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Freshwater Immersion to Control the Clubbed Tunicate, *Styela clava*

Background

It is recommended that mussel seed being transferred from an area infested with the colonial tunicates, Botryllus schlosseri and Botrylloides violaceus, to an area free of these tunicates be treated with a 24 hr freshwater immersion (continuous flow) before transport to the new area. As some of the areas infested with these two colonial species have neighbouring populations of Styela clava and/or Ciona intestinalis within the area, there is concern that there is potential for growers to inadvertently introduce these highly invasive species to areas not already affected. This study was a follow-up to work completed investigating the impact of freshwater exposure on the colonial and vase tunicates to determine if the 24 hr freshwater immersion for colonial tunicates is also sufficient to cause mortality in S. clava, in the event that this species remained undetected, but was inadvertantly transported to a "clean" area.



The clubbed tunicate, S. clava.

Methods

A quantity of clubbed tunicates were collected from Darnley Basin on September 20th, 2012. The tunicates were separated into clusters of approximately 20-25 individuals, which included both juveniles and adults, and were placed in seawater for a two hour period to recover from the handling. Three clusters of tunicates were used in each of the four exposure groups (3, 6, 12 and 24 hrs), in addition to the five clusters for the seawater control group. The tunicates were exposed to the freshwater treatment in a 20 L bucket.



S. clava exposed to freshwater treatment

Following treatment, they were placed into a cage with individual compartments and tied to the side of the Malpeque Harbour wharf. The cage was collected on September 27th and the final assessment was completed. Similar to the freshwater treatment trial completed on *Ciona intestialis* (AquaInfo Note 26.2015), mortality was assessed by evidence of filtration and colour change.



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Results



S. clava 1-week post-treatment: seawater control



S. clava 1-week post-treatment: 3 hr freshwater



S. clava 1-week post-treatment: 6 hr freshwater



S. clava 1-week post-treatment: 12 hr freshwater



S. clava 1-week post-treatment: 24 hr freshwater

Similar to the freshwater treatment trial with C. intestinalis, as soon as the tunicates were exposed to the freshwater treatment, the tunic contracted and the organism ceased to filter water and expel faeces. This was evident on the bottom of the treatment bucket. Small brown fecal pellets were noted on the bottom of the seawater control bucket, but the bottom of the freshwater treatment buckets remained clean for the duration of the freshwater exposure. Based on visual observations, 100% tunicate mortality was observed in all freshwater treatment groups. In many cases, 1week post treatment, the internal organs of the tunicates were absent; only tunic and holdfast remained. In other cases, the siphons were still intact, holding the internal organs within the tunic. When a small amount of pressure was applied to the organism, the internal content of the organism was released through the siphons. The seawater control group continued to siphon.

Conclusions and Recommendations

Based on the results from this trial, a minimum of a three hour immersion in freshwater is sufficient to cause 100% mortality in both juvenile and adult clubbed tunicates. It is possible that shorter immersion durations in freshwater would cause mortality in the clubbed tunicate, but they were not considered in this trial because in previous work it was determined that a minimum of a 3-hour freshwater exposure was required to cause mortality in *C. intestinalis*.

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