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Responses of *Mitrella lunata* and *Caprella* spp., potential tunicate micropredators, in Prince Edward Island estuaries to acetic acid anti-fouling treatments

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ABSTRACT

In Prince Edward Island, Canada, acetic acid treatments that are used to control the clubbed tunicate (*Styela clava*), a fouling pest on mussel lines, may also affect other epifaunal mussel sock species, including potential tunicate predators. We studied the effect of acetic acid treatment on two potential predators of the tunicates, the gastropod *Mitrella lunata* (lunar dove shell) and the amphipod *Caprella* spp. (caprellids) in a preliminary lab study and more intensive field study. In the laboratory, caprellids and gastropods were allowed to attach to sections of rope in saltwater. The ropes were lifted and sprayed with 5% acetic acid or saltwater and organisms were monitored for 5–9 days. The acetic acid spray killed all amphipods whereas gastropods were mostly unaffected by the treatment. Gastropods were more affected by the lifting process than amphipods. In the field, gastropod and amphipod populations were compared over the short (5 days) and long-term (5–6 weeks) between mussel socks that were lifted from the water and sprayed with 5% acetic acid (simulating commercially used control methods) and control socks which were lifted from the water but not sprayed, or neither lifted nor sprayed. Gastropod populations were not affected by acid treatment after 5 days, but acid-sprayed populations were significantly lower than untouched control populations 5–6 weeks after treatment. Lifting of the mussel socks without acid spraying did not decrease gastropod populations significantly. Amphipod populations on acid treated mussel socks were lower than those on socks that were simply lifted from the water in both the short-term and the long-term.

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1. Introduction

Mussel mariculture is a major industry in Prince Edward Island (PEI), Canada, with a value of more than $36 million to the province's GDP (DFO, 2006). Large numbers of production units, consisting of buoyed lines and "socks" containing growing mussels, are suspended in the protected waters of PEI estuaries. These production units are also home to a diverse community of epifaunal species, including potential fouling organisms that affect mussel growth (Ellis et al., 2002). The 1998 introduction and subsequent proliferation of the clubbed tunicate (*Styela clava* Herdman), an aquatic invasive species on mussels and aquaculture equipment, prompted mussel growers to search for methods to control this fouling organism. Field trials have shown that spraying with 5% acetic acid (vinegar) has been successful in reducing tunicate numbers (Neil MacNair, PEI Department of Agriculture, Fisheries, Aquaculture, and Forestry, Charlottetown, PEI, Personal Communication).

However, acetic acid treatment could also have detrimental effects on the epifauna of mussel socks, including potential epifaunal predators of tunicates in PEI such as the lunar dove shell gastropod (*Mitrella lunata* Say) and the caprellid amphipod (*Caprella* spp., including *C. mutica* and *C. linearis*). *M. lunata* preys on the larvae of various tunicate species in East Long Island Sound, NY (Osman et al., 1992; Osman and Whitlatch, 1995; Lee-Miles Rogers, 1998; Osman and Whitlatch, 2004), and caprellids prey on small invertebrates in the water column (Gerhard Pohle, Huntsman Marine institute, St. Andrew's, NB, Personal Communication) including ascidian larvae (Robert Whitlatch, University of Connecticut, Groton, CT, Personal Communication). In addition, PEI mussel growers have noted high numbers of caprellids in areas of high *S. clava* infestation compared to areas without *S. clava* (Jeff Davidson, unpublished data) and this settling behaviour has also been observed in the North Sea (Buschbaum and Gutow, 2005). Preferential settling of caprellids near *S. clava* could be another indication that caprellids prey on tunicate larvae in PEI.

Predators such as *M. lunata* and caprellids that target the newly settled larvae of sessile species like tunicates can change the community structure of an epifaunal community. In a series of experiments on subtidal epifaunal communities in southern New England (Osman et al., 1992; Osman and Whitlatch, 1998), predation by *M. lunata* and other micropredators eliminated or reduced tunicate recruitment (including *S. clava*), and was a major factor in shaping the marine benthic

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communities. The presence of potential micropredators should be taken into account when considering long-term control strategies for aquatic invasive species such as *S. clava* in PEI estuaries. Control programs including chemical or mechanical anti-fouling treatments could have negative effects on micropredators, eliminating the opportunity for these natural predators to become established. The status of *M. lunata* and caprellids as predators of *S. clava* remains to be investigated in PEI, but their effectiveness in other locations suggests that they should be included in invasive tunicate management programs in PEI.

The most effective type of management strategy should integrate anti-fouling treatments with natural control mechanisms like predation by regulating the timing of anti-fouling treatments to minimize harm on beneficial species. Since the main control strategy currently used for clubbed tunicates involves lifting mussel socks from the water and treating them with acetic acid, implementing an integrated control strategy requires information on acetic acid toxicity as well as the mechanical effects of lifting socks from the water on potential micropredators in the PEI ecosystem. Therefore, the objectives of this study were to investigate the direct effect of 5% acetic acid on *M. lunata* and *Caprella* spp. individuals, and to determine the effect of acetic acid treatment on *M. lunata* and *Caprella* spp. field populations over time. We tested the null hypothesis that the anti-tunicate treatment used by PEI mussel growers would have no effect on the *M. lunata* and *Caprella* spp. populations living on mussel socks, either in the short-term (5 days) or in the long-term (5–6 weeks). We predicted that acetic acid would have a negative effect on both species in the short-term, since they are likely not adapted to acute changes in pH due to the buffering capacity of sea water (Dobson and Frid, 1998). We also predicted that populations would recover in the long-term by repopulating acetic acid treated mussel socks.

2. Methods

2.1. Sampling sites

This study had two components: a preliminary laboratory exposure study and a field treatment study. For both components, mussel sock samples were collected from commercial mussel leases in two PEI estuaries: Tracadie Bay (46.408°N, 62.992°W) and Murray River (46.031°N, 62.521°W). Tracadie Bay socks were tunicate-free and had high numbers of *M. lunata* (lunar dove shells, hereafter referred to as "*Mitrella*" or "gastropod"), and Murray River socks were infested with *S. clava* tunicates and populated by high numbers of *Caprella* spp. (hereafter referred to as "caprellids" or "amphipod").

2.2. Laboratory exposure study

Gastropods and amphipods were collected in June and July 2003, and transported to the laboratory for a series of experiments designed to approximate field treatments. Individuals (75 gastropods and 129 amphipods) were allowed to acclimate in flow-through saltwater tanks at the same temperature at which they were collected (10 °C), for a minimum of 3 days before the start of the experiments. Two laboratory trials were conducted for each species, each one including a saltwater control (28 ppt; the measured salinity of PEI estuaries) and an acetic acid treatment. Sample sizes varied depending upon the number of specimens available. For the gastropods, 29–30 animals were used for the control and acid spray (respectively) in the first trial, and only eight per treatment in the second trial. For the caprellids, the sample sizes were 23 and 44 for the first trial, and 32 and 30 for the second. The animals were placed onto a piece of rope representing the mussel sock, and placed in a 1.5 L jar freshly filled with aerated saltwater (10 °C) for 5 min or until all animals had attached to the piece of rope. The rope was then lifted from the water and sprayed for 5–10 s with either acetic acid or with saltwater (28 ppt; Instant Ocean®). Ropes were kept out of the water for 45 s to simulate the time that mussel socks are exposed to air during commercial acetic acid treatment, then they were lowered back into the jars. Following the initial observations (at 5 and 30 min post-exposure), jars were covered with 1 mm screening and placed into a flow-through saltwater tank except when being observed as indicated below.

The reactions of amphipods and gastropods to the saltwater or acetic acid sprays, the air exposure and the placement in water were observed in each trial. Observations on activity and mortality were recorded 5 min, 30 min, 2–3 h and again 5–9 days post-treatment). For the latter two observations, animals were transferred to a petri dish filled with saltwater and observed in more detail under the dissecting microscope. The animals were classified qualitatively as active (gastropods: moving; amphipods: crawling/swimming, gills moving), inactive (gastropods: no protrusion out of the shell; amphipods: twitching/no activity) or dead (gastropods: no reaction to prodding into the shell; amphipods: floating/no gill activity).

2.3. Field treatment population study

During sampling, mussel sock sections of 0.3 m or 0.15 m in length (in Tracadie Bay and Murray River, respectively) were collected from the bottoms of adjacent mussel socks on each sampling day and placed into 1 mm mesh onion bags underwater to avoid loss of specimens. Baseline data of amphipod and gastropod population sizes were collected by sampling twenty adjacent socks before treatment. On the treatment day, ten socks were designated as lifted/sprayed socks. These socks were lifted out of the water, sprayed with acetic acid for 10–20 s, and exposed to air for 1 min before being returned to the water. Ten socks were designated as lifted socks and were lifted out of the water but not sprayed with acetic acid. Lifted socks were designated at upstream locations to minimize contamination of these socks with acetic acid from the adjacent lifted and sprayed socks. The same twenty socks were sampled again 5 days after treatment (short-term effect) and 5–6 weeks after treatment (long-term effect). Eight or ten socks (depending on availability) located upstream of the other twenty were designated as control socks and neither lifted or sprayed during the experiment. These control socks were only sampled at the last sample date (long-term). Sampling and treatment dates are summarized in Table 1.

Samples from Tracadie Bay were stored at −20 °C until processing when each sample was washed through a series of sieves (mesh size 17 mm, 3 mm, 870 μm) and *Mitrella* were identified and counted. Caprellids did not withstand freezing well, so samples from Murray River were maintained in 2 L plastic containers covered with mesh (1 mm) and placed in flow-through saltwater tanks at 10 °C up to 5 days. Processing of Murray River samples consisted of spreading each sample in a tray and manually collecting and counting the caprellids.

Recruitment of both study species occurred between the second and third sampling date, causing a dramatic increase in numbers, so some Murray River samples had to be preserved (−20 °C) for later processing, and samples from both estuaries were subsampled to estimate population numbers. Tracadie Bay samples were subsampled

Table 1
Sampling dates before and after treatment of mussel socks in two Prince Edward Island estuaries in 2003 and the number of adjacent socks sampled at each time point

Sampling/ treatment	Sampled socks	Tracadie Bay	Murray River
Before treatment	20 (undesignated)	21 July	29 July
Treatment (TRT)		24 July	8 August
Short-term post-TRT	10 lifted, 10 lifted/sprayed	29 July	13 August
Long-term post-TRT	8 (Tracadie B.) or 10 (Murray R.) control, 10 lifted, 10 lifted/sprayed	4 September	11 September

Treatment consisted of lifting the socks out of the water or lifting and spraying them with 5% acetic acid.

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