Aqua Info
AQUACULTURE NOTES

AIN 28.2022

# Lime and Brine Treatment to Control the Spread of the Vase Tunicate, *Ciona intestinalis*

## Background

The use of hydrated lime and brine solutions have successfully been used for controlling predators and fouling organisms by the aquaculture industry in Prince Edward Island for many years. Currently, it is a requirement under a federal condition of licence to treat oysters with a 4% hydrated lime and 30% salt or brine mixture prior to transferring them from an area with clubbed tunicates, *Styela clava*, to an area without that species to minimize the risk of unintentional spread. Over the past couple of years, another tunicate species, the vase tunicate, *Ciona intestinalis*, has been detected in several new areas throughout PEI, including oyster seed collection and grow-out areas. At present, shellfish are not permitted to be transferred from areas where the vase tunicate (Figure 1) is present to areas without that species. Currently, there is no accepted treatment for oysters to minimize the risk of unintentional spread of the vase tunicate. A trial was setup and completed in the fall of 2021 to confirm that the current recommended treatment for the clubbed tunicate would also be effective on the vase tunicate for the purpose of movement of oysters.

**Figure 1. The vase tunicate underwater.**

## Methods

Seven treatment groups, including an untreated control group, were assessed in this trial. The immersion duration and dry times were selected based upon previous recommendation for the treatment for oysters coming from a clubbed tunicate area.

| | Immersion Time* | Dry Time |
|---|---|---|
| **1** | 0 | 0 |
| **2** | 30 seconds | 0 |
| **3** | 30 seconds | 30 minutes |
| **4** | 30 seconds | 1 hour |
| **5** | 1 minute | 0 |
| **6** | 1 minute | 30 minutes |
| **7** | 1 minute | 1 hour |

* 4% hydrated lime and 30% salt solution.

Vase tunicates (average length of 57 mm, range of 29-75mm) were collected from the Lower Montague wharf on October 25, 2021 (water temperature 13.3$^{o}$C). Approximately 15 tunicates were placed in each of 21 tagged PVC holding units (Figure 2); three holding units for each of the seven treatment groups.

**Figure 2. A staff member setting up and tagging PVC holding units to allow for varying exposure and air-dry times.**

Prince Edward Island CANADA
**Fisheries and Communities**

The treatment units were held in sea water until the time of treatment. The treatment commenced when the tunicates were immersed in the treatment mixture which was 40g of hydrated lime and 270g of salt in 1L of seawater; scaled up to 600g of hydrated lime and 4050 grams of salt in 15L of seawater for this trial (Figure 3). The immersion was followed by an air-dry (none, 30 minutes or 1 hour).

**Figure 3. Hydrated lime (4%) and brine (30% salt) mixture prepared for the treatment trial; 600g of hydrated lime and 4050 grams of salt in 15L of seawater.**

Following each of the treatments, the experimental units were held in seawater until all the treatments were completed. Once all the treatments were completed, the experimental units were placed in buckets of seawater (Figure 4) for transport to a mussel line and held for a period of two weeks (Figure 5). The experimental units were collected on November 8, 2021 and transported to the Aquaculture Division's laboratory for final assessment and determination of mortality.

**Figure 4. PVC holding units being transferred to mussel line in buckets of seawater.**

**Figure 5. PVC holding units held on mussel line in Montague River for 2-week period following treatment.**

## Results

Upon return to the Aquaculture Division's laboratory, the tunicates from each treatment group were removed from the PVC holding unit and placed in a stryofoam cooler filled with seawater for observation. The control group and the two immersion treatments of 30 seconds and 1 minute that were not followed with any air dry time had no observable tunicate mortality (Figures 6; one minute immersion followed by no air dry time used as representative picture for the three treatment groups). The presence of small faecal pellets in these groups is also noteworthy; an indication of survival and health of the organisms (Figure 7).

**Figure 6. One minute immersion followed by no air-dry (Similar results observed in control group and 30 second immersion followed by no air dry time).**

**Figure 7. Faecal pellets present in some treatment groups; an indication that tunicates are healthy and filtering water.**

High tunicate mortality was observable in both of the 30 second treatment groups that were followed by an air dry period of 30 minutes and 1 hour; however, there were some tunicates that survived the treatment (Figures 8 & 9).

**Figure 8. Thirty second immersion followed by thirty minute air-dry.**

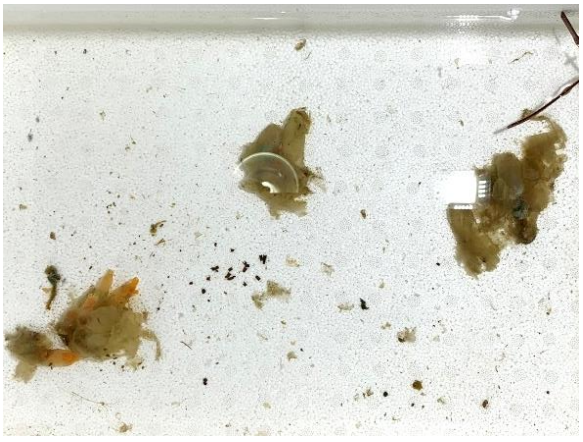

**Figure 9. Thirty second immersion followed by one hour air-dry.**

The tunicates that did survive the 30 second immersion followed by either the 30 minute or 1 hour air dry did not appear healthy. Their siphons remained partially contracted, as compared to the tunicates in the untreated control group and the immersion treatments that had no air dry time (Figure 10).

**Figure 10. Close up of the vase tunicate *Ciona intestinalis* in water after the trial was complete. Several tunicates remain viable, but look unhealthy, as indicated by the partially contracted siphons.**

Based on visual observation, no tunicates survived the one minute immersion followed by either a thirty minute or one hour air-dry (Figure 11).

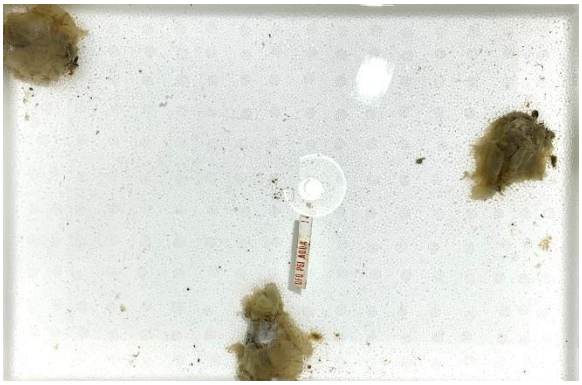

**Figure 11. One minute immersion followed by thirty minute air-dry (similar results observed in the one minute immersion followed by 1 hour air dry treatment).**

The final results are summarized in Figure 12, with all three replicates for each treatment combined as either live or dead tunicates, based on appearance.

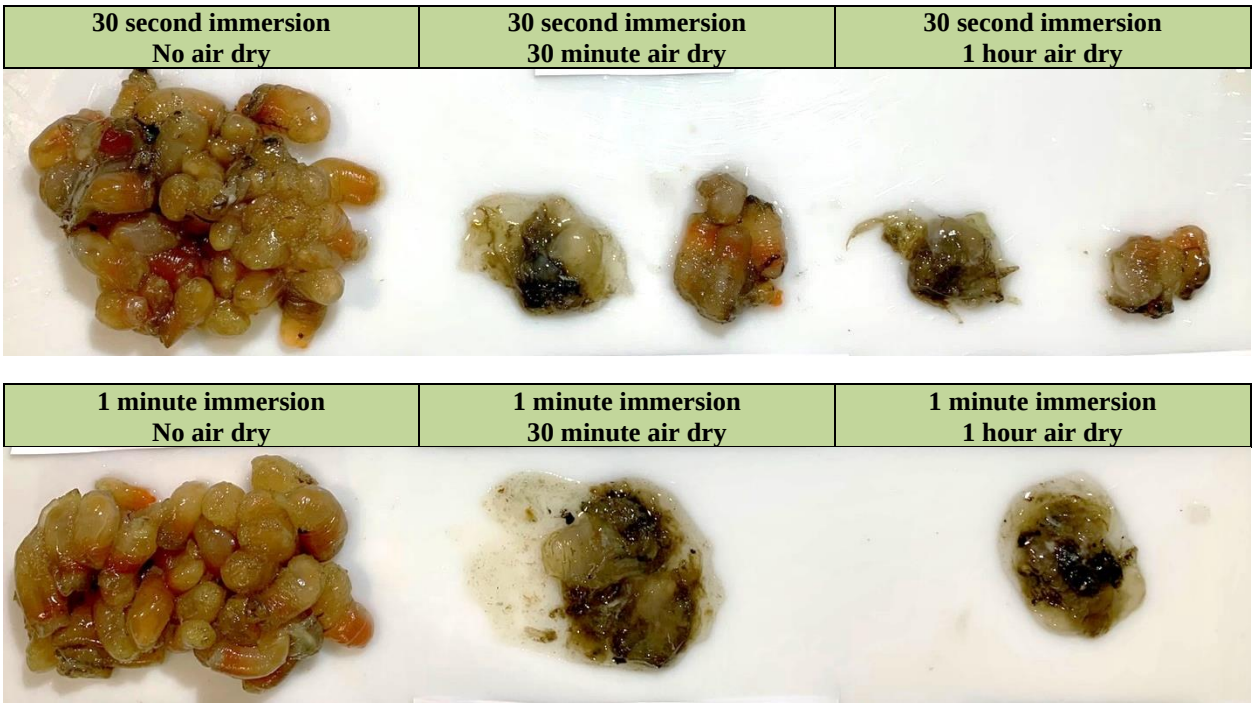


**Figure 12. Final results of the treatment trial. The tunicates for each treatment are grouped as dead or live.**

## Conclusion and Recommendations

The results of this trial show that a ***minimum of a 1 minute immersion in a mixed 4% hydrated lime and a 30% salt solution followed by a minimum of a 30 minute air dry is required for 100% vase tunicate mortality***. The 30 second immersion followed by the air dry results in a significant reduction in vase tunicate biomass, but there are still tunicates present that are filtering and excreting faeces, even with the 1 hour air dry time. This result shows the importance of both components of the treatment: immersion time and dry time.

The information gathered in this treatment trial could be applied in the context of shellfish movements through Fisheries and Oceans Canada's Introdutions and Transfers process to reduce the risk of unintentional spread of the vase tunicate from areas that it is present to areas that it is not presently found. However, additional treatment and exposure times should be explored for on-farm management of the species. Knowledge on longer exposure times and shorter air-dry time would be useful for the oyster aquaculture industry for vase tunicate control on spat collectors.

For further information contact:

PEI Fisheries & Communities
Aquaculture Division
11 Kent Street
Charlottetown, PEI
C1A 7N8