Aqua Info
AQUACULTURE NOTES

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Freshwater Immersion to Control the Vase Tunicate, *Ciona intestinalis*

Background

It is recommended that mussel seed being transferred from an area infested with colonial tunicates (golden star, *Botryllus schlosseri* and violet, *Botrylloides violaceus*) to an area free of these tunicates be treated with a 24 hr freshwater immersion (continuous flow) before transferring to the new area. As some of the areas infested with these two colonial species have neighbouring populations of the vase tunicate, *Ciona intestinalis* (*Fig. 1*) or have had unconfirmed reports of *C. intestinalis* within the area, there is the concern that there is the potential for growers to inadvertently introduce this highly invasive species to areas where it has not been detected. This study was conducted to determine if the 24 hr freshwater immersion for colonial tunicates is also sufficient to cause mortality in *C. intestinalis*, in the event that this species remained undetected, but nevertheless, was transported to a "clean" area. A series of trials were completed in 2009 and 2010 to determine the effectiveness of freshwater immersion to cause 100% mortality in *C. intestinalis*.

Figure 1. The vase tunicate, *C. intestinalis.*

Methods

Live specimens were collected from the field and transported in aerated seawater to the PEI Aquaculture Division's laboratory. Water quality was routinely checked. The specimens were acclimatized overnight to ensure they were still viable and filtering, and then subjected to different exposure periods of freshwater. A cluster of tunicates was placed in PVC holding units (*Fig. 2*), tagged, and placed in an exposure group (three units per exposure).

Figure 2. Tunicates being placed in PVC holding units.

The untreated control group was left in seawater for the duration of the experiment and the other groups were subjected to freshwater treatment (*Figs. 3 & 4*). Once the predetermined freshwater exposure time was

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complete the experimental units were removed and placed in the seawater holding tank with the untreated controls (*Fig. 3*).

Figure 3. Specimens were held in seawater prior to and after the treatment exposure.

Figure 4. Experimental units were fully immersed in fresh water for a predetermined exposure.

Trial #1. Live specimens were collected from Cardigan on November 24th (water temperature ~ 7 °C). All of the tunicates were greater than 50 mm in length. They were held overnight in seawater (temperature = 6.1 °C, salinity = 22.3 ppt) and placed in the respective exposure group, in triplicate, on the following day. The following freshwater exposures (temperature = 11.7 °C, salinity = 0.2 ppt) were used: 1, 3, 6, 12, 24, and 48 hrs, along with an untreated control group. On November 27th, 2009, the lab trials were completed and the experimental units were transported to Georgetown Wharf and tied to the side (water temperature = 8.2 °C, salinity = 23.5 ppt). The experimental units were retrieved on December 2nd, 2009, transported back to the lab and visually inspected to determine the level of mortality.

Trial #2. Based on the results from the first trial, an additional study was initiated to determine if freshwater exposures less than three hours would be sufficient to cause mortality in *C. intestinalis*. On October 18th, 2010, vase tunicate specimens were collected from the floating docks in Lower Montague (temperature = 11.9 °C, salinity = 25.1 ppt). They were held overnight in seawater (temperature = 10.6 °C, salinity = 21.7 ppt) and placed in their respective exposure group, in triplicate, on the following day. The following freshwater exposures (temperature = 11.7 °C, salinity = 0.2 ppt) were used: 15 min, 30 min, 1 hr, 3 hrs, 6 hrs, 12 hrs and 24 hrs, as well as an untreated control group. Upon completion of the lab trial, on October 20th, 2010, the experimental units were transported to Montague River (temperature = 10.7 °C, salinity = 26.0 ppt) and tied onto a mussel longline. The experimental units were retrieved on November 9th, 2010, transported back to the lab and visually inspected to determine the level of mortality.

Results

Trial #1. As soon as the tunicates were exposed to the freshwater treatment, the tunic contracted and the organism ceased to filter water and expel faeces (*Fig. 5*).

Figure 5. *C. intestinalis* exposed to freshwater treatment.

The freshwater treatment would be analogous to a person holding their breath under water. The tunicates in the untreated control group (seawater) continued to filter and expel faeces. After the tunicates had been retrieved from Georgetown Wharf, the freshwater treated organisms had a greenish coloration and no longer had the ability to filter water (*Fig. 6*). In addition, no tactile response was evident in the organism.

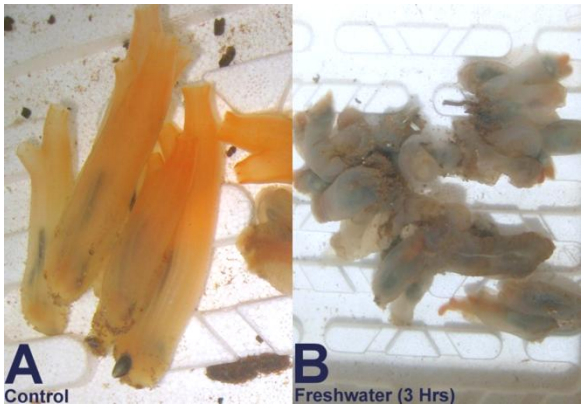

Figure 6. Comparison of *C. intestinalis* from untreated control (seawater) and freshwater treatment (3 hrs), one week post-treatment.

Based upon visual observation, all freshwater exposures of three hours or more caused 100% mortality in adult vase tunicates. Mortality was confirmed by the colouration of the tunicates and the inability of the tunicate to filter (see Figure 7). Results were similar in all treatment groups.

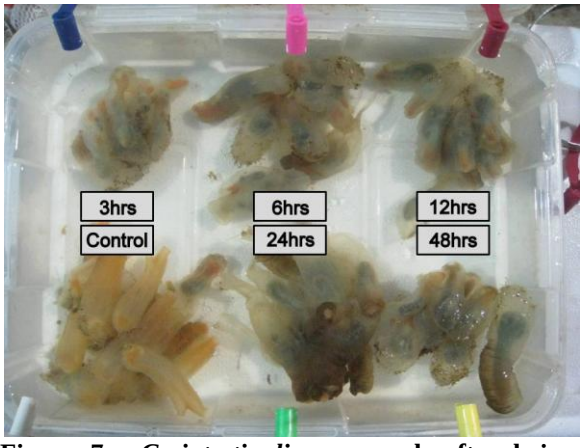

Figure 7. *C. intestinalis* one week after being subjected to various freshwater exposure, as indicated by labels (seawater control, 3 hrs, 6 hrs, 12 hrs, 24 hrs and 48 hrs).

Trial #2. Similar results were observed in the follow-up trial in the fall of 2010 (*Fig. 8*). All freshwater exposures of three hours or more resulted in 100% mortality of the vase tunicate, *C. intestinalis*. However, the shorter exposures tested in this trial did not result in tunicate mortality. There appeared to be some mortality (approximately 50%) in the one hour exposure group, but negligible difference was observed between the control group and the two freshwater exposures less than one hour (15 and 30 minutes). The tunicates exhibited a tunic contraction and a stop in filtration when exposed to freshwater immersion, but they were able to recover from the lower exposure times and would relax their tunic and resume filtration when they were placed back in seawater (results shown below).

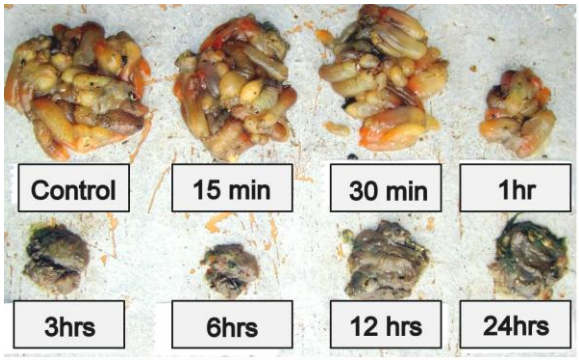

Figure 8. *C. intestinalis* three weeks after being subjected to various freshwater exposures, as indicated by labels (seawater control, 15 min, 30 min, 1 hr, 3 hrs, 6 hrs, 12 hrs and 24 hrs).

The photo shown on the following page is the remaining tunicate mass from the 12 hour exposure group, three weeks following the freshwater treatment (*Fig. 9*). The tunicates in the higher exposure groups were reduced to a mass of decomposing material, making individual tunicates difficult to distinguish

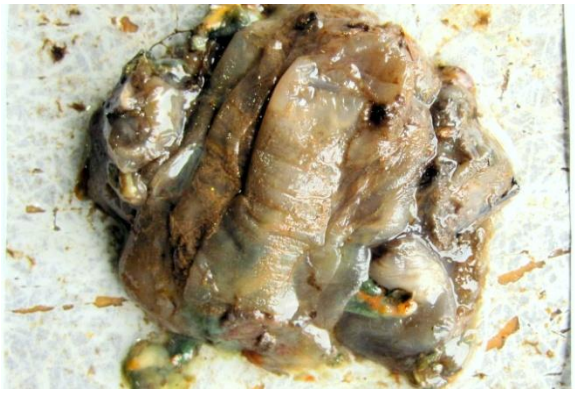

Figure 9. *C. intestinalis* three weeks following a 12 hour immersion in freshwater.

Conclusions and Recommendations

At the time of the trial there were still several large water bodies where colonial tunicates had not been detected. However, since that time, colonial tunicates (*B. schlosseri and B. violaceus*) have been detected in most water bodies around PEI. Freshwater immersion for 24 hours remains the recommended treatment when transferring mussel seed from areas with colonial tunicates present to areas where they have not been detected, but it is no longer common practice with colonial tunicates being distributes throughout PEI.

Currently, the industry is not willing to accept the risk associated with transferring mussel seed from *C. intestinalis* positive areas to areas where it is not present, even with a form of treatment. This is because the impact of vase tunicate fouling is too high to consider even the smallest risk of an unintentional transfer.

Based on the results from this trial, a minimum of a three hour immersion in freshwater is sufficient to cause 100% mortality in adult tunicates. The larval and juvenile life stages of the vase tunicate were not considered in this trial; however, it is presumed that at earlier life stages the vase tunicate would be more vulnerable to some form of treatment, including freshwater immersion.

Freshwater treatment for vase tunicate fouling on mussel crop was never considered a viable management option on the farm. This is because access to freshwater is limited and the exposure time required (3 hours) exceeds the treatment time that would be considered acceptable by the industry. This is a treatment that is specific to controlling the spread of the vase tunicate on mussel seed transfers in the event that that practice ever becomes necessary.

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