799) which experienced effects at 52 mg/L acetic acid (Spencer and

Ksander 1995; Armstrong et al. 1996; Spencer and Ksander 1997; Spencer and Ksander 1999; Armstrong and Armstrong 2001).

The results of the Microtox test and field experiment on *Ciona* confirm previous indications that acetic acid is a more effective biocide than other acids adjusted to the same pH (e.g., Forrest et al. 2007; MacNair pers. comm.), thus the effects of the hydrogen ion alone do not explain its toxicity. One likely mechanism is the toxicity of the acetate ion. At pH<6.5, sodium acetate acts to uncouple cellular communication by disrupting the gap junction channels between cells (Germain and Anctil 1996). If tunicates were being managed with acetic acid, we predict that any toxic action of acetate would be limited to a small footprint and short time-frame around the treatment site, as a substantial quantity of acetic acid would be required to decrease the pH of estuarine water by almost two units from its normal pH to 6.5. While we do not expect that treatments with acetic acid would decrease the pH this far, we were not able to confirm this empirically because we could not locate any growers spraying acetic acid during our study.

As was the case with hydrated lime, organisms show a wide range of susceptibility to acetic acid. Acetic acid at 4-5%, the concentration used to treat tunicates in PEI, is lethal to macroalgae (*Cladophora* sp., *Undaria pinnatifida* (Harvey, 1873), spat and to a lesser extent adults of blue mussel, bryozoans, and polychaetes at exposures between 15 sec and 4 min (Figure 2; Sharp et al. 2006; Forrest et al. 2007). With dilution of the acetic acid the time required for a lethal exposure increases substantially; for example, all *Styela clava* died after 1 min immersion in 4% acetic acid, but the lethal exposure in 1% acetic acid was 10 min (Coutts and Forrest 2005). Exposure to 1% acetic acid inside wrapped pontoons for 12 hr caused almost complete mortality of most non-target taxa as well as *Styela clava*, but Pacific oysters *Crassostrea gigas* (Thunberg, 1793) and calcareous tubeworms *Pomatoceros terraenovae* Benham, 1927 survived the 12 hr treatment. (Coutts and Forrest 2005). One issue with the use of acetic acid to control tunicates on mussel aquaculture in PEI is its toxicity to the crop species, blue mussel (LeBlanc et al. 2007). Immersion in 5% acetic acid for only 30 s caused 74% reduction in mussel biomass, from the combined effects of mortality and loss of attachment. The species used in mussel aquaculture in New Zealand, the green mussel *Perna canaliculus* Gmelin, 1791, was less sensitive to acetic acid than blue mussel, with >88% attachment and >83% survival across a range of concentrations (1-8%) and exposure times (1-20 min) as long as mussels were rinsed after treatment (Forrest et al. 2007).

Fish are apparently able to detect and avoid acids at levels that do not cause short term harmful effects. For example, sand smelt, *Atherina boyeri* Risso, 1810, was able to detect changes of <1 pH unit and showed significant avoidance at pH 6.5-6.6 which was not yet toxic to the fish (Davies 1991). It is possible that, given the opportunity, the threespine sticklebacks we tested would have avoided acetic acid at toxic levels, which occurred at a pH between 4.2 and 7.0 (Table 4).

Management implications for invasive tunicate control

Hydrated lime and acetic acid are often perceived as environmentally safe chemicals for the management of invasive tunicates because they are natural products found in the environment, but this in turn means that anthropogenic additions have the potential to cause imbalances in natural processes. Both chemicals have the ability to alter pH, which is a key factor affecting the rates of many important biological and geochemical processes like photosynthesis, metabolic processes, sediment diagenesis, calcium carbonate sedimentation or dissolution (Schröder et al. 2005). Canadian water quality guidelines mandate that the pH of marine and estuarine waters should remain between 7.0-8.7 pH units (CCME 1999). Treatments used for tunicates in PEI appear unlikely to alter pH outside these limits (at the present or anticipated levels of use) except in the immediate vicinity of treatment sites and for short durations.

Treatments with natural chemicals such as hydrated lime and acetic acid are part of an emerging suite of approaches to the management of fouling tunicates. These treatments are important elements of the toolkit for management of bivalve aquaculture in the increasingly tunicate-infested environment of PEI and elsewhere in the world. Decisions about whether to use these treatments must balance the economic and potential environmental costs of treatment against the known consequences of unmanaged tunicate biofouling. The shift to use of pressure washing by many growers in PEI will reduce the

potential for impacts associated with chemical treatments, but may not be feasible in all estuaries. Specifically, pressure washing is not very effective against *Styela clava*, so chemical treatments may continue as the methods of choice in estuaries where this is the dominant tunicate.

Timing of treatments may need to take into account the seasonal cycles of potentially susceptible organisms such as the larvae of flatfishes and lobsters. While elevated pH resulting from hydrated lime treatments appears to be a short-term phenomenon confined to the immediate vicinity of the treatment, there remains the possibility that the same oceanographic features that retain planktonic larvae within the estuary (Hudon and Fradette 1993) could also retain water masses containing lime. In PEI, winter flounder larvae are unlikely to be exposed to hydrated lime, as larvae metamorphose by the end of June in nearby New Brunswick estuaries (Locke and Courtenay 1995). Larvae of other flatfishes, e.g., windowpane *Scophthalmus aquosus* (Mitchill, 1815), are found in estuaries until late September. Their susceptibility to hydrated lime is unknown. American lobster larvae are known to be intolerant of quicklime (Loosanoff and Engle 1942) and the susceptibility and likely degree of exposure of this important commercial species to hydrated lime is presently being investigated. Lobster larvae are distributed in coastal PEI waters from early or mid-June to mid-September (Harding et al. 1982; Scarratt 1964), but what proportion of the population occurs inside the estuaries is unknown. Only the larval stage is potentially susceptible to hydrated lime; Needler (1940) and Shumway et al. (1988) found that quicklime did not harm lobster juveniles and adults. Thus, protection of lobster stocks in the event that larvae are affected by hydrated lime and are found in significant numbers inside estuaries where lime is used may be as simple as scheduling tunicate treatments to start no earlier than mid-September. Land-based disposal of the hydrated lime remaining in the trough following treatment would substantially reduce the amounts entering the water column and any potential for non-target impacts on sensitive biota.

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