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Short communication

In situ growth of the colonial ascidian *Didemnum vexillum* under different environmental conditions

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Abstract

We assessed the relative growth of *Didemnum vexillum* under different environmental conditions. Pouches containing pre-weighed *D. vexillum* colonies were deployed at three different depths and in three different salinity regimes for two weeks. Upon recovery, colonies were reweighed to determine their change in biomass. *D. vexillum* grew fastest in shallow water (1.0 m) and in high salinity areas (26-30 ppt).

Key words: *Didemnum vexillum*, depth, growth rate, invasion, salinity

The colonial ascidian *Didemnum vexillum* Kott, 2002 is rapidly spreading on both coasts of North America and in many parts of the world (Bullard et al. 2007a). There is growing concern about the ecological and economic impacts that the species may have on infested regions (e.g., Coutts and Forrest 2007; Valentine et al. 2007). To help determine which coastal habitats are most at risk of *D. vexillum* colonization, we assessed its relative growth under different environmental conditions (water depth and salinity levels).

Pre-weighed *D. vexillum* colony fragments were deployed in plastic mesh pouches in different habitats near Groton, Connecticut during July and August 2006. Seawater surface temperatures during this period ranged from ~18-26°C. For each experiment 25-50 separate colonies were collected and torn into ~1-2 g pieces. Each fragment (hereafter referred to as "colony") was weighed and placed in a Vexar pouch (~15 x 15 cm, 0.6 cm mesh). Pouches were held in flow-through seawater tables for 48-72 h to facilitate colony attachment and to ensure colony viability (e.g., Bullard et al. 2007b), they were then transported to study sites and deployed for two weeks. Upon recovery, *D. vexillum* colonies were re-weighed. The change in biomass for each colony was determined by dividing its ending biomass by beginning biomass. Some *D. vexillum* colonies disappeared from pouches during deployments; a few (<10%) probably washed through ruptures in the pouches, but most died during deployment. Data from missing colonies were excluded from relative growth comparisons. Differences in *D. vexillum* colony survivorship within each experi-ment were examined using one-way ANOVAs on the arcsine transformed mean percentage of remaining colonies from each treatment.

For the depth experiment, twelve pouches were deployed at three depths at three separate sites (108 total pouches deployed; each site ~100 m apart). Depths were 1.0 m, 2.5 m and 4.0 m below the surface. Pouches were attached to PVC racks suspended from a weighted buoy line. Because the buoy line rose and fell with the tide, racks always remained the same relative distance from the surface. A nested two-factor ANOVA was used to examine differences in *D. vexillum* growth, with site and depth being factors and depth being nested within site.

For the salinity experiment twelve pouches were deployed at each of nine sites (108 total

pouches deployed) in the Thames River estuary. These sites experienced different salinity regimes: high = 26-30 ppt (mouth of the river; n =3), medium = 15-28 ppt (~6 km upstream; n =3), low = 10-26 ppt (~12 km upstream; n =3). Salinity values represent the measured salinity range (bottom water measured with a refractometer). Relatively few salinity measurements were taken so each area may have experienced a wider salinity range during experiments. Each site was ~1.0-1.5 m depth at low tide and all were at least 100 m apart. Pouches were secured to fixed weights and held ~0.3 m off the bottom. A one-way ANOVA compared mean growth rates among salinities (n = 3 sites for each salinity).

Different environmental conditions greatly affected the growth of *D. vexillum*. In the depth experiment, significant differences were detected in *D. vexillum* growth at different depths ($P < 0.001$) and at different sites ($P = 0.002$) (Figure 1). Growth rates were significantly higher in high salinity areas (Figure 2). Two week survivorship of *D. vexillum* was not significantly different among salinities or depths (Table 1), but most remaining *D. vexillum* colonies in low

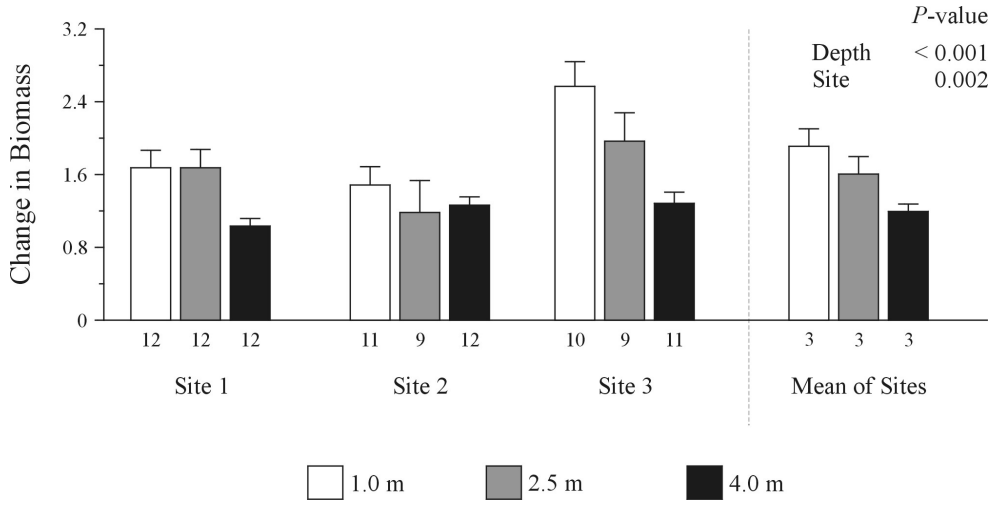

Figure 1. Relative growth of *Didemnum vexillum* at different depths. Numbers at the base of the histograms indicate the number of colonies remaining at the end of the experiment (n = 12 initially deployed at all sites). *P*-values are from a nested two-factor ANOVA with depth and site as factors and depth nested within site.

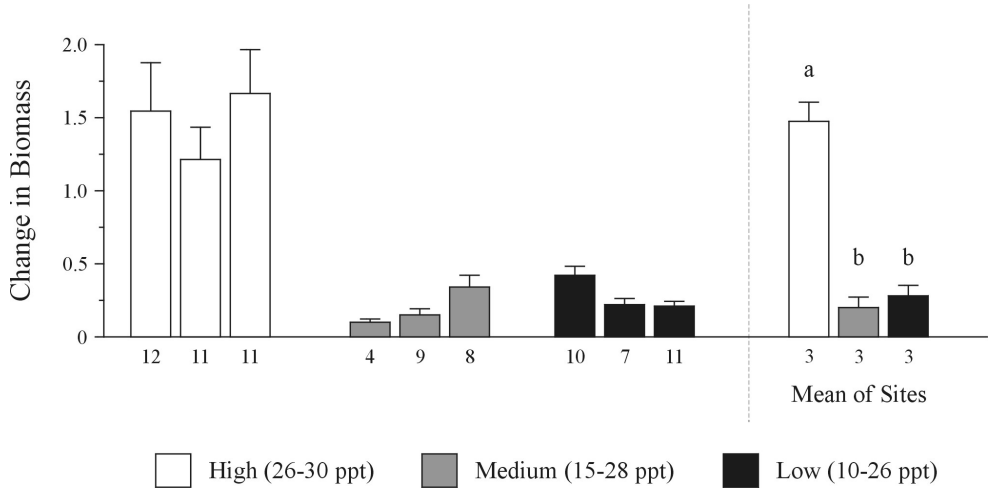

Figure 2. Relative growth of *Didemnum vexillum* at different salinities. Numbers at the base of the histograms indicate the number of colonies remaining at the end of the experiment (n = 12 initially deployed at all sites). Letters indicate significant differences (Tukey-Kramer test).

Table 1. Mean survivorship (± 1 SE) of *Didemnum vexillum*. *P*-values are the results from ANOVAs. n = 3 for all treatments.

Experiment	Treatment			*P*-value
Depth	Shallow (1.0m)	Mid (2.5m)	Deep (4.0m)	
Survivorship	92 ± 5%	83 ± 14%	97 ± 5%	0.469
Salinity	High (26-30 ppt)	Med (15-28 ppt)	Low (10-26 ppt)	
Survivorship	94 ± 3%	58 ± 13%	78 ± 10%	0.061

and medium salinity areas were bloated, discolored (bright orange) and appeared to be dying.

It is unclear why *D. vexillum* grew faster in shallow water, but it does not appear to be due to temperature differences. Temperature loggers indicated that the 1.0 m depth was only 0.55°C warmer on average than the 4.0 m depth and all depths experienced similar measured ranges of temperatures (from ~20.0 to 25.6°C). It is more likely that other factors, such as food availability, allowed *D. vexillum* to grow faster in shallow water. Phytoplankton are a major source of food for ascidians (e.g., Seiderer and Newell 1988; Petersen and Riisgard 1992) and are likely more abundant in shallow water, even in well mixed nearshore waters. If more food was available at shallower depths, shallow colonies may have been able to grow faster than deeper colonies. Clearly, additional work is needed to specifically determine what factors contributed these observed *D. vexillum* growth patterns.

The results of our depth experiment were somewhat unexpected because many of the largest *D. vexillum* populations have been found at depths >30 m (Bullard et al. 2007a). Thus, one might predict that the species would grow faster in deeper water. However, while *D. vexillum* grew statistically faster in shallow water during our experiments, it grew well at all of the depths examined. In two weeks its mean biomass increased by 60% at 2.5 m and 19% at 4.0 m. Relatively rapid deep water growth coupled with a reduction in spatial competitors at deep sites may explain its dominance in deep water areas (e.g., Bullard et al. 2007a; Osman and Whitlatch 2007). At the same time, rapid shallow water growth may facilitate the ability of this species to quickly colonize shallow water locations. This may be especially true when other competitors are absent such as after disturbance events, due to seasonal changes in species abundance, or from the construction of new artificial surfaces (e.g., Altman and Whitlatch 2007; Osman and Whitlatch 2007; Locke et al. 2007).

D. vexillum grew best in high salinity areas; it grew poorly and appeared to be dying in lower salinity areas (Figure 2, Table 1). This is not unexpected given that ascidians do not generally grow well in low salinity areas (Vazquez and Young 2000; Thiyagarajan and Qian 2003). For example, in an extensive survey of thirty-three Connecticut and Rhode Island docks conducted during fall 2003, we never found ascidians in areas with salinities of <20 ppt (R. Whitlatch unpub. data). Similarly, in the laboratory most *D. vexillum* colonies appeared dying after one week in 20 ppt seawater while those in 30 ppt seemed healthy (S. Bullard unpub. data).

The rapid grow rates of *D. vexillum* in shallow, high salinity areas suggests that many coastal sites are susceptible to *D. vexillum* colonization. Additional work is needed to identify other factors that may ecologically limit *D. vexillum,* such as temperature tolerances (e.g., McCarthy et al. 2007). Work is also need to determine how to slow the spread of the species and to control it once it has invaded an area.

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References

Altman S, Whitlatch RB (2007) Space invaders: the effect of small-scale disturbance on invasion success in marine communities. *Journal of Experimental Marine Biology and Ecology* 342: 15-29, doi:10.1016/j.jembe.2006.10.011

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 (2007a) The colonial ascidian *Didemnum* sp. A: current distribution, basic biology, and potential threat to marine communities of the northeast and west coasts of North America. *Journal of Experimental Marine Biology and Ecology* 342: 99-108, doi:10.1016/j.jembe.2006.10.020

Bullard SG, Sedlack B, Reinhardt JF, Litty C, Gareau K, Whitlatch RB (2007b) Fragmentation of colonial ascidians: differences in reattachment capability among species. *Journal of Experimental Marine Biology and Ecology* 342: 166-168, doi:10.1016/j.jembe.2006.10.034

Coutts ADM, Forrest BM (2007) Development and application of tools for incursion response: lessons learned from the management of the fouling pest *Didemnum vexillum*. *Journal of Experimental Marine Biology and Ecology* 342: 154-162, doi:10.1016/j.jembe.2006.10.042

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

McCarthy A, Osman RW, Whitlatch RB (2007) Effects of temperature on growth rates of colonial ascidians: a comparision of *Didemnum* sp. to *Botryllus schlosseri* and *Botrylloides violaceus*. *Journal of Experimental Marine Biology and Ecology* 342: 172-174, doi:10.1016/j.jembe.2006.10.036

Osman RW, Whitlatch RB (2007) Variation in the ability of *Didemnum* sp. to invade established communities. *Journal of Experimental Marine Biology and Ecology* 342: 40-53, doi:10.1016/j.jembe.2006.10.013

Petersen JK, Riisgard HU (1992) Filtration capacity of the ascidian *Ciona intestinalis* and its grazing impact in a shallow fjord. *Marine Ecology Progress Series* 88: 9-17, doi:10.3354/meps088009

Seiderer LJ, Newell RC (1988) Exploitation of phytoplankton as a food resource by the kelp bed ascidian *Pyura stolonifera*. *Marine Ecology Progress Series* 50: 107-115, doi:10.3354/meps050107

Thiyagarajan V, Qian PY (2003) Effect of temperature, salinity and delayed attachment on development of the solitary ascidian *Styela plicata* (Lesueur). *Journal of Experimental Marine Biology and Ecology* 290: 133-146, doi:10.1016/S0022-0981(03)00071-6

Valentine PC, Collie JS, Reid RN, Asch RG, Guida VG, Blackwood DS (2007) 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

Vazquez E, Young CM (2000) Effects of low salinity on metamorphosis in estuarine colonial ascidians. *Invertebrate Biology* 119: 433-444