

**Hydrated Lime Application by the
PEI Aquaculture Industry**

Technical Report #253

Report prepared by:

Aaron Ramsay, Kim Gill, Allan Morrison & Neil M^{ac}Nair

**PEI Department of Fisheries, Aquaculture and Rural Development
Aquaculture Division
P.O. Box 1180
548 Main Street, Montague
Prince Edward Island
C0A 1R0**

August 2014

Acknowledgements

The authors would like to thank the mussel growers in March Water and Darnley Basin for their help with field sampling over the three years that the research was conducted. We would also like to thank Fisheries and Oceans Canada – Charlottetown for providing a beach seine to collect specimens for the project. In addition, we would like to thank Ken Doe (Environment Canada – Moncton) for providing his data on preliminary findings on the impact of hydrated lime on lobster larvae and Andrea Locke (Fisheries & Oceans Canada – Moncton) for providing her unpublished data on lobster larvae response to lime exposure.

Executive Summary

Hydrated lime (also known as slaked lime) is used in both the mussel and oyster aquaculture industries as a tool to control predators and fouling organisms that impact cultured shellfish. In some Island estuaries the use of hydrated lime has increased due to the need of mussel producers to reduce the fouling of the invasive tunicate, *Styela clava*, on their crop. Treatment with hydrated lime is an effective management strategy for mussel growers to utilize for the removal of *S. clava* from their cultured mussels and gear.

The Department of Fisheries, Aquaculture and Rural Development (DFARD), Fisheries and Oceans Canada (DFO) and Environment Canada (EC) have cooperated on a series of research and development projects to investigate potential impacts related to the use of lime. The potential impacts on water quality, the benthos and on non-target organisms were investigated and the findings are discussed within this report. Although this report is written by staff of DFARD, it also incorporates findings of investigations conducted by researchers from DFO and EC. The detailed results of the work completed by DFO and EC can be found by referring to Locke *et al.* (2009 & unpublished data; see references).

A variety of tests have been completed over multiple years and by multiple departments, at both the provincial and federal level. Testing has been conducted in both a controlled laboratory setting and an industry applicable field setting. The results show that the footprint of hydrated lime application is very small with respect to water quality and benthic impacts. Based on the results of this research, the use of hydrated lime by the aquaculture industry, at the current

application rate, has not shown to have a negative impact on water quality, the benthic environment or to non-target organisms.

Table of Contents

ACKNOWLEDGEMENTS	I
EXECUTIVE SUMMARY	II
TABLE OF CONTENTS	IV
LIST OF FIGURES	V
LIST OF TABLES	VI
INTRODUCTION.....	1
CHEMISTRY OF LIME.....	2
INDUSTRY APPLICATION AND USE	5
<i>MUSSEL INDUSTRY</i>	<i>5</i>
<i>OYSTER INDUSTRY.....</i>	<i>7</i>
FIELD-BASED EVALUATION OF THE EFFECTS OF HYDRATED LIME ON THE ENVIRONMENT.....	8
<i>NON-TARGET ORGANISMS</i>	<i>11</i>
<i>BENTHIC ENVIRONMENT</i>	<i>19</i>
LAB-BASED EVALUATION OF THE EFFECTS OF HYDRATED LIME ON THE ENVIRONMENT.....	20
<i>LC50 FOR NON-TARGET ORGANISMS</i>	<i>21</i>
<i>LOBSTER LARVAE BEHAVIOURAL RESPONSE</i>	<i>23</i>
DISCUSSION	24
REFERENCES.....	27

List of Figures

Figure 1. Mussel producers using a lime trough to treat mussel socks fouled with solitary tunicates.	2
Figure 2. The lime cycle.	3
Figure 3. Bags of hydrated lime (calcium hydroxide).	4
Figure 4. Hydrated lime in solution, in a mixing tank, onboard a mussel boat (<i>left</i>) and mussel socks being sprayed with a lime solution (<i>right</i>).	6
Figure 5. Lime spray system being used to control clubbed tunicate, <i>S. clava</i> , fouling on mussel socks.	6
Figure 6. Submerged bucket (with holes) filled with hydrated lime to simulate the slow release of hydrated lime.	9
Figure 7. DFARD staff measuring pH at varying distances from a liming operation. Clockwise from top left: tank with lime solution, 1 m away from application site, 3 m away from application site, and greater than 10 m away from application site.	11
Figure 8. Spraying the bottom and top halves of mussel socks fouled with the clubbed tunicate, <i>S. clava</i>	13
Figure 9. Sticklebacks held in PVC pipes.	15
Figure 10. Sticklebacks being placed into experimental cage (15 per cage, <i>left</i>) and sand shrimp being transferred to experimental cage (1 per cage, <i>right</i>).	17
Figure 11. Experimental animals being subjected to a hydrated lime solution from an aquaculture operation.	17
Figure 12. Mussel long-line with experimental units (sticklebacks and sand shrimp) attached, after exposure to lime treatment.	18
Figure 13. SCUBA diver in the water in the vicinity of a liming operation to qualitatively assess the impact of liming on the benthic environment.	20
Figure 14. Hydrated lime test solutions used in sand shrimp and lobster larvae tests (K Doe). .	21

List of Tables

Table 1. Results of exposure of various species to a suspension of hydrated lime applied to a cultured mussel line on November 4 th , 2008.	15
Table 2. Results of exposure of various species to a solution of hydrated lime applied to a cultured mussel line on November 27 th , 2008.	16
Table 3. Results of exposure of sticklebacks and sand shrimp to a solution of hydrated lime applied to a cultured mussel line on October 7 th , 2009.....	18
Table 4. Results of hydrated lime exposure tests on sticklebacks, sand shrimp and lobster larvae.	22

Introduction

Hydrated lime (also known as slaked lime) is used in both the mussel and oyster aquaculture industries for controlling predators and fouling organisms that impact the culture of shellfish. In the mussel industry, hydrated lime is used to control starfish and fouling tunicates on mussel collectors, mussel socks and aquaculture gear, such as buoys and backlines. Lime is very effective for controlling echinoderms (starfish). There is a long history of the use of lime in North America, particularly for the control of starfish on oyster beds (Wood, 1908; Needler, 1940; MacKenzie, 1977). In addition, hydrated lime has been used to remove starfish from mussel collectors on PEI since the beginning of the mussel aquaculture industry. Hydrated lime is also now used to effectively control invasive tunicates, including the clubbed tunicate, *Styela clava*, on mussel crop and gear (see Figure 1). Currently, the use of hydrated lime to control *C. intestinalis* fouling on mussel crop has been replaced by the use of high-pressure water, which is a more effective and efficient control treatment for *C. intestinalis*. However, hydrated lime is still used to control *C. intestinalis* on mussel collectors. Lime is still effectively used as a control agent in areas where the clubbed tunicate, *S. clava*, has an impact on mussel culture, as the high-pressure spray system is ineffective in controlling the clubbed tunicate. Hydrated lime is also used in the cultured oyster industry to reduce fouling organisms (*i.e.* bryozoans and sea grapes, *Mogula sp.*) and to remove starfish from spat collectors. Hydrated lime has also been used to remove the nuisance algae, *Codium fragile* (oyster thief), from oysters to prevent the algae from being introduced to unaffected areas. It is a general practice for oyster growers to treat their oyster seed with hydrated lime prior to transferring it to new areas to ensure that no unwanted fouling organisms are transferred with the oyster seed. A condition of the DFO contaminated oyster spat collection license and a condition commonly used on DFO Introduction and Transfer

licenses requires immersion in hydrated lime prior to transfer to new water bodies. Processors commonly treat shellfish with hydrated lime prior to transfer to their own leases. Lime treatment minimizes the risk of unwanted pests and predators being moved with the oysters when they are relayed during the spring contaminated fishery to clean areas for cleansing.



Figure 1. Mussel producers using a lime trough to treat mussel socks fouled with solitary tunicates.

Chemistry of Lime

There are two main types of lime that are utilized to control aquatic pests and predators in North America; quicklime, or calcium oxide (CaO) and hydrated lime, or calcium hydroxide (Ca(OH)_2). Both of these substances are by-products that result when limestone or calcium carbonate (CaCO_3) is heated to very high temperatures. Limestone is a naturally occurring sedimentary rock that consists of calcium and/or magnesium carbonate, and/or dolomite, and small amounts of other minerals. When limestone is heated to temperatures greater than 1000 °C

in special ovens (lime kilns), the limestone loses carbon dioxide (CO_2) and is converted to quicklime. Hydrated lime is created by adding water to the quicklime. When hydrated lime comes in contact with carbon dioxide, either in the atmosphere or in water, it reverts back to limestone. The process is displayed graphically below in Figure 2.

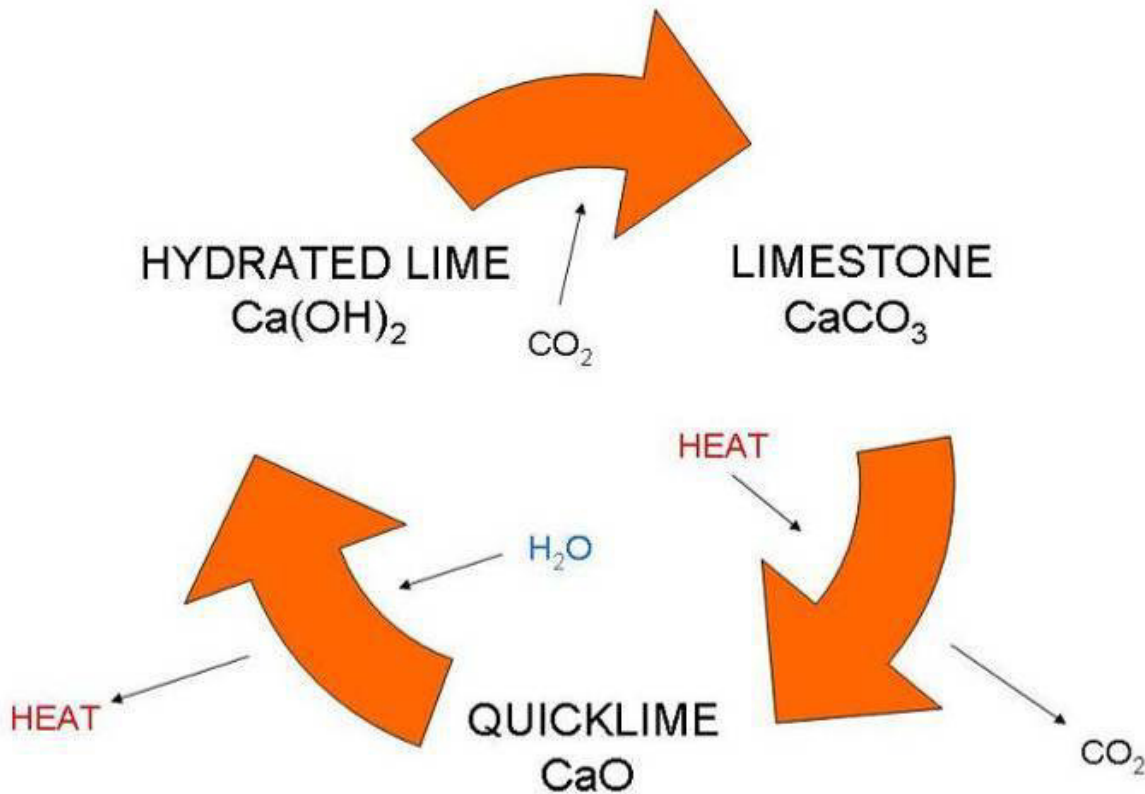


Figure 2. The lime cycle.

The process of burning limestone to produce quicklime and hydrated lime has been practiced for centuries. Numerous factors can affect the quality of the quick or hydrated lime, ranging from the temperature of the kilns to natural impurities in the limestone. It is important to note that the main component of bivalve shells is limestone. Hydrated lime is more stable than quicklime; therefore, quicklime is often hydrated with water to form hydrated lime, to allow for ease of storage. Hydration with water increases the weight by 25.3 %. Hydrated lime is shipped as a

fine powder in paper bags and will convert back to limestone within the bags when carbon dioxide in the atmosphere passes through the bag and contacts the lime (Figure 3). As a result, there is a shelf life for hydrated lime. Industry experience has shown that the effectiveness of hydrated lime is reduced as a control agent if too much of the lime has been converted back to limestone before use.



Figure 3. Bags of hydrated lime (calcium hydroxide).

In the PEI aquaculture industry, hydrated lime is mixed with seawater to create a suspension at an approximate concentration of 4%. This is the equivalent of 40 g of hydrated lime in 960 mL (for practicality, 1 L is used for measurement) of seawater. The lime/seawater suspension is highly alkaline with a pH of approximately 12.7 (pH of a saturated solution should be between 12.3 and 12.8 according to the National Lime Association, 2007)

Industry Application and Use

Mussel Industry

In the mussel industry, the hydrated lime suspension is applied as either an immersion treatment or by spraying it onto crop and gear. For the immersion method, mussel seed collectors and grow-out socks are immersed in large troughs that are attached to the side of mussel boats. The troughs are filled with seawater (see Figure 1) and the hydrated lime is added to the water to make the suspension. Mussel seed collectors are immersed in the trough for approximately 30 seconds (typically for removal of starfish and tunicate fouling). Though not a common industry practice anymore, mussel socks can be immersed for 1 to 2 minutes in the lime solution to cause tunicate mortality.

The spray method is utilized primarily on grow-out mussel socks and is currently the standard industry method used for the control of *S. clava* fouling. The lime/seawater suspension is mixed in large tanks (see Figure 4) and pumped (approximately 50 L/min) through a garden hose. The suspension is lightly sprayed on the socks as they are lifted from the water (approximately 20 seconds air dry prior to being sprayed with lime solution) using a crane and hauler system. After being sprayed, the socks are slowly re-immersed into the water, allowing for an approximate 45 second air exposure. Buoys are also treated at the same time as the socks are being sprayed. Typically, the buoys are removed, immersed in a hydrated lime solution, and air dried in the boat for later use. The air exposure following lime application is an important step in the process and is required to ensure high tunicate mortality. This activity typically occurs in late July, six weeks after tunicate larvae are identified in water samples collected and analyzed by FARDs Mussel Monitoring Program staff. This information is reported to industry on a weekly basis.

For the immersion of mussel socks, one to two bags (50 lbs/bag) are used per 600 ft line (approximately 400 socks). It takes about an hour to treat one line. At this rate, growers can treat from six to ten lines per day using a total of 450 to 1000 lbs of lime over the work day (average = 600 lbs per day). Similar application rates are observed with growers using a spray application.



Figure 4. Hydrated lime in solution, in a mixing tank, onboard a mussel boat (*left*) and mussel socks being sprayed with a lime solution (*right*).

Over the last several years, new systems for the application of lime spray have been developed. Many mussel growers are now using low-pressure spray systems to control the clubbed tunicate fouling on their mussel crop (Figure 5). The main components are a mixing tank, a booth with multiple low-pressure nozzles (that hangs over the side of the boat) and a recovery system (for the collection of unused lime solution). The amount of lime being applied is more controlled with these systems.



Figure 5. Lime spray system being used to control clubbed tunicate, *S. clava*, fouling on mussel socks.

Oyster Industry

In the United States, there is a long history of the use of quicklime in the oyster industry. Quicklime was used continuously for 40 years in the Connecticut oyster industry to control starfish (Shumway, 1988). It has been applied to the oyster beds on the substrate at a rate of 1500 to 2000 lbs/acre. Quicklime has also been used to control sea urchins in waters off the west coast of the US (Bernstein and Welsford, 1982).

In the 1980's, quicklime was experimented with in PEI in trials designed to reduce starfish populations on wild oyster beds in the Summerside Harbour area, where there is an important oyster fishery. The lime was spread at a rate of 2000 lbs/acre, over a 3-6 acre area. This method was similar to the methods that were being used routinely to control starfish populations on oyster beds in Chesapeake Bay (MacKenzie, 1977; Needler, 1940). The quicklime applications were completed in less than an hour at slack tide. Diver surveys were conducted on the application area immediately following the application to make observations on any impacts as a result of the lime application. Divers observed that mussels on the bottom were filtering and sea lettuce and other algae species did not appear to be affected by the quicklime. Starfish that came in contact with several particles of lime were killed, although those starfish that were protected by seaweed or shell were not affected.

Currently, the PEI oyster industry utilizes hydrated lime/seawater suspensions to treat pests and predators on oyster collectors using the immersion technique. Collectors are dipped in the lime/seawater suspension to remove fouling organisms and predators such as bryozoans, algae,

sea squirts (*i.e.* *S. clava*, *Molgula sp.*) and starfish. Collectors are often treated shortly after oyster larvae have set and repeat treatments may be applied, if required, to reduce fouling.

Field-Based Evaluation of the Effects of Hydrated Lime on the Environment

The Department of Fisheries, Aquaculture and Rural Development has conducted numerous trials to test the efficacy of hydrated lime to control tunicate fouling. Hydrated lime was found to be very effective for treatment of the clubbed tunicate, *S. clava*. Since 2007, specific trials using hydrated lime have been conducted in order to affirm its use by the industry and support the theory that lime has limited impact outside its intended purpose. Lime quickly converts to the inert CaCO_3 (limestone) when it comes in contact with the CO_2 in the seawater. Studies have been conducted on water quality and the footprint related to the release of lime into the water column during the application of a treatment. Studies of the effect on non-target organisms and the potential of the impact of lime on the benthos were also conducted.

Impact to Water Quality

The conversion process of hydrated lime to calcium carbonate (CaCO_3) and the footprint of potential impact from hydrated lime were monitored. Both the duration of time that it took the treated water to return to a normal pH and distance at which the pH of the ambient water returned to normal were monitored. A study was developed in which hydrated lime was slowly released into the water column using a bucket and current flow (see Figure 6). The bucket, with holes placed in the bottom, was filled with powdered hydrated lime. It was aligned in the water column in a vertical position that allowed the water current to flow through the mouth of the bucket and out the holes in the bottom. Small amounts of lime were dispersed into the water

column behind the bucket, simulating a treatment scenario where there is a small but constant release of lime into the water column. The pH measurements were taken of the ambient water (pH = 7.9 - 8.3), behind the bucket and into the current, as the cloud of lime and calcium carbonate dissociated with seawater. The pH of the water mixing with the lime was 12.5 directly adjacent to the bottom of the bucket where the lime was being released, and the pH levels quickly decreased to 8.4 - 8.7 just three meters away from the area where the lime was being released.

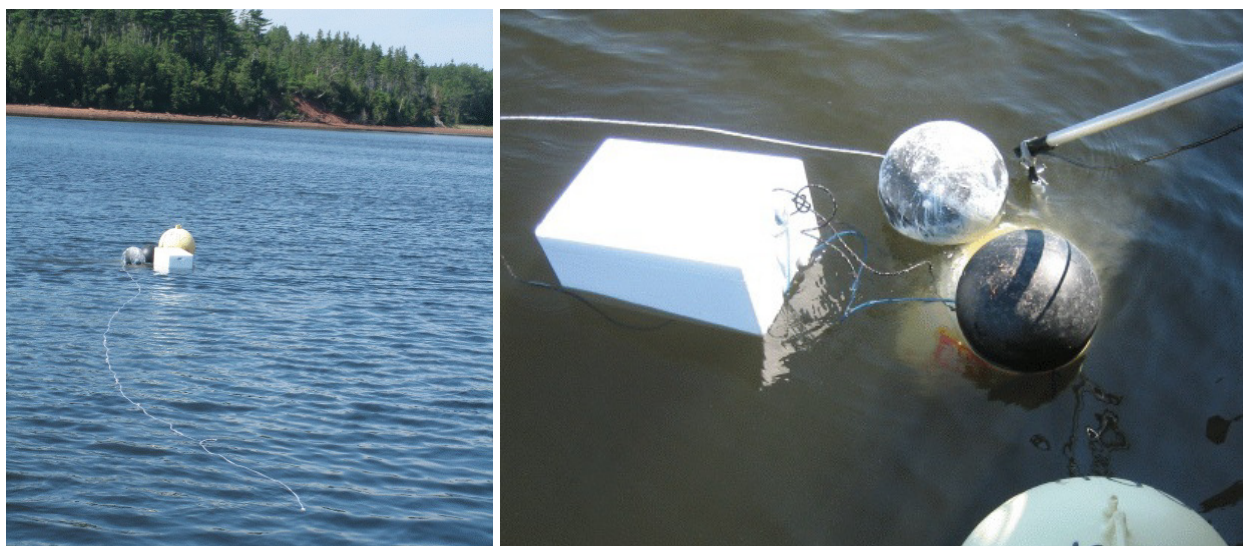


Figure 6. Submerged bucket (with holes) filled with hydrated lime to simulate the slow release of hydrated lime.

In 2007, trials were conducted on potential impact from the use of hydrated lime on mussel socks that was applied by the immersion technique utilizing a treatment trough. The purpose of these trials was to determine the effect of the lime suspension on the pH levels of the adjacent water during treatment. According to the literature, seawater has a pH between 7.8 and 8.3 (Sverdrup *et al.*, 1942; Pelejero *et al.*, 2005). The seawater in Murray River, at the location where the trial was conducted, had a pH of 8.1. The suspension of hydrated lime in the treatment trough had a pH of 12.6. The seawater immediately outside the treatment trough, within the plume of lime

that dissociated from the trough and from the harvest socks, had a pH as high as 9.0. Water approximately 10 m from the trough, directly above the treated line, had a pH between 8.2 – 8.3. The readings dropped rapidly to 8.1 in areas adjacent to the treated line.

In the fall of 2008, staff from DFARD measured the pH of the water around boats that were spraying mussel socks with hydrated lime solutions in Malpeque Bay. The pH readings of the ambient water adjacent to a boat treating mussel socks on September 11th showed much variation. The pH of the seawater prior to the lime application was 8.1 with a surface water temperature of 18.2 °C. The pH readings in and around the white plume of lime that was disassociating from the treated socks and drifting from the boat had a pH range of 8.25 to 9.2. In a second observation trial conducted on November 4th, the pH of the hydrated lime suspension in the mixing tank was 13.1. The pH of the water prior to treatment was 8.23 and the surface water temperature was 5.8 °C. pH readings adjacent to the boat fluctuated widely depending on depth and location, from 8.20 to a high of 9.63, recorded in one location directly below where the hydrated lime solution was sprayed on the mussel socks. Readings were greatly reduced within two metres of the area where the lime was being applied.

Similar studies were conducted again, in October 2009, adjacent to a lime treatment operation in Darnley Basin. Measurements of the pH of the ambient water were conducted and were found to be 7.43 several hundred metres from the liming site. The pH readings ranged from 7.5 to 9.6 in the vicinity of the cloud of hydrated lime suspension drifting from the vessel (the 9.6 reading was only observed in a small localized area directly under where the lime was sprayed on the surface of the water). The pH readings taken 8 to 15 m away from the spraying activity, within

the cloud of lime particles, ranged from 7.4 to 7.7. The lime solution in the tank had a pH reading of 12.3 (see Figure 7). The pH of the lime in suspension in the trough remains high because there is only a limited amount of dissolved carbon dioxide in seawater within the treatment trough, which quickly becomes utilized in the conversion of hydrated lime to calcium carbonate.



Figure 7. DFARD staff measuring pH at varying distances from a liming operation. Clockwise from top left: tank with lime solution, 1 m away from application site, 3 m away from application site, and greater than 10 m away from application site.

Non-Target Organisms

In 2007, an observation trial was conducted to evaluate the effect of the hydrated lime suspension on non-target organisms where the hydrated lime was applied by the immersion

technique utilizing treatment troughs. While the socks are being immersed and pulled through the treatment trough, the organisms that are on the socks also are immersed in the lime suspension. Several green crabs, *Carcinus maenas*, were removed from the socks after they had been in the trough for two minutes. The crabs were then held in cages at the site and monitored weekly for several weeks to determine whether the lime affected the crabs. All of the green crabs survived the exposure to the lime suspension. Observations were also made on other species that came off the mussel sock and were immersed in the lime suspension in the trough. Small fish species, such as cunners, *Tautogolabrus adspersus*; rock gunnels, *Pholis gunnellus* and sculpins, *Myoxocephalus sp.* did not survive the long-term exposure to the hydrated lime suspension. As these fish fall off the sock and tend to remain in the trough they have long exposure times. The crabs were capable of holding onto the socks and exit with the sock and are then re-submerged in ambient sea water. It should be noted that most of the mobile fish species leave the sock as it is being pulled up and out of the water, before it enters the trough. Only a very small number remain on the sock and enter the lime trough. The mortality rate of tunicates fouling the socks was also studied and was found to be approximately 80 % following the immersion treatment.

In 2008, the impact of hydrated lime treatment on various non-target marine organisms normally found in Malpeque Bay was assessed in two different trials where hydrated lime was applied utilizing the spray technique. The trial was conducted with industry, utilizing industry methods and application techniques. The first treatment trial was conducted on November 4th, using the following species: rock crab, *Cancer irroratus*; common and purple starfish, *Asterias sp.*; sand shrimp, *Crangon septemspinosa*; mummichog, *Fundulus heteroclitus* and rockweed,

Ascophyllum nodosum. On November 27th, a second trial was carried out using *C. septemspinosus*; stickleback, *Gasterosteus* sp.; blue mussel, *Mytilus edulis* and eastern oyster, *Crassostrea virginica*. For both treatment trials, the hydrated lime was mixed with seawater to form a lime suspension in a 1000 litre tank equipped with a mechanical agitator. Two 22.7 kg (50 lb) bags of hydrated lime (Limo) were added to the tank of seawater (approximately 800 litres). The mussel line was lifted out of the water with a hydraulic boom and the long-line was placed on a starwheel near the stern of the vessel. The disk hauler on the end of the boom was moved forward along the side of the vessel toward the bow to lift the mussel socks out of the water near the middle of the boat. Two people sprayed the mussel socks with the hydrated lime suspension as the boat moved along the line (Figure 8). One person sprayed the bottom half of the sock first, since it would be out of the water for a shorter time period than the top portion, and was followed by the second person, who sprayed the long-line and the top of the socks.



Figure 8. Spraying the bottom and top halves of mussel socks fouled with the clubbed tunicate, *S. clava*.

In the first trial (November 4), the starfish, sand shrimp and mummichogs were placed in oyster bags (4 mm mesh size; 88 cm long, 45 cm wide, 8 cm high). The rock crabs were placed in plastic coated wire cages (12 mm mesh size; 44 cm long, 39 cm wide, 8 cm high). All the species being utilized in the trial were collected on October 24th and held in cages on a mussel long-line. They were provided with crushed mussels as a source of food prior to the trial to ensure there were no unrelated mortalities. On November 4, the cages were collected and held in tanks of seawater aboard the boat until they were suspended from the treated mussel line. The control cages were also held aboard the boat in tanks of water for the same time period as the treated cages. Additional crushed mussels were placed in each cage at this time to provide a food source for the remainder of the trial. The treatment cages were placed in the water so that they were directly in the “cloud” of hydrated lime that was disassociating from the treated mussel socks into the surrounding seawater. The cages holding the various species were tied onto the treated long-line just after the socks were sprayed. Measurements (pH) were taken around the vessel during the application (see *Impact to Water Quality* section). On November 10th, six days following the treatment application, the organisms in each cage were examined and counted. They were then returned to the cages and re-examined and counted on November 18, fourteen days after the initial treatment. Some of the sand shrimp and the mummichogs had escaped from their cages. It is possible that wave action may have allowed the smaller animals to escape through the folds in the ends of the bags. As a result, the sand shrimp and mummichog were excluded from the analysis (see Table 1). New cages, with an improved enclosure design, were constructed for the second, follow-up trial.

Table 1. Results of exposure of various species to a suspension of hydrated lime applied to a cultured mussel line on November 4th, 2008.

Treatment	# of Experimental Units	Mortality (%) <i>Nov 10</i>	Mortality (%) <i>Nov 18</i>
Rock Crab			
<i>Treated</i>	18	0	11.1
<i>Control</i>	18	0	0
Starfish			
<i>Treated</i>	30	0	0
<i>Control</i>	30	0	0
<i>Adjacent to Tx line</i>	60	0	2.0
Rockweed			
<i>Treated</i>	3-6 plants	Healthy	Healthy
<i>Control</i>	3-6 plants	Healthy	Healthy

The second treatment trial was conducted on November 27, using cages with an improved enclosure design and a larger number of organisms per cage, than was used in the initial trial. Also, the various species used in this trial were placed in the cages the day of the trial or the day prior to the treatment trial. The non-target species used in the second trial were sand shrimp, sticklebacks, mussels and oysters. The sand shrimp were placed in plastic mesh bags (20 cm long, 11 cm wide, 8 cm high). The sticklebacks were placed in a cage made from a 20 cm length of 6 inch (15.2 cm) diameter PVC pipe that was sealed at both ends with 2 mm mesh netting (Figure 9).

**Figure 9. Sticklebacks held in PVC pipes.**

The mussels and oysters were placed in oyster bags (4 mm mesh size; 88 cm long, 45 cm wide, 8 cm high). This trial used the same treatment procedure that was used in the November 4th trial. Following the treatment on November 27, the cages were left out until December 5, when they were retrieved and the results analyzed (see Table 2 for results).

Table 2. Results of exposure of various species to a solution of hydrated lime applied to a cultured mussel line on November 27th, 2008.

Treatment	# of Experimental Units	Mortality (%)
Sand Shrimp		
<i>Treated</i>	240	33.0
<i>Control</i>	240	37.5
Stickleback		
<i>Treated</i>	45	0
<i>Control</i>	45	0
Mussel		
<i>Treated</i>	40	0
<i>Control</i>	40	0
Oyster		
<i>Treatment</i>	40	0
<i>Control</i>	40	0

On October 7th, 2009 another trial to expose sand shrimp and sticklebacks to a hydrated lime suspension was initiated. Arrangements were made with a grower in Darnley Basin to carry out this trial while they were treating their mussels and gear with lime to control clubbed tunicates. The sand shrimp and sticklebacks were collected from the East River adjacent to Glenfinnan Island using a beach seine the day prior to the treatment trials (October 6th, 2009). These organisms were held in containers of seawater until the treatment trials were carried out on the following day. The shrimp and sticklebacks were placed in cages before being attached to the mussel line, one half for control (not exposed to lime) and the remaining half for treatment.

Individual shrimp were placed in cages made from a 4 in. (10.2 cm) section of 4 in. (10.2 cm) diameter PVC pipe. The sticklebacks were placed in cages made from 8 in. (20.3 cm) section of 6 in. (15.2 cm) diameter PVC pipe. Each cage held 15 sticklebacks (see Figure 10).



Figure 10. Sticklebacks being placed into experimental cage (15 per cage, *left*) and sand shrimp being transferred to experimental cage (1 per cage, *right*).

Both ends of the tubing were covered with a mesh material. The cages containing the control organisms were tied on a mussel long line several hundred meters away from the lime treatment area. The remaining cages holding the shrimp and the sticklebacks were dropped into the lime cloud directly under the long-line (see Figure 11).



Figure 11. Experimental animals being subjected to a hydrated lime solution from an aquaculture operation.

These cages were tied to the mussel long-line and were air exposed for approximately 1 minute, which is consistent with industry practice, before re-entering the water (see Figure 12). Two days following the treatment the cages were examined and on October 20th, 2009 all the cages were collected and the number of live shrimp and sticklebacks recorded (see Table 3).



Figure 12. Mussel long-line with experimental units (sticklebacks and sand shrimp) attached, after exposure to lime treatment.

Table 3. Results of exposure of sticklebacks and sand shrimp to a solution of hydrated lime applied to a cultured mussel line on October 7th, 2009.

Treatment	# of Experimental Units	Mortality (%) Oct 9	Mortality (%) Oct 20
Stickleback			
Treated	45	0	0
Control	45	0	0
Sand Shrimp			
Treated	20	0	0
Control	20	0	0

Benthic Environment

On October 7th, 2009 a dive survey was conducted in Darnley Basin while mussel socks infested with clubbed tunicate were treated with a hydrated lime solution. Near the area of treatment application the divers observed the lime in solution and described it as appearing as snow falling underwater. This is assumed to be the hydrated lime reacting with the carbon dioxide in the water to revert the chemical back to flakes of calcium carbonate or inert limestone. The divers were tasked with giving a qualitative assessment of the impacts of hydrated lime usage on the benthos. Directly below the area of liming several fish species were observed and appeared to be unaware of the lime application above them. Small crabs and other crustaceans were observed on the bottom substrate. After several days of liming in this bay, there was no evidence that limestone was accumulating in the bottom sediments. The sediments were a brownish-red colouration, typical of PEI rivers and estuaries.

Similar observations were made by divers in Murray River where hydrated lime was released from a lime trough and the “lime cloud” was followed by divers to observe the reaction of animals on submerged mussel long-lines and on the bottom that came directly in contact with the limestone particles. At the surface, where the hydrated lime was entering the water column, the divers observed the small particles of lime changing to large flakes that were described as similar to snow underwater. The divers observed, and videoed, the flakes becoming directly in contact with a variety of organisms including fish species, crabs and tunicates, all which were apparently unaware of the presence of limestone flakes, and continued to feed undisturbed. Once the limestone flakes reached the bottom, they dissociated into the sediments leaving no evidence of their presence. Despite the liming treatments that had recently occurred in the area, there was no

lime build up on the bottom observed by the divers. Additionally, it should be noted that in the many years of underwater observations in mussel growing areas department staff have never observed lime deposits on the substrate.



Figure 13. SCUBA diver in the water in the vicinity of a liming operation to qualitatively assess the impact of liming on the benthic environment.

Lab-Based Evaluation of the Effects of Hydrated Lime on the Environment

In 2008 and 2009, laboratory-based trials were conducted by DFO and EC to compliment the findings from the field-based trials conducted by DFARD. These trials were completed on sand shrimp, *C. septemspinosa*, and threespine stickleback, *Gasterosteus aculeatus*, as well as lobster (*Homarus americanus*) larvae. Organisms were exposed to a variety of hydrated lime concentrations and multiple exposures. Researchers calculated LC50's (concentration required to cause 50% mortality) for each of the species and exposure types. In addition, behavioural responses to hydrated lime exposure were documented. The methods and results of these studies are summarized here; however, additional information can be found in Locke *et al.*, 2009; Locke (*unpublished data*) and Reeb *et al.*, 2011.

LC50 for Non-Target Organisms

Threespine stickleback were exposed to several concentrations of hydrated lime solution (32, 100, 320, 1000 and 3200 mg/L) and to a seawater control for a 96 hr exposure period. Ten fish were introduced into each of the test concentrations and the seawater control. Water quality (temperature, dissolved oxygen, salinity and pH) was routinely measured to ensure accuracy of results. The experiment was routinely checked for dead fish, with any dead fish being removed from the experiment. The results of this experiment indicate that 50 % mortality of sticklebacks can be expected when exposed to a pH of 10.47 for 96 hrs (see Table 4).

Sand shrimp, which are similar to lobster larvae, were exposed to several concentrations of hydrated lime solution (5, 50, 500, 5000 and 50000 mg/L; see Figure 14) and a seawater control for a 96 hr exposure period. One sand shrimp was placed in each of 10 replicate 1 L mason jars for each concentration. Again, water quality was measured and dead sand shrimp were removed from experiment when observed.

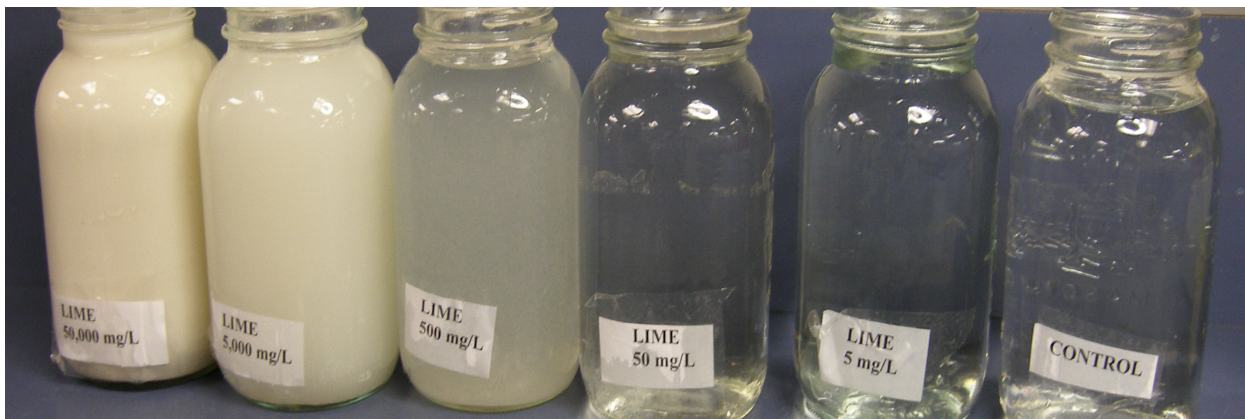


Figure 14. Hydrated lime test solutions used in sand shrimp and lobster larvae tests (K Doe).

The results of this experiment indicate that 50 % mortality of sand shrimp can be expected when exposed to a pH of 9.7 for 96 hrs (see Table 4). An additional trial was conducted on sand shrimp using a 14 day exposure. Twenty sand shrimp were exposed to each of the following concentrations; 3.2, 10, 32, 100 and 320 mg/L. The results of this experiment indicate that 50 % mortality of sand shrimp can be expected when exposed to a pH of 9.2 for 14 days (see Table 4). Lobster larvae (Stage III) were exposed to several concentrations of hydrated lime (5, 50, 500, 5000 and 50000 mg/L; see Figure 14) and a seawater control for each of the following three exposure periods; (1) 96 hrs, (2) 1 hr pulse and (3) 1 hr pulse on three consecutive days. In the pulse exposure trials the experiment lasted a total of 12 days (including exposure period). A total of 20 replicates were used for each concentration in each of the three exposure trials. The results of this experiment indicate that 50 % mortality of lobster larvae can be expected when exposed to one of the following conditions: a pH of 9.73 for 96 hrs, a pH of 10.6 for 1 hr or a pH of 10.5 for 1 hr on three consecutive days (see Table 4).

Table 4. Results of hydrated lime exposure tests on sticklebacks, sand shrimp and lobster larvae.

Species	Exposure	Median Effective Concentration *		No Effect Concentration **	
		Concentration (mg/L)	pH	Concentration (mg/L)	pH
<i>Stickleback</i>	96 hr	457	10.47	100	9.54
<i>Sand Shrimp</i>	96 hr	158	9.7	50	8.58
	14 days	53.1	9.2	32	8.17
<i>Lobster Larvae</i>	96 hr	121	9.73	N/A	N/A
	1 hr	965	10.6	N/A	N/A
	3 * 1 hr	606	10.5	N/A	N/A

* Concentration required to cause mortality in 50% of the test subjects (LC50).

** Highest concentration that will cause 0% mortality in the test subjects.

Lobster Larvae Behavioural Response

Several experiments were conducted to examine the impact of various hydrated lime concentrations and exposures on lobster larvae (stage IV) in 2008 and 2009 (A. Locke, *unpublished data*). The goal of the experiments was to determine whether stage IV lobster larvae exhibit a tail flick when exposed to hydrated lime solutions. The tail flick is a typical response when they encounter a situation that could be potentially hazardous.

The five relevant experimental groups (and their sample size) included: control (10), CaCO_3 5 mg/L (10) and 50 mg/L (9), hydrated lime 5 mg/L (9) and 50 mg/L (9). The observation period consisted of two consecutive 5 min periods immediately before the injection and three consecutive 5-min periods after. There was no increase in tail flicks following the injection of control seawater, CaCO_3 5 mg/L, CaCO_3 50 mg/L and hydrated lime 5 mg/L. The larvae increased their tail flick rates in response to the hydrated lime 50 mg/L. The reaction was strong during the first five minutes following the injection, but subsided back to control levels thereafter. Almost all tail flicks took place at or near the bottom of the jars. Since the larvae did not react to CaCO_3 , even at 50 mg/L, suggests that the tail flick increase observed in the high concentration hydrated lime group is due to the caustic nature of the particles encountered, not to their particulate form.

The final experiment investigated the affect of hydrated lime on lobster larvae settling behaviour. The goal of the experiment was to determine, in the laboratory, whether stage IV lobster larvae avoid areas that have been exposed to lime. A series of aquaria were set-up that gave lobster larvae the option of either settling on an area that had been limed versus an area that was only

sand-covered. The position and behaviour of the larvae were noted at 24 and 48 hours. The hydrated lime injections left a carpet of white flocculent material over the sand of the limed areas. The larvae did not avoid this residue, as multiple walking tracks could be seen on the flocculent carpets 24 hours post injection. Twenty-four hours post-injection, the larvae did not tend to settle away from the injected side in either the control, CaCO_3 50 mg/L, CaCO_3 500 mg/L and hydrated lime 500 mg/L. They did settle away from the hydrated lime 50 mg/L. Forty-eight hours post injection, no significant avoidance of the limed (hydrated lime) area was observed.

Discussion

A variety of tests have been completed over multiple years and by multiple departments, at both the provincial and federal level. Testing has been conducted in a controlled laboratory setting and an industry applicable field setting. The results show that the footprint of hydrated lime application is very small with respect to water quality and benthic impacts. The pH of the hydrated lime solution in mixing tanks on the boats is greater than 12, but during application it is rapidly reduced to ambient levels in the environment, even as close as 1m away from the application site. Dive surveys conducted beneath lime applications observed no adverse effects. Benthic organisms were undisturbed and there was no evidence that there was an accumulation of limestone on the bottom sediments.

In the field studies conducted by DFARD, sticklebacks, sand shrimp, rock crabs, rock weed, starfish, mussels and oysters were all subjected to hydrated lime suspensions under realistic conditions and exposures. In all cases there was either no mortality or comparable mortality to

the control subjects. The laboratory studies by DFO and EC showed that pH greater than 9 would result in 50 % mortality of sticklebacks, sand shrimp and lobster larvae, but exposure was required for a considerable amount of time (96 hrs). The water quality results show that the pH rapidly decreases from 12 to less than 9 within the first metre of the application site; therefore, hydrated lime application is not expected to cause any significant harm to non-target organisms. Hydrated lime converts to limestone quite rapidly (within several minutes) and laboratory studies used exposures of 1 hr, 96 hrs and 14 days.

Locke *et al.* (2009) speculate that at the ecosystem level, hydrated lime addition to PEI estuaries may have two positive consequences. Firstly, it may counter the acidification of ocean waters. Within the past two centuries, surface waters of the world have experienced a pH reduction of about 0.1 units. The researchers hypothesize that the application of hydrated lime may be locally beneficial, in this respect. Secondly, hydrated lime addition may improve water quality in eutrophic systems. Raymond *et al.* (2002) indicate that hydrated lime may improve water quality in estuaries that experience anaerobic events (not a common occurrence in mussel growing areas) due to excess nutrient levels, which have become common in PEI over the past few decades.

In conclusion, the use of hydrated lime by the aquaculture industry, at the current application rate, has not shown to have a negative impact on water quality, the benthic environment or to non-target organisms. With more research and development an alternate treatment for pests and predators may be developed; however, hydrated lime application is currently the only solution to cause mortality of the clubbed tunicate, *S. clava*. This information presented in this report

should be reassuring to all stakeholders of the minimal impact of hydrated lime on the environment and there are no concerns regarding its use by the aquaculture industry.

References

- Bernstein BB, Welsford RW (1982) An assessment of feasibility of using high-calcium quicklime as an experimental tool for research into kelp bed/sea urchin ecosystems in Nova Scotia. Canadian Technical Report of Fisheries and Aquatic Sciences 968.
- Doe K, Locke A, Fairchild W, Reese S (2009) Toxicity of hydrated lime suspensions to larval american lobster: Preliminary results from 2008. Presentation at the Technical Briefing on Use of Hydrated Lime to Control Fouling of Cultured Mussels and Oysters. March 13, 2009. Charlottetown, PEI, Canada.
- Locke A. Response of lobster larvae to lime exposure. *Unpublished data*.
- Locke A, Doe KG, Fairchild WL, Jackman PM, Reese EJ (2009) Preliminary evaluation of effects of invasive tunicate management with acetic acid and calcium hydroxide on non-target marine organisms in Prince Edward Island, Canada. *Aquatic Invasions* 4 (1): 221-236.
- MacKenzie CL (1977) Use of quicklime to increase oyster seed production. *Aquaculture* 10: 45-51.
- National Lime Association (2007) Lime fact sheet: Lime terminology, standards and properties. January 2007. 10p. <http://www.lime.org>.
- Needler, AWH (1940) Use of quicklime for killing starfish on oyster grounds. Fisheries Research Board of Canada, Ottawa. Oyster Farming Circular 40-FRB-OFC11.
- Pelejero C, Calvo E, McCulloch MT, Marshall JF, Gagan MK, Lough JM, Opdyke BN (2005) Preindustrial to modern interdecadal variability in coral reef pH. *Science* 309: 2204-2206.
- Raymond BG, Crane CS, Cairns DK (2002) Nutrient and chlorophyll trends in Prince Edward Island estuaries. In: Cairns DK (ed) Effects of land use practices on fish, shellfish and their habitats on Prince Edward Island. Canadian Technical Report of Fisheries and Aquatic Sciences 2408: 142-153.
- Reebs SG, Jackman PM, Locke A, Fairchild WL (2011) Avoidance by sand shrimp, *Crangon septemspinosa*, of sandy patches covered by hydrated lime (calcium hydroxide) deposits. Can. Tech. Rep. Fish. Aquat. Sci. 2938: iv + 7 p.
- Shumway SE, Card D, Getchell R, Newell C (1988) Effects of calcium oxide (quicklime) on non-target organisms in mussel beds. *Bulletin of Environmental Contamination and Toxicology* 40: 503-509.
- Sverdrup HU, Johnson MW, Fleming RH (1942) The oceans, their physics, chemistry and general biology. Prentice-Hall, NY.

Wood FB (1908) Enemies and perils of the oyster. Connecticut Shell-Fishery Commission, Report 1907-1908, Document 30, Appendix: 94-98.