

## Freshwater Treatment as a Management Tool to Control the Spread of Colonial Tunicates

### Background

The feasibility of treating mussel seed from a colonial tunicate (golden star, *Botryllus schlosseri* & violet, *Botrylloides violaceus*) infested area to allow for transfer to an area free of colonial tunicates was investigated in three separate trials completed in four different years. The mussel industry was requesting a solution that would reduce the risk of inadvertently transferring colonial tunicates from seed collection areas to grow-out areas. A treatment that would cause 100% mortality of colonial tunicates (*Fig. 1*) without harming mussel seed was requested. The PEI Aquaculture Division conducted research trials aimed at developing a freshwater treatment protocol to enable the safe transfer of seed from colonial-infested waters.



**Figure 1.** Colonial tunicates fouling mussel gear.

Three separate trials were completed. The goal of the original trial was to find an effective exposure period to cause significant mortality of the colonial tunicates. Subsequent trials were completed to optimize the treatment and to potentially reduce the freshwater exposure period required. Trials were both lab based

and at an industry scale; being applied to the colonial tunicates and mussel seed.

**Trial 1.** Mussels fouled with colonial tunicates were collected from farms in Savage Harbour on June 30, 2008. They were placed in mesh sleeves and distributed throughout three Xactic tanks (three treatment units at the top, middle and bottom of each tank) that were filled with mussel seed. The following treatments were applied to each of the three tanks: (1) 24 hrs of flow-through freshwater (2) 24 hrs of standing freshwater and (3) 1 hr of flow-through freshwater. Water temperature ranged from 11-14 °C, while salinity ranged from 0.4-0.6 ppt. Additionally, a control group was placed in the water at Savage Harbour. The mussel seed was soaked after the freshwater exposure time was complete. Mussels and tunicates were observed for one week following the treatment.

Based on visual observation, tunicate mortality appeared to be 100% in both 24 hour freshwater treatments (standing water and flow-through, see *Fig. 2*). No mortality was observed in the colonial tunicates exposed to the 1 hour freshwater treatment (flow-through, see *Fig. 3*). The colonial tunicates had turned a purplish coloration following the 24 hr freshwater treatments. Individual zooids were no longer tightly held within the colony. Most of the decaying tunicate mass was no longer present four days after the 24 hr freshwater treatment.



**Figure 2.** Colonial tunicate mass before 24hr flow-through and standing freshwater (top), 1hr following 24hr standing freshwater exposure (middle left), 4 days following 24hr standing freshwater exposure (middle right), 1hr following 24hr flow-through freshwater exposure (bottom left) and 4 days following 24hr flow-through freshwater (bottom right).



**Figure 3.** Colonial tunicate mass before 1hr flow-through freshwater (top), 1hr following exposure (bottom left) and 4 days following treatment (bottom right).

**Trial 2A.** On November 3, 2009, mussels fouled with colonial tunicates (collected from St. Peter's Bay) were placed in the mesh bags and were distributed throughout six Xactic tanks that were filled with mussel seed from St. Peter's Bay (three bags were placed near the bottom of each of the tanks, four in the middle layer and three near the top; *Figs. 4&5*). The mesh bags had a string tied to them and an identification tag so that it could be retrieved at the end of the treatment. No treatment was applied to three of the tanks (control) and a six hour flow-through freshwater (temperature = 7.7 °C, salinity = 0.2 ppt) treatment was applied to the remaining three tanks. Following the treatment, the seed was socked, tagged and deployed in St. Peter's Bay (*Fig. 6*). Socks were assessed two weeks after deployment.



**Figure 4.** Mesh bags filled with mussels fouled with colonial tunicates being distributed in one layer of tank filled with mussel seed.



**Figure 5.** Tanks filled with mussel seed and the treatment units are treated with flow-through freshwater for six hours.





**Figure 6.** Socks and mesh bags are deployed in St. Peter's Bay for a two-week period.

High mortality of both species of colonial tunicate was observed 2 weeks post-treatment, estimated at approximately 95% based on visual observation. In the untreated control group an estimated 65% mortality was observed in colonial tunicates. Live tunicate tissue was removed from all experimental units and combined to show the relative level of survival in the treated group versus the untreated control group (*Fig. 7*). Much greater mortality was observed in the treated group. Based on visual observations there were no negative impacts on mussel health associated with the freshwater treatment. Mussels in both the treated group and the untreated control group were showing signs of coming out of the socking material and producing byssal threads for attachment.



**Figure 7.** Total live tunicate tissue from all treatment units two weeks following exposure.

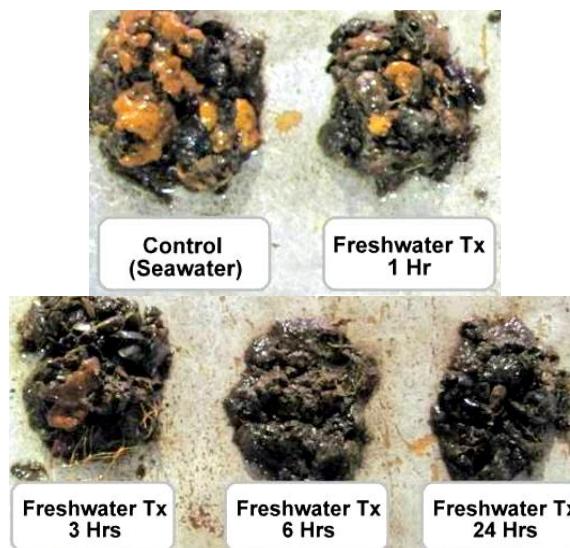
**Trial 2B.** Colonial tunicates (removed from mussels) were also collected and placed in 15 PVC pipes (10 cm length, 10 cm diameter) with the two ends closed with a mesh material for the second part of the experiment to determine length of exposure necessary to cause 100% mortality on the colonial tunicates

(*Fig. 8*). Three experimental units were exposed to a seawater control as well as four freshwater exposures (1, 3, 6 and 24 hrs). After the freshwater exposure, the experimental units were held overnight in water from St. Peter's Bay (temperature = 7.4 °C, salinity = 22.4 ppt). Water quality was checked the following morning (temperature = 7.9 °C, salinity = 26.1 ppt and dissolved oxygen = 6.95 mg/L).



**Figure 8.** Treatment units exposed to freshwater immersion.

The untreated control group sustained minimal mortality, with the violet tunicate maintaining its vibrant orange colour. Approximately 50% of the tunicates exhibited signs of mortality from a 1 hr freshwater treatment and after a 3 hr treatment approximately 75% mortality was observed in the tunicates. After a 6 hr freshwater treatment there was no sign of living tissue. No live tissue was identified in the 24 hr treatment group (*Fig. 9*).



**Figure 9.** Colonial tunicate tissue two weeks after freshwater immersion treatment.

**Trial 3.** On October 13, 2010 both species of colonial tunicates were collected from a mussel lease in Savage Harbour. Using a similar experimental design to previous trials, three mesh bags filled with colonial tunicates were placed at the top, middle and bottom layer of tanks filled with mussel seed. Two tanks were used for each of three treatment groups: (1) declumped seed in 12 hrs of freshwater flow-through (2) not declumped seed in 12 hrs of freshwater flow-through and (3) untreated control (no freshwater or seawater, not declumped). The following day the mussel seed was declumped, socked and deployed on a mussel lease. The mesh bags were also tied onto the line. On October 25, 2010 all experimental units were collected and analyzed.

Significant tunicate mortality resulted from the 12 hr freshwater treatment (both declumped and not declumped); however, several small colonies survived (*Fig. 10*). Visual observation of the freshwater treated mussel socks show no indication of being adversely affected by the treatment. This is consistent with previous findings by DFARD and within industry. It was interesting to note that the socks filled with mussels that were exposed to freshwater immersion were not floating (*Fig. 11*). This means that the socks do not need to be soaked in seawater prior to deployment on the mussel lease, which is usually the case. Freshwater immersion is sufficient to cause the socks to sink, which prevents the socks from wrapping around the line when it is released from the boat.



**Figure 10.** Small colonial tunicate colony on mussel two weeks after freshwater treatment.



**Figure 11.** Two socks filled with mussel seed from the untreated control group (*left*) and one sock filled with mussel seed from the 12 freshwater treatment group.

### Conclusions and Recommendations

- It is recommended that mussel seed from colonial tunicate infested areas be exposed to a 24 hour freshwater immersion (flow-through) if they are being transferred to non-infested area.
- Near 100% mortality resulted in colonial tunicates exposed to a minimum 6 hour freshwater immersion at the lab scale.
- The 1, 6 and 12 hour freshwater exposures were all unsuccessful in causing near 100% mortality in colonial tunicates at an industry scale. Presumably, there are small pockets of seawater in the tanks filled with mussels which enable small colonial tunicate colonies to survive.
- It is important to use flow-through freshwater with a stand pipe (*Fig. 5*) when treating to ensure complete exposure to freshwater in the tanks.

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