Aquatic Invasions (2009) Volume 4, Issue 1: 95-103
This is an Open Access article; doi: 10.3391/ai. 2009.4.1.10

Special issue "Proceedings of the 2nd International Invasive Sea Squirt Conference"
(October 2-4, 2007, Prince Edward Island, Canada)
Andrea Locke and Mary Carman (Guest Editors)

Research article

Settlement and potential for transport of clubbed tunicate (*Styela clava*) on boat hulls

Emily A. Darbyson[1,2], John Mark Hanson[3*], Andrea Locke[3] and J. H. Martin Willison[1]

[1]*Department of Biology, Dalhousie University, Halifax, Nova Scotia, B3H 4J1 Canada*
[2]*Present address: Department of Biology, McGill University, Montreal, Quebec, H3A 1B1 Canada*
[3]*Gulf Fisheries Centre, Fisheries and Oceans Canada, P.O. Box 5030, Moncton, New Brunswick, E1C 9B6 Canada*

**Corresponding author*
E-mail: HansonM@dfo-mpo.gc.ca

Received 19 December 2007; accepted for special issue 18 April 2008; accepted in revised form 4 December 2008; published online 16 January 2009

Abstract

The invasive clubbed tunicate (*Styela clava*) was first identified in Georgetown harbor, Prince Edward Island (PEI), Canada, in 1998 and has since spread to other estuaries in PEI. Worldwide, the dispersal of this species is attributed to hull fouling and contamination of bivalve aquaculture products. We tested how clubbed tunicate settlement patterns differed among the most common boat hull surfaces and colors as well as the ability of these tunicates to survive extended atmospheric exposure similar to that of boats being transported on trailers during summer months. Untreated hulls made of fiberglass, painted wood, and bare aluminum were quickly colonized by larval tunicates with hulls painted black attracting significantly more colonists compared to white-painted hulls. Use of anti-fouling paint kept colonization to a minimum, even after 12 weeks in the water. Bare aluminum hull material attracted the highest numbers of tunicates, which is a problem because boats operating around bivalve aquaculture sites are mainly constructed of aluminum and cannot be painted with anti-fouling paint according to industry codes of practice. Aluminum and fiberglass hull material that had one-year-old tunicates growing on them were exposed to open air for 48 h during September (mean daytime high temperature 29.7˚C, night-time low 8.5˚C). Nearly all tunicates were alive after 8 hours with only 10 to 11 % mortality after 48 h. Based on a 48 h survival time, viable tunicates on boats removed from infested waters of PEI could be spread on boats transported on trailers to any waters within 1600-2000 km of PEI – a distance encompassing the entire Atlantic seaboard of Canada.

Key words: invasive species, secondary spread, estuaries, Gulf of Saint Lawrence, exposure to air, bivalve aquaculture

Introduction

Until recently, negative effects of marine invasive species were not a major concern in the southern Gulf of St. Lawrence (sGSL). This changed abruptly in the mid-1990s with the arrival of a number of alien invaders including several species of ascidians (Locke et al. 2007). The clubbed tunicate (*Styela clava* Herdman, 1881), a solitary species, was first reported in Prince Edward Island (PEI), in 1997. The clubbed tunicate is thought to have arrived in the sGSL as a fouling organism on the hull of an ocean-going vessel coming from an international location because there are no other populations of this species in Canadian waters (Locke et al. 2007). Clubbed tunicate is not able to spread long distances by most natural vectors because it has a short planktonic larval phase of 12 to 36 hours (Stachowicz et al. 2002). Given the prevailing currents and semi-enclosed nature of the sGSL, and its remoteness from possible source populations, it is unlikely that metamorphosed juvenile or adult clubbed tunicates entered the sGSL attached to drifting seaweed or woody debris.

The clubbed tunicate, like most ascidians, survives best in locations that are protected from strong wave action, e.g., harbors and embayments (Osman and Whitlatch 1999; Davis and Davis 2007). In the sGSL, this species is only found in protected estuaries. Invasive tunicates typically thrive on artificial substrates such as floating docks, pilings, buoys, and boat hulls (Glasby and Connell 1999; Lambert 2002). The clubbed tunicate is no exception and is often very abundant on artificial substrates and at much higher densities than on natural substrates

(Lützen 1999). In sGSL estuaries, there is almost no natural hard substrate available and clubbed tunicate is primarily found on mussel-culture gear, such as ropes, socks, buoys, and anchors (Bourque et al. 2005; LeBlanc et al. 2007; Locke et al. 2007).

Areas protected from strong wave action that enhance the survival of ascidians are also often chosen by humans as locations for harbors and marinas. The increase in world-wide shipping and recreational boating during the last several decades and the associated facilities needed to dock these vessels has led to a dramatic rise in the amount of substrate available to fouling species for settlement and growth (Floerl and Inglis 2003; Minchin et al. 2006). Consequently, alien tunicates are now the dominant filter-feeders found in many marinas and harbors, worldwide. These structures also facilitate the spread of invasive ascidians (Lambert and Lambert 1998; Lambert 2002) because tunicate-infested harbors act as the source of new invasions to other local areas, with boats acting as one of the primary vectors (Osman and Whitlatch 1999).

Many vectors that spread alien species in marine systems from their initial colonization sites are associated with boating and aquaculture activities. For example, the areas of south-eastern PEI that have been heavily invaded by the clubbed tunicate are also the locations of many harbors, marinas, and intense bivalve aquaculture operations; therefore, it is likely that this invader is being transported between locations by a variety of vessel types. In addition, some of these boats are transported overland (trip durations of hours to days) to other locations in eastern Canada or the northeastern United States. The overland transportation of invaders could allow them to spread more rapidly and to more distant locations than by water-based mechanisms alone; unfortunately, little is known about this species' ability to survive exposure to air aside from the observation that they survive for short periods in tropical locations (Holmes 1976).

The goal of this study was to assess the potential for the clubbed tunicate to be spread by boating activities. The first part of this study tested whether clubbed tunicate settlement differed among the most common boat-hull materials; the effectiveness of antifouling paints against tunicate settlement; and the effect of hull color on settlement rate. The second part of this study tested the ability of the clubbed tunicate to withstand atmospheric exposure under Atlantic Maritime summer conditions similar to those that would be experienced on boats being transported overland on trailers.

Methods

Study site

The clubbed tunicates used in this study were collected on settlement plates deployed on 18 June 2003 in Murray River, PEI (46°01.609'N; 62°33.750'W). Summer water temperature often exceeds 25°C, and salinity in the lower estuary varies between 20 and 30‰ (Drinkwater and Petrie 1988). A large amount of blue mussel, *Mytilus edulis*, aquaculture occurs in Murray River, most of which was heavily infested with clubbed tunicates at the time of the study.

Settlement on hull materials

To determine the susceptibility of commonly used hull materials to colonization by the clubbed tunicate, 180 settlement plates (10 X 10 cm) were constructed of either fiberglass, wood (white spruce), or aluminum. These substrates were chosen as representative boat hull materials following consultation with the major boat builders on PEI. Antifouling and untreated exterior paints were used to test the effectiveness of the antifouling paint in preventing settlement. We compared black and white paint to determine if there was a preference for dark- *versus* light-colored hulls.

Following the normal practice of boat builders, the fiberglass plates were treated with a de-waxer before being painted, and the aluminum plates were left unpainted. All painted plates were given two coats of paints in accordance with the manufacturer's instructions. There were 20 plates of each of the following treatments: (1) fiberglass + black antifouling paint; (2) fiberglass + white antifouling paint; (3) fiberglass + black exterior paint; (4) fiberglass + white exterior paint; (5) wood + black anti-fouling paint; (6) wood + white antifouling paint; (7) wood + black exterior paint; (8) wood + white exterior paint; and (9) bare aluminum. The black antifouling paint used was Nautical Komposition KL-990™; the white antifouling paint was Interlux Tri-Lux II™. Both were copper-based ablative (i.e., designed to slough off over time) antifouling paints manufactured by International Paint Inc., Union, NJ, USA.

These were the antifouling paints most commonly used by boaters on PEI, as determined from an informal survey of boat builders and boating supply stores. The black and white exterior (house) paints were Dutch Boy[TM] exterior oil with gloss enamel. We have observed similar exterior house paints used in various locations around Atlantic Canada on small wooden vessels, such as fishing scows, reapplied annually as an alternative to more expensive boat paints.

One plate of each treatment type was attached in a random order to ropes that were 1.6 cm in diameter and 3 m long (Figure 1). The ropes were attached to a mussel line normally used to hang mussel socks in Murray River. The plates were left in the water for two weeks to accumulate a biofilm before the experiment commenced. Plates were collected 1, 2, 4, 8 and 12 weeks after this initial period with the last set of plates being removed on 22 September 2003. Four replicates of each treatment were collected during each sampling period. The treated side of each plate was scraped clean of all attached organisms upon removal from the water, and the contents were stored in 5% seawater-formalin. These samples were examined in the laboratory, and the number of clubbed tunicates was recorded.

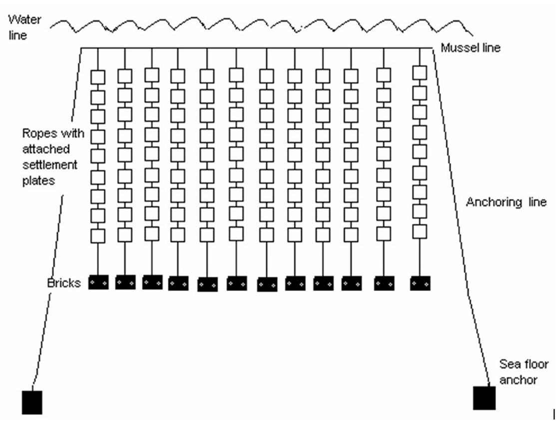

Figure 1. Tunicate settlement: experimental setup, showing how lines of settlement plates were suspended from a buoyed line normally used for mussel aquaculture.

Tolerance to atmospheric exposure

A random-block design was used for the exposure experiment. The 10 X 10 cm plates (24 of each) used for this experiment were: (1) fiberglass + black exterior paint; and (2) bare aluminum. The settlement plates used to collect tunicates for this experiment were left in the water for over a year to allow tunicates time to settle and grow. Settlement plates were removed from the water on 1 September 2004, 440 days after being placed in the water. The plates were immediately transported to the exposure location (an unshaded area on a lawn in Charlottetown, PEI) and placed on a tarpaulin to be exposed to the atmosphere. Three replicates per treatment were examined for dead tunicates after 1, 2, 4, 8, 16, 32, and 48 hours of exposure to air.

Mortality was determined by the use of the vital stain, Janus Green B. The stain was dissolved in seawater to form a 0.05% solution. After the allotted exposure time, the tunicates were placed in the staining solution for 30 minutes to give the tunicates time to open their siphons and begin to filter water. After staining, the tunicates were placed in jars containing a 5% seawater-formalin solution. Preserved specimens were transported to the laboratory where each tunicate was cut open to check for the presence of living pharyngeal tissue, which the Janus Green B stained a purplish color. Mortality was defined as the absence of purple-hued pharyngeal tissue.

Statistical analysis

The weekly abundance data from the settlement experiment had heterogeneous variances that could not be corrected by transformation; therefore, these data were analyzed using a Kruskal-Wallis analysis of variance to evaluate settlement on hull materials. A separate Kruskal-Wallis test was performed to test for the effect of painted versus unpainted substrates and to examine the effect of paint color. The Mann-Whitney test was used for *a posteriori* comparisons of means.

For the exposure experiment, the variances of the percentage of living tunicates were heterogeneous even after transformation. Consequently, the Kruskal-Wallis test was used to test for the effects of exposure time and substrate type. All *a posteriori* comparisons were done using the Mann-Whitney test. All statistical analyses were carried out using SPSS v.13 (SPSS, 2004).

Results

Settlement on hull materials

There was no settlement of tunicates on any of the treatment plates during weeks 1 and 2 of the experiment. During week 4, a few tunicates were found on three of the aluminum plates, and one tunicate on each of a fiberglass + black exterior paint plate and a wood + white exterior paint plate. Due to low numbers of tunicates in all treatments in weeks 1 through 4, only data from weeks 8 and 12 were included in subsequent analyses.

Tunicate settlers were most abundant (~ 400 individuals/100 cm^2) on the aluminum plates (weeks 8 and 12) followed by wood and fiberglass plates treated with black exterior paint (Table 1). All hull materials treated with antifouling paint had very low densities of tunicates on them (average < 5 individuals/100 cm^2). Due to low number of settlers, all antifouling-treatment data were omitted from further analyses (i.e., the anti-fouling paint was effective throughout the 12-week experiment). There was no significant difference (P > 0.05) in the number of settled tunicates on painted wood and fiberglass plates (Table 2). There was, however, a significant difference (P < 0.01) in the number of settlers between weeks, with the number of settlers in week 12 being far greater than in week 8. There were also significantly more (P < 0.01) settlers on the plates treated with black exterior paint compared to white exterior paint.

Because there was no significant difference in the number of tunicate settlers on fiberglass and wood plates, the data for these materials were combined into two categories based on color. There was a significant difference (χ^2 = 17.418; df = 2; P < 0.001) in the number of settlers on bare aluminum, black painted, and white painted plates. The number of settlers on the bare aluminum plates and black painted plates did not differ, but there were significantly more (P < 0.001) settlers on the aluminum plates compared to those plates painted white and significantly more (P = 0.003) settlers on the plates painted black compared to white (Figure 2). These results were the same for both weeks.

Tolerance to atmospheric exposure

The average density of tunicates on the plates exposed to air was 64.7 individuals/100cm^2. The tunicates ranged in length from 2 to 10 cm. The weather during the air-exposure experiment was mainly sunny and clear with a few showers at about 8 hours of exposure (Figure 3). The mean daytime high was 29.7°C and night-time low was 8.5^{o}C. The mean day-time humidity was 92.5% compared with 33.5% at night.

The majority of tunicates survived the 48 hour exposure period regardless of the treatment (Figure 4). The overall survival rate after 48 h was almost 90%. The treatment with the fewest survivors (±SE) was aluminum after 32 hrs of exposure (89.3 ± 8.9% survival) while there were no deaths on fiberglass after 2 hours of exposure and aluminum after 8 hours exposure.

Based on percentage of tunicates surviving, there was no effect of plate type but there was a small effect of exposure time (Table 3), although

Table 1. Mean (± SE) number of individuals/100cm^2 of clubbed tunicate settlers on settlement plates by hull surface type and paint treatments.

Treatment	Week				
(Substrate + Paint)	1	2	4	8	12
Aluminum	0	0	1.7 (±0.71)	370.5 (±87.91)	422.8 (±39.04)
Fiberglass + Black Antifouling	0	0	0	2.1 (±0.48)	4.7 (±2.52)
Fiberglass + White Antifouling	0	0.3 (±0.27)	0	2.8 (±1.08)	3.2 (±0.88)
Fiberglass + Black Exterior	0	0	0.2 (±0.25)	232.0 (±70.95)	328.4 (±66.89)
Fiberglass + White Exterior	0	0	0	90.8 (±27.33)	182.6 (±38.87)
Wood + Black Antifouling	0	0	0	3.6 (±1.85)	1.3 (±1.28)
Wood + White Antifouling	0	0	0	0.7 (±0.46)	3.8 (±3.42)
Wood + Black Exterior	0	0	0	155.8 (±75.23)	347.9 (±34.74)
Wood + White Exterior	0	0	0.2 (±0.24)	28.1 (±6.62)	202.5 (±13.55)

Table 2. Results of Kruskal-Wallis analysis for the effects of week, plate type, and plate color on clubbed tunicate settlement.

Source of Variation	df	χ^2	*P*
Week	1	9.320	0.002
Plate Type	1	0.364	0.546
Plate Color	1	8.642	0.003

Table 4. Results of pair-wise comparisons (Mann-Whitney U-test) for the effect of atmospheric exposure time on clubbed tunicate survival. Differences in survival at all times between 0 and 8 hours were non-significant, NS (P>0.05).

	16 h	32 h	48 h
0 h	<0.005	<0.01	<0.05
1 h	NS	<0.05	NS
2 h	<0.005	<0.005	<0.01
4 h	<0.005	<0.005	<0.01
8 h	<0.05	<0.05	0.05
16 h		1	NS
32 h			NS

this is not relevant from a practical standpoint given the large number of tunicates that survived. Statistically, there were significantly fewer tuni-cates alive at 16 and 32 h compared to exposure times of 0, 2, 4 and 8 h (Table 4). There were also significantly more survivors at exposure times 0, 2 and 4 h compared to 48 h. Finally, there were significantly more survivors at time 1 h compared to time 32 h.

Discussion

Antifouling paints were effective in minimizing tunicate settlement on the treated plates throughout the experiment, as compared to the heavy infestations that developed on other surfaces. By week 8 settlers were abundant on the bare aluminum plates, and by week 12 tuni-cates were abundant on all plates not treated with antifouling paint. The earlier colonization of aluminum as opposed to wood or fiberglass contradicted the findings of Anderson and Underwood (1994), who observed greater recruitment of oysters and barnacles onto marine plywood than onto fiberglass or aluminum after four weeks of immersion. However, the wood used by Anderson and Underwood (1994) was unpainted and differed from the wood we used in

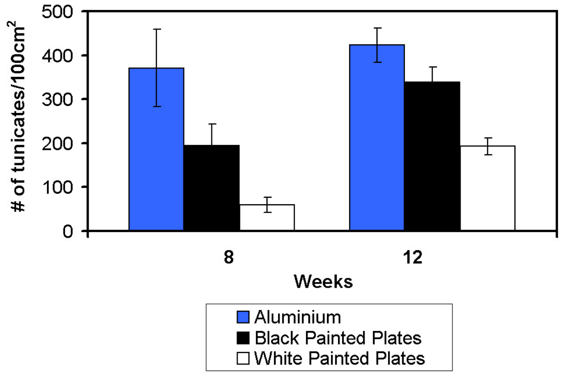

Figure 2. Mean (± SE) number of clubbed tunicate settlers on painted and unpainted settlement plates during weeks 8 and 12.

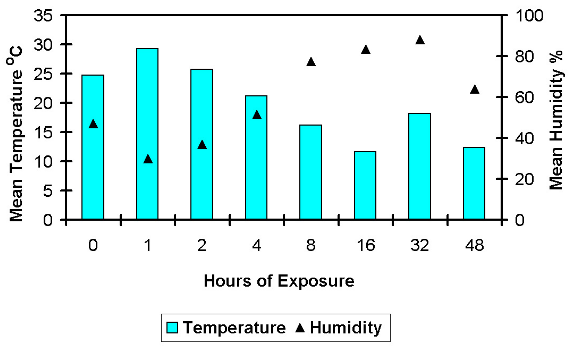

Figure 3. Mean temperature and humidity experienced by clubbed tunicates during atmospheric exposure experiment. Values are presented for each time interval between mortality checks. An asterisk under the hour of exposure indicates a rain event.

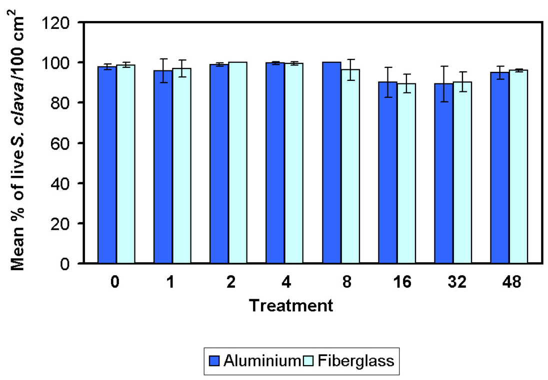

Figure 4. Survival of the clubbed tunicate to atmospheric exposure over a period of 48 hours. Error bars represent ±SE for % of surviving tunicates.

two important respects. First, the unpainted wood would have had more grooves and a rougher surface than our painted wood. Settlement in grooves is advantageous because it protects new recruits from becoming dislodged (Crisp and Barnes 1954). Second, our use of painted surfaces for all but the aluminum plates, because we were trying to reproduce the treatment given to vessel hulls in our study area, may have prevented tunicate larvae from settling as the newly painted surfaces release chemicals while they cure (Pomerat and Weiss 1946). By week 12 there was no difference in the number of tunicates on the black exterior-painted plates and the unpainted aluminum plates, which may be due to chemical residues from the paint having dissipated and the smoothness of the surface diminished due to corrosion by seawater.

Antifouling paints were fully effective against clubbed tunicates for at least two months after application but, by week 12, a very low level of infestation was visible on the plates. This is similar to observations in Australian waters where antifouling paints were effective for about two months (Floerl et al. 2005). The antifouling paint we used is ablative; i.e., it is designed to slough off over time and consequently needs to be re-applied regularly, although the manufacturer's instructions indicate the paint could be effective for up to two years. On the other hand, our test conditions (and those of Floerl et al. 2005) may not have been representative of the effectiveness of antifouling paint on a moving boat. The relatively low velocity of water past the mooring, compared to that experienced by most areas on a ship hull, may compromise the effectiveness of the coating to ablate and keep the test surface clean (Floerl et al. 2005; Ostrom 1995). Modern self-cleaning paints are designed for use on vessels that are sailing at least 70% of the time (Floerl et al. 2005). Nevertheless, while antifouling paints have been found to be effective against some invasive species (e.g., zebra mussels (*Dreissena polymorpha*); Sayder et al. 1991), they have previously been shown to be less effective against the settlement of ascidians than against settlement of organisms such as barnacles and bivalves (Floerl et al. 2005).

This study demonstrated that antifouling paints could reduce the risk of spreading tunicates, but hull materials not treated with antifouling paint are likely to be fouled by tunicates and could disperse them to new locations. This is particularly important for the bivalve aquaculture industry in PEI because many of the vessels involved in mussel culture have aluminum hulls that are not treated with antifouling chemicals in order to avoid harming the mussels being cultured. These vessels are often operating for long periods in areas where there is a high abundance of clubbed tunicates and offer near-ideal conditions for clubbed tunicate settlement because, as off-bottom substrates, they are largely inaccessible to predators (Osman and Whitlatch 1999). The cause for concern is that these vessels are often moved between PEI estuaries because mussel growers often lease sites in more than one estuary. Aquaculture operators acknowledge that equipment that was not cleaned properly before being moved has transported tunicates between estuaries (Locke et al. 2007), but at present there is no evidence for or against the transport of tunicates on their boat hulls as opposed to transport on other kinds of gear or on bivalve shells.

Boats used in bivalve aquaculture are not alone in their potential to spread invaders. Recreational boats are also of concern because many are only used once or twice a week and are left docked for days at a time, giving tunicates ample time to settle (Darbyson et al. 2009, this issue). Towed hulls such as barges, especially those used for dredging or other marine construction in harbors, are often heavily fouled (Locke, pers. obs.) and slowly towed hulls have been implicated in the dispersal of tunicates in other parts of the world (Lambert 2002).

Tunicates in this study recruited in greatest numbers to dark colored surfaces during weeks 8 and 12, which is consistent with findings for other fouling organisms such as spirorbid polychaetes and barnacles (Pomerat and Reiner 1942; James and Underwood 1994). Invertebrate larvae, including tunicates, use diffuse light as the major information source for orientation and settlement (Svane and Dolmer 1995). Clubbed tunicate larvae detect light with a small ocellus, which is made up of a pigmented retinal cell and a lens cell (Wallace 1961). Tunicate larvae initially swim upward toward the light and later swim or sink toward the bottom (Grave 1920, Mast 1921, Grave and Woodbridge 1924, Grave 1926). The sinking phase is critical for larval settlement, and is invoked by a decrease in light intensity; larvae swim to darker areas of the substrate and settle on either the vertical or under-surfaces of rocks and other substrates (Millar 1971). Young and Chia (1984) found that juvenile ascidians select shaded surfaces possibly to avoid the major sources of mortality (grazing herbivores, silt, algal overgrowth) associated with unshaded substrates. The larger number of clubbed tunicate recruits on dark coloured boat hull surfaces suggests that the use of lighter coloured paint may limit the numbers settling on boat hulls (for a short period) and, as a direct consequence, fewer are transported to new areas. In practice, this difference is of little consequence because it only resulted in about a 43% reduction in settlement after 12 weeks – there were still >180 individuals/100 cm^2 on white hull material. Consequently, the use of light colored boat hulls will have but a minor effect as a measure to slow the spread of this species.

This was the first study to examine the ability of the clubbed tunicate to survive atmospheric exposure. The low number of mortalities observed after 48 h of exposure was somewhat unexpected given that tunicates are soft bodied organisms that, despite their leathery tunic, would not appear to be well adapted to atmospheric exposure. However, this species does have the ability to survive and recover from short periods of intertidal exposure at tropical temperatures (Holmes 1976). It survives these temporary periods of exposure by closing its siphons to create a suitable microhabitat (Sims 1984). While it was thought that tunicates could survive a short length of exposure under sGSL summer conditions, we expected that most of the tunicates would have perished after a few hours. The results of this experiment indicate otherwise and, while the number of deaths did increase towards the end of the experiment, total mortality was quite low - no more than 11% died in any treatment.

The low mortality rate may be a result of several factors, one of which is that the tunicates occurred in large clumps on the plates. Ricciardi et al. (1995) found that zebra mussels also survive atmospheric exposure better in clumps. Further enhancing the probability of their survival is the fact that the animals used in these experiments were adults (age 1+) and their larger size and tougher tunic may have helped to minimize water loss relative to what might be expected from smaller individuals. Ricciardi et al. (1995) had similar findings for the zebra mussel where thermal tolerance and desiccation resistance increased with mussel size. Nevertheless, a recent study has concluded that air-exposure for 40 h (21°C, relative humidity 34%) is an ineffective method to kill all sizes of post-larval clubbed tunicates infesting mussel socks (LeBlanc et al. 2007); suggesting small-sized individuals may be almost as resistant to desiccation. Tunicates of the size used in the present study would most likely be found on aquaculture equipment which remains in the water for more than a season, whereas smaller individuals are more likely to be found on boats that are removed annually from the water during the winter months.

The ability of an invasive species to withstand exposure and survive overland transport is a key element in the development of dispersal models to predict the rate of spread of organisms from infested to un-infested areas, and even more importantly in the management of invasive species. Given that the tunicates in this study survived at least two days out of water in summer conditions, the potential range of over-land transport covers all of Atlantic Canada and much of the northeast of the USA. It is almost inevitable that commercial and recreational boats traveling by water will spread the clubbed tunicate to new locations in Atlantic Canada in the next decade or two, but the ability of the species to survive out of water on trailered boats greatly increases the potential range and rate of spread.

Management of trailered boats as a vector in the dispersal of invasive species has most often been attempted against freshwater organisms (e.g., zebra mussel and Eurasian milfoil, *Myriophyllum spicatum*; Johnson et al. 2001), but could also benefit the marine environment and associated industries. Prevention of spread of tunicates around PEI is a major challenge because boats are transported by land and water from a large number of sources and carried to many locations. It is unlikely, and almost impossible to enforce, that all boats moving between ports by water would be thoroughly cleaned before removal from an infested estuary. Given that the only group severely affected by invasive tunicates at the present time is the bivalve aquaculture industry, there is no incentive for recreational boat operators or commercial fishermen to absorb the cost of removing their boats from the water for cleaning before traveling by water between ports. On the other hand, inspection of boats and trailers departing from tunicate-infested ports, or codes of practice and public education for the cleaning of boats before re-launching elsewhere, could reduce the likelihood of tunicate transport to new sites in PEI by the overland vector. Preventing the overland transport of alien species off PEI could be as simple as establishing control points at the two points of egress from PEI: the Confederation Bridge and Wood Island-Caribou Ferry. Slowing the spread of invasive species by land transport might help to give the aquaculture industry operators the time to adapt to the almost inevitable spread of new species to all aquaculture leases on PEI and, eventually, to all suitable waters of the sGSL. The relative importance of overland transport, as compared to waterborne boat-based transport and indeed to other vectors (e.g., transport on crabs and lobsters, see Bernier et al., this issue) remains to be investigated with respect to the effectiveness of any vector-control management.

Acknowledgements

This work was funded by an Enviromental Sciences Strategic Research Fund (Fisheries and Oceans Canada) grant to AL and JMH.ED received additional support from the Lett Fund (Dalhousie University). Technical support was provided by R. Bernier and K. Ellis. Statistical advice was provided by R. Scheibling.

References

Anderson MJ, Underwood AJ (1994) Effects of substratum on the recruitment and development of an intertidal estuarine fouling assemblage. *Journal of Experimental Marine Biology and Ecology* 184: 217-236, doi:10.1016/0022-0981(94)90006-X

Bernier RY, Locke A, Hanson JM (2009) Lobsters and crabs as potential vectors for tunicate dispersal in the southern Gulf of St. Lawrence, Canada. *Aquatic Invasions* 4: 107-112, doi:10.3391/ai.2009.4.1.11

Bourque D, MacNair N, Leblanc A, Landry T, Miron G (2005) Preliminary study of the diel variation of ascidian larvae concentrations in Prince Edward Island. Canadian Technical Report of Fisheries and Aquatic Sciences No. 2571

Crisp DJ, Barnes H (1954) The orientation and distribution of barnacles at settlement with particular reference to surface contour. *Journal of Animal Ecology* 23: 142-162, doi:10.2307/1664

Darbyson E, Locke A, Hanson JM, Willison JHM (2009) Marine boating habits and the spread of invasive species in the Gulf of St. Lawrence. *Aquatic Invasions* 4: 87-94, doi:10.3391/ai.2009.4.1.9

Davis MH, Davis ME (2007) The distribution of *Styela clava* (Tunicata, Ascidiacea) in European waters. *Journal of Experimental Marine Biology and Ecology* 342: 182-184, doi:10.1016/j.jembe.2006.10.039

Drinkwater K, Petrie B (1988) Physical oceanography observations in the Cardigan Bay region of Prince Edward Island 1982-1987. Canadian Technical Report of Hydrography and Ocean Sciences 110

Floerl O, Inglis GJ (2003) Boat harbour design can exacerbate hull fouling. *Austral Ecology* 28: 116-127, doi:10.1046/j.1442-9993.2003.01254.x

Floerl O, Inglis GJ, Marsh HM (2005) Selectivity in vector management: an investigation of the effectiveness of measures used to prevent transport of non-indigenous species. *Biological Invasions* 7: 459-475, doi:10.1007/s10530-004-4863-5

Glasby TM, Connell SD (1999) Urban structures as marine habitats. *Ambio* 28: 595-598

Grave C (1920) *Amaroucium pellucidum* (Leidy) form *constellatum* (Verrill). I. The activities and reactions of the tadpole larva. *Journal of Experimental Marine Biology and Ecology* 30: 239-257

Grave C (1926) *Molgula citrina* (Alder and Hancock). Activities and structure of the free-swimming larva. *Journal of Morphology* 42: 453-471, doi:10.1002/jmor.1050420205

Grave C, Woodbridge H (1924) *Botryllus schlosseri* (Pallas): the behaviour and morphology of the free-swimming larva. *Journal of Morphology* 39: 207-247, doi:10.1002/jmor.1050390107

Holmes N (1976) Occurrence of the ascidian *Styela clava* Herdman in Hobsons Bay, Victoria: A new record for the southern hemisphere. *Proceedings of the Royal Society of Victoria* 88: 115-116

James RJ, Underwood AJ (1994) Influence of colour of substratum on recruitment of spirorbid tubeworms to different types of intertidal boulders. *Journal of Experimental Marine Biology and Ecology* 181: 105-115, doi:10.1016/0022-0981(94)90107-4

Johnson LE, Ricciardi A, Carlton JT (2001) Overland dispersal of aquatic invasive species: a risk assessment of transient recreational boating. *Ecological Applications* 11: 1789-1799, doi:10.1890/1051-0761(2001)011[1789:ODOAIS]2.0.CO;2

Lambert CC, Lambert G (1998) Non-indigenous ascidians in southern California harbors and marinas. *Marine Biology* 130: 675-688, doi:10.1007/s002270050289

Lambert G (2002) Nonindigenous ascidians in tropical waters. *Pacific Science* 3: 291-298, doi:10.1353/psc.2002.0026

LeBlanc N, Davidson J, Tremblay R, McNiven M, Landry T (2007) The effect of anti-fouling treatments for the clubbed tunicate on the blue mussel, *Mytilus edulis*. *Aquaculture* 264: 205-213, doi:10.1016/j.aquaculture.2006.12.027

Locke A, Hanson JM, Ellis KM, Thompson J, Rochette R (2007) Invasion of the southern Gulf of St. Lawrence by the clubbed tunicate (*Styela clava* Herdman): Potential mechanisms for invasions of Prince Edward Island estuaries. *Journal of Experimental Marine Biology and Ecology* 342: 69-77, doi:10.1016/j.jembe.2006.10.016

Lützen J (1999) *Styela clava* Herdman (Urochordata, Ascidea), a successful immigrant to North West Europe: Ecology, propagation and chronology of spread. *Helgoland Marine Research* 52: 383-391

Mast SO (1921) Reactions to light in the larvae of the ascidians, *Amaroucium constellatum*, *Amaroucium pellucidum* with special reference to photic orientation. *Journal of Experimental Zoology* 34: 149-187, doi:10.1002/jez.1400340204

Millar RH (1971) The biology of ascidians. *Advances in Marine Biology* 9: 1-100, doi:10.1016/S0065-2881(08)60341-7

Minchin D, Floerl O, Savini D, Occhipinti-Ambrogi A (2006) Small craft and the spread of exotic species. In: Davenport J, Davenport JL (eds) The ecology of transportation: managing mobility for the environment. Springer, The Netherlands, pp 99-118, doi:10.1007/1-4020-4504-2_6

Osman RW, Whitlatch RB (1999) Ecological interactions of invading ascidians within epifaunal communities of southern New England. In: Pedersen J (ed) Marine Bioinvasions: Proceedings of the first national conference, pp 164-174

Ostrom W (1995) Field testing of antifouling coatings. Woods Hole Oceanographic Institute Upper Ocean Processes Group Technical Note 9508, 4 pp

Pomerat CM, Reiner ER (1942) The influence of surface angle and of light on the attachment of barnacles and other sedentary organisms. *Biological Bulletin* 82: 14-25, doi:10.2307/1537933

Pomerat CM, Weiss CM (1946) The influence of texture and composition of surface in the attachment of sedentary marine organisms. *Biological Bulletin* 91: 57-65, doi:10.2307/1538033

Ricciardi A, Serrouya R, Whoriskey FG (1995) Aerial exposure of zebra and quagga mussels (Bivalvia: Dreissenidae): implications for overland dispersal. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 470-477, doi:10.1139/f95-048

Sayder FL, Kelch DO, Lichtkoppler FR (1991) Field performance of selected commercial antifouling coatings against the zebra mussel (*Dreissena polymorpha*). *Journal of Shellfish Research* 10: 254-255

Sims LL (1984) Osmoregulatory capabilities of three macrosympatric stolidobranch ascidians, *Styela clava* Herdman, *S. plicata* (Lesueur), and *S. montereyensis* (Dall). *Journal of Experimental Marine Biology and Ecology* 82: 117-129, doi:10.1016/0022-0981(84)90098-4

SPSS (2004) SPSS statistical analysis package, Volume 13. 233 S. Wacker Drive, 11th floor, Chicago, Illinois 60606: SPSS Inc.

Stachowicz JJ, Fried H, Osman RW, Whitlatch RB (2002) Biodiversity, invasion resistance and marine ecosystem function: reconciling pattern and process. *Ecology* 83: 2575-2590, doi:10.1890/0012-9658(2002)083[2575:BIRAME]2.0.CO;2

Svane I, Dolmer P (1995) Perception of light at settlement: a comparative study of two invertebrate larvae, a scyphozoan planula and a simple ascidian tadpole. *Journal of Experimental Marine Biology and Ecology* 187: 51-61, doi:10.1016/0022-0981(94)00171-9

Wallace H (1961) The breeding and development of *Styela mammiculata* Carlisle. *Journal of the Marine Biological Association of the United Kingdom* 41: 187-190, doi:10.1017/S0025315400001636

Young CM, Chia FS (1984) Microhabitat-associated variability in survival and growth of subtidal solitary ascidians during the first 21 days after settlement. *Marine Biology* 81: 61-68, doi:10.1007/BF00397626