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Short communication

Limited value of the common periwinkle snail *Littorina littorea* as a biological control for the invasive tunicate *Didemnum vexillum*

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Abstract

Didemnum vexillum is an invasive tunicate that aggressively grows on and fouls all manner of substrates in coastal New England habitats. Most alarmingly, *D. vexillum* acts as a shellfish pest and is capable of completely encapsulating and smothering bivalves, causing them to have reduced growth or be killed. Fouling by *D. vexillum* on aquaculture gear and product requires remediation. While there are numerous manual eradication methods, they are labor intensive and expensive. We investigated whether the common periwinkle snail *Littorina littorea* can be utilized as a biological control for *D. vexillum*. The only known predator of senescing *D. vexillum* is the snail *L. littorea* and there are no known predators of healthy *D. vexillum*. Field observations indicated that *L. littorea* may be consuming, scouring, or otherwise removing stressed *D. vexillum* from rocks in intertidal pools at Sandwich, Massachusetts, during all seasons. We used two methods to investigate whether *L. littorea* could be used as a biological control by either consuming or scouring *D. vexillum* off shellfish. We examined *L. littorea*'s fecal pellets and conducted a laboratory experiment to determine if the snails would "clean" unhealthy *D. vexillum* from aquaculture product. Fecal pellets from *L. littorea* collected on unhealthy *D. vexillum* contained the characteristic spicules of this tunicate, thus confirming that *L. littorea* consumes *D. vexillum* under field conditions. The laboratory experiment indicated that *L. littorea* did not notably consume or scour *D. vexillum* from shellfish under the conditions we provided. At this time, we recommend that manual eradication methods be considered the primary defense for shellfish aquaculturists and others interested in controlling *D. vexillum* and that *L. littorea* should merely be considered as a supplement to these more reliable control methods.

Key words: Shellfish aquaculture, invasive tunicate, *Littorina littorea*, *Didemnum vexillum*, biological control

Didemnum vexillum Kott, 2002 is an invasive tunicate that first appeared in New England at Damariscotta, Maine in 1988 (Bullard et al. 2007). The first record of this species in Massachusetts was in 1993 at Sandwich, near the east entrance to the Cape Cod Canal (Carman and Roscoe 2003). *Didemnum vexillum* is continuing to spread in New England (Bullard et al. 2007) and elsewhere (Minchin and Sides 2006) and is becoming an increasingly global problem (Lambert 2005). *Didemnum vexillum* attaches to the shells of dead and living mollusks (Dalby and Young 1992) and other hard substrates. In the near shore, *D. vexillum* has been observed attached to blue mussels *Mytilus edulis* Linnaeus, 1758 and eastern oysters *Crassostrea virginica* (Gmelin, 1791). In offshore waters, it grew on sea scallops *Placopecten magellanicus* (Gmelin, 1791) at Georges Bank (Dijkstra et al. 2007; Valentine et al. 2007a; Valentine et al. 2007b). *Didemnum vexillum* grows rapidly and is capable of completely overgrowing a cluster of shells in a few weeks (Valentine et al. 2007a). Infestation by *Didemnum* sp. can lead to bivalves that have reduced growth rates or are misshapen or dead (Guenther et al. 2006). Further, tunicate build-up on the outside of aquaculture nets and bags restricts water flow and food availability for shellfish (Carver et al. 2003).

Didemnum sp. fouls oysters and aquaculture equipment and thus causes economic hardship for commercial shellfishermen. In Shakespeare Bay, New Zealand, the estimated recent income loss to the green mussel *Perna canaliculus* (Gmelin, 1791) industry caused by *D. vexillum* over five years was $807,000 (Sinner and Coutts

2003). The shellfish industry in New England and Atlantic Canada could similarly experience substantial economic losses if *D. vexillum* continues to increase in abundance.

Although there are no known measures for preventing invasion by *D. vexillum*, there may be control measures for managing the species. Because oysters can tolerate exposure to air for a longer period of time than *Didemnum* sp., Japanese aquaculture researchers recommend emersion for killing *Didemnum* sp. on cultured oysters (Katayama and Ikeda 1987). Other non-biological methods of removing tunicates on shellfish include high-pressure spray, hand brushing, freshwater rinse, saline dips, vinegar spray, hot water, and dilute bleach dips (Katayama and Ikeda 1987; Debrosse and Allen 1993; Denny 2007). Removal of fouling organisms by manual cleaning costs up to 30% of operational expenses (Guenther et al. 2006) thus other control methods are highly desirable.

Biological control, utilizing a natural enemy of the pest species to control or eradicate the pest from a geographic area, is considered a priority control method of control by invasion ecologists, for both economic and evolutionary reasons (Ehler 1998). Biological controls have been demonstrated as effective for removing tunicates from shellfish (Kuris and Lafferty 1999). The common periwinkle snail *Littorina littorea* (Linnaeus, 1758) may be useful as a biological control for *D. vexillum*. This species has already been effective at removing *Ciona intestinalis* (Linnaeus, 1767) from infested oysters under laboratory conditions (Carver et al. 2003). *Littorina littorea* has been observed consuming or scouring through stressed colonies of *D. vexillum* attached to rocks throughout the year (Valentine et al. 2007a). The goal of our study was to determine if *L. littorea* is consuming *D. vexillum* or at least if it can help dislodge the tunicate from mussels shells. We also investigated the potential for use of *L. littorea* as a biological control for *D. vexillum* that have colonized *M. edulis*, a commercially important shellfish species.

Methods

Fecal pellet examination. Only August 2, 2007, specimens of *L. littorea* found on three microhabitats: (i) unhealthy *D. vexillum*, (ii) boulders, and (iii) *Ascophyllum nodosum* (Linnaeus) LeJolis, 1863 seaweed, were collected at the rocky, intertidal pools in Sandwich, MA, USA. Ten *L. littorea* from each microhabitat were placed in one of three small plastic bottles with seawater. After an hour, fecal pellets were withdrawn from each bottle using a pipette. The fecal pellets were placed in a Petri dish with seawater and examined under the microscope and photographed.

Laboratory experiment. *Mytilus edulis* covered with *D. vexillum* specimens were collected from Iselin dock, Woods Hole, MA, USA, on July 9, 2007, transferred to flowing seawater tanks at WHOI Redfield Lab, and placed in numbered flow-through containers. The containers were distri-buted randomly among two flowing sea-water tanks that provided ambient temperature and salinity conditions. On July 10, five healthy specimens of *D. vexillum* were stressed by exposure to 1.5 hours of air (low stress), five exposed to 2.5 hours of air (medium stress), five exposed to 3.5 hours of air (high stress) and five were not stressed (control). Also on July 10, *L. littorea* were collected from unhealthy *D. vexillum* at Sandwich and transported in a cooler to Redfield Lab. We placed 15 *L. littorea* in four of each level of the stressed didemnid and non-stressed didemnid flow-through containers. The containers were covered to keep the snails from emigrating. We checked each container twice weekly for a total of three weeks. During each observation period, we recorded whether *L. littorea* was in contact and potentially consuming *D. vexillum*. On July 18, 2007, we further stressed the three levels of didemnids with a freshwater bath because the *D. vexillum* was not senescing as expected due to the air exposure. The 1.5-hour air exposure group was exposed to freshwater for 10 minutes; 2.5-hour group for 20 minutes; the 3.5-hour group for 30 minutes. On July 24, a black plastic cover was placed over the tank to prevent algal growth. Although no algae were observed growing on the containers, the black plastic cover ensured that the snails only had senescing didemnids as a food source. We used a Chi-square test to analyze the differences between treatments and then subdivided the contingency table by dropping the highest Chi square value and repeating the analysis.

Results

Fecal pellet examination. Examination of the fecal pellets from the three different micro-habitats revealed that those from *L. littorea* on *D. vexillum* were different than the fecal pellets

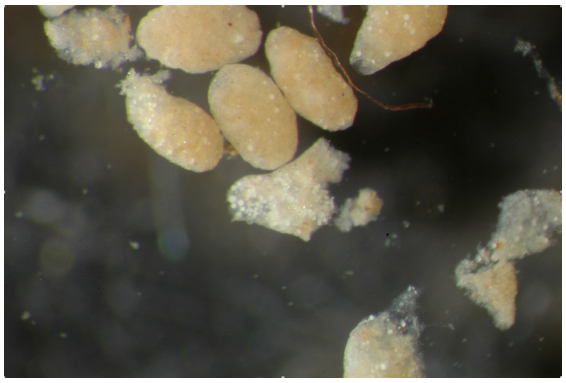

Figure 1. Fecal pellets of *Littorina littorea* collected from senescent *Didemnum vexillum* at Sandwich, Massachusetts (pellets are about 0.5 mm in length). The numerous opaque white specks in the center pellet are spicules of *Didemnum vexillum*.

Figure 2. Fecal pellets of *Littorina littorea* collected from granite boulders at Sandwich, Massachusetts (pellets are about 0.5 mm in length).

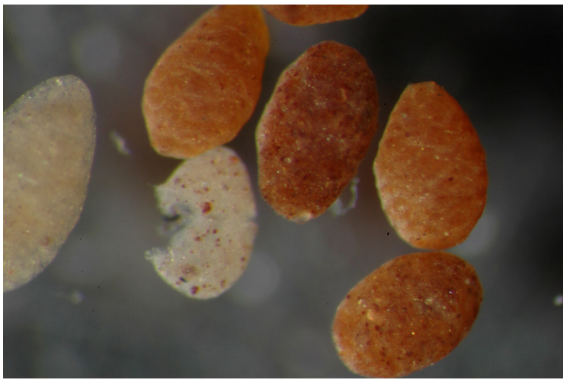

Figure 3. Fecal pellets of *Littorina littorea* collected from seaweed *Ascophyllum nodosum* at Sandwich, Massachusetts (pellets are about 0.5 mm in length).

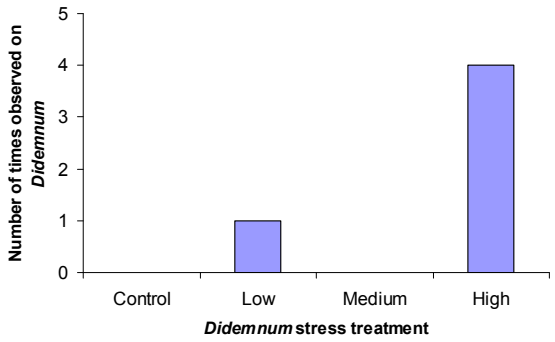

Figure 4. Total number of times *Littorina littorea* were observed on *Didenmum vexillum* in the laboratory experiment.

of *L. littorea* on rocks or on *A. nodosum* (Figures 1, 2, 3). Many didemnid spicules were present in the fecal pellets of *L. littorea* that had been on *D. vexillum*. The distinctive calcareous (aragonite) spicules of Didemnidae are apparently not digestible and pass through the snail's digestive tract. The presence of didemnid spicules in fecal pellets confirms that *L. littorea* consumes *D. vexillum*.

Laboratory experiment. Air exposure and freshwater baths stressed *D. vexillum* as evidenced by senescence of some of the tissue in the colonies. However the *M. edulis* survived the air and freshwater exposures. The *D. vexillum* in the control (non-stressed) treatment survived the laboratory conditions and *L. littorea* were never observed on the healthy colonies of *D. vexillum,* although they were also never observed on the *D. vexillum* that received the medium stress treatment either (Figure 4). *Littorina littorea* were most commonly observed on the *D. vexillum* in the high stress treatment. The Chi square test indicated that the difference between treatments was significant (X^2=7.84, df=3, p=0.049) and when the high stress treatment was dropped because it had the highest Chi square value, there was no longer a significant difference between the treatments (p>0.05). Stressed *D. vexillum* became filmy and rotten after the second and third weeks, but was not notably consumed or scoured away by the snails.

Discussion

Littorina littorea is not considered a predator of *D. vexillum* in the true sense of the definition of "predator," nor is it a "natural enemy" (species capable of reducing the population of another

species through one or more methods such as interference, parasitism, competition, etc.) (e.g., Ehler 1998). Instead *L. littorea* may act as a scavenger on stressed *D. vexillum*. It does not graze or crawl on healthy *Didemnum* sp., but does spend more time on stressed *D. vexillum* in the laboratory as well as in field conditions (Valentine et al. 2007a). *Littorina littorea* may be cleaning or scouring senescent *D. vexillum* from a substrate that may otherwise be covered with algae. Alternatively, if space is available during the active didemnid larval recruitment period, rocky substrate grazed clean of epibionts by *L. littorea* may become occupied by a new colony of *D. vexillum*.

The results of this study suggest that *L. littorea* is probably not useful as a biological control for *D. vexillum* in aquaculture settings. It appears that *L. littorea* will not voraciously consume or scour *D. vexillum* from shellfish under the conditions we furnished in the laboratory. While the fecal pellet results indicate that *L. littorea* consumes *D. vexillum* under field conditions, at this time we recommend that non-biological control methods may be more reliable for aquaculturists and others interested in controlling *D. vexillum* fouling on shellfish. Addition of *L. littorea* could be considered as a supplemental method with limited value to control the spread of *D. vexillum* in aquaculture settings.

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References

Bullard SG, Lambert G, Carman MR, Byrnes J, Whitlatch RB, Ruiz G, Miller RJ, Harris L, Valentine PC, Collie JS, Pederson J, McNaught DC, Cohen AN, Asch RG, Dijkstra J, Heinonen K (2007) The invasive colonial ascidian *Didemnum* sp. A: current distribution, basic biology, and potential threat to marine communities of the northeast and west coasts of the United States. *Journal of Experimental Marine Biology and Ecology* 342: 99-108, doi:10.1016/j.jembe.2006.10.020

Carman MR, Roscoe LS (2003) The didemnid mystery. *Massachusetts Wildlife* 53: 2-7

Carver CE, Chisholm A, Mallet A (2003) Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production. *Journal of Shellfish Research* 22: 621-631

Dalby Jr JE, Young CM (1992) Role of early post-settlement mortality in setting the upper depth limit of ascidians in Florida epifaunal communities. *Marine Ecology Progress Series* 80: 221-228, doi:10.3354/meps080221

Debrosse GA, Allen SK (1993) Control of overset on cultured oysters using brine solutions. *Journal of Shellfish Research* 12: 29-33

Denny C (2007) Techniques to treat seed mussels (*Perna canaliculus*) on farms infected with the invasive sea squirt (*Didemnum vexillum*) in the Marlborough Sounds, New Zealand (abstract). Fifth International Conference on Marine Bioinvasions Proceedings, May 21, 2007, Massachusetts Institute of Technology, Cambridge, Massachusetts

Dijkstra J, Harris L, Westerman E (2007) Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. *Journal of Experimental Marine Biology and Ecology* 342: 61-68, doi:10.1016/j.jembe.2006.10.015

Ehler LE (1998) Invasion biology and biological control. *Biological Control* 13: 127-133, doi:10.1006/bcon.1998.0649

Guenther J, Southgate PC, deNys R (2006) The effect of age and shell size on accumulation of fouling organisms on the Akoya pearl oyster *Pinctata fucata* (Gould). *Aquaculture* 253: 366-373, doi:10.1016/j.aquaculture.2005.08.003

Katayama K, Ikeda Z (1987) Tolerance of fresh water, hot water, and sun-drying by *Didemnum moseleyi*, fouling organisms attached to culture oyster. *Bulletin of the Fisheries Experiment Station, Okayama Prefecture* 2: 104-106

Kuris AM, Lafferty KD (1999) Can biological control be developed as a safe and effective mitigation against established introduced marine pests? In: Pederson J (ed), Marine Bioinvasions: Proceedings of the First National Conference, MIT, Cambridge, MA, pp 102-106

Lambert G (2005) Ecology and natural history of the protochordates. *Canadian Journal of Zoology* 83: 34-50, doi:10.1139/z04-156

Minchin D, Sides E (2006) Appearance of a cryptogenic tunicate, a *Didemnum* sp. fouling marina pontoons and leisure craft in Ireland. *Aquatic Invasions* 1: 143-147, doi:10.3391/ai.2006.1.3.8

Sinner J, Coutts ADM (2003) Benefit-cost analysis of management options for *Didemnum vexillum* in Shakespeare Bay. Cawthron Report 924, Cawthron Institute, Nelson, New Zealand, 12 pp

Valentine PC, Carman MR, Blackwood D, Heffron E (2007a) Ecological observations on the colonial ascidian *Didemnum* sp. in a New England tide pool habitat. Journal of Experimental *Marine Biology and Ecology* 342: 109-121, doi:10.1016/j.jembe.2006.10.021

Valentine PC, Collie JS, Reid RN, Asch RG, Guida VG, Blackwood DS (2007b) The occurrence of the colonial ascidian *Didemnum* sp. on Georges Bank gravel habitat - Ecological observations and potential effects on groundfish and scallop fisheries. *Journal of Experimental Marine Biology and Ecology* 342: 179-181, doi:10.1016/j.jembe.2006.10.038