Ecological observations on the colonial ascidian *Didemnum* sp. in a New England tide pool habitat ☆

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Abstract

The colonial ascidian *Didemnum* sp. has colonized northwestern Atlantic coastal habitats from southern Long Island, New York, to Eastport, Maine. It is also present in offshore habitats of the Georges Bank fishing grounds. It threatens to alter fisheries habitats and shellfish aquacultures.

Observations in a tide pool at Sandwich, MA from December 2003 to February 2006 show that *Didemnum* sp. tolerates water temperatures ranging from ≤1 to >24 °C, with daily changes of up to 11 °C. It attaches to pebbles, cobbles, and boulders, and it overgrows other tunicates, seaweeds, sponges, and bivalves. From May to mid July, colonies appear as small patches on the bottoms of rocks. Colonies grow rapidly from July to September, with some growth into December, and they range in color from pink to pale yellow to pale orange. Colony health declines from October through April, presumably in response to changes in water temperatures, and this degenerative process is manifested by color changes, by the appearance of small dark brown spots that represent clumps of fecal pellets in the colony, by scavenging by periwinkles, and by a peeling-away of colonies from the sides of cobbles and boulders. At Sandwich, colonies died that were exposed to air at low tide. The species does not exhibit this seasonal cycle of growth and decline in subtidal habitats (40–65 m) on the Georges Bank fishing grounds where the daily climate is relatively stable and annual water temperatures range from 4 to 15 °C. Experiments in the tide pool with small colony fragments (5 to 9 cm²) show they re-attach and grow rapidly by asexual budding, increasing in size 6- to 11-fold in the first 15 days. *Didemnum* sp. at Sandwich has no known predators except for common periwinkles (*Littorina littorea*) that graze on degenerating colonies in the October to April time period and whenever colonies are stressed by desiccation.

The tendencies of the ascidian (1) to attach to firm substrates, (2) to rapidly overgrow other species, (3) to tolerate a wide temperature range, (4) to be free from predation, and (5) to spread by colony fragmentation combine to make it a potential threat to benthic marine habitats and aquacultures. *Didemnum* sp. is known to overgrow mussels, oysters, and sea scallops, and it likely envelops other bivalves too.

1. Introduction

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Competitively aggressive colonial ascidians of the genus *Didemnum* occur in the coastal waters of the northeastern United States, from Long Island, New York, to Eastport, Maine, and in New England offshore

waters on the Georges Bank fishing grounds (Carman and Roscoe, 2003; Bullard et al., 2006-this volume). The earliest coastal records of these organisms are an anecdotal report from the Damariscotta River in Maine in 1988 and a documented occurrence in 1993; and they were documented on Georges Bank in 2002 and further recorded there in 2003, 2004, and 2005 (U.S. Geological Survey, 2006).

Within the past few years, several species of the colonial tunicate genus *Didemnum* from different parts of the world have drawn attention as nuisance species because they reproduce rapidly and foul ship's hulls, maritime structures, and marine habitats (including shellfish aquacultures and fishing grounds). Two species have been formally named: *Didemnum vexillum* described from the North and South Islands of New Zealand (Kott, 2002); and *Didemnum vestum*, described from Portsmouth Harbor, New Hampshire, USA (Kott, 2004). Species of the genus *Didemnum* that are possibly different but related to *D. vexillum* and *D. vestum* have been recorded from Europe, the U.S. west coast, British Columbia, and from coastal and offshore regions of New England other than New Hampshire. These organisms (including those in the tide pool at Sandwich, Massachusetts) have not yet been formally compared to either

D. vexillum or *D. vestum* in the published literature, and here they are provisionally called *Didemnum* sp. for purposes of communication.

In New England, these organisms reproduce rapidly and overgrow sessile plant and animal species and non-living substrates such as rocks, wood, plastic, and metal; healthy colonies have no known predators. They grow at water depths that range from lowest intertidal to at least 65 m. *Didemnum* sp. colonies are composed of many small individual zooids that filter seawater for food particles. Zooids are approximately 0.2 mm wide and 1 mm long (Kott, 2004, Figs. 2, 4). Seawater and food enters each zooid through its oral siphon. Fecal pellets and larvae exit a zooid through its cloacal siphon into a cloacal canal system that leads to cloacal apertures on the colony's surface. Colonies vary in color from pink to pale yellow to pale orange, and their growth form is plastic because they respond to the dynamics of the habitat. In quiet waters, *Didemnum* sp. colonies are lobed and beard-like; and when growing on steep surfaces or the undersides of docks and ship hulls, the colonies form long tendrils, which pinch off and fall to the seabed to form a new colony if the substrate is suitable. In currents, the species produces encrusting, warty mats that conform to the surface of the substrate.
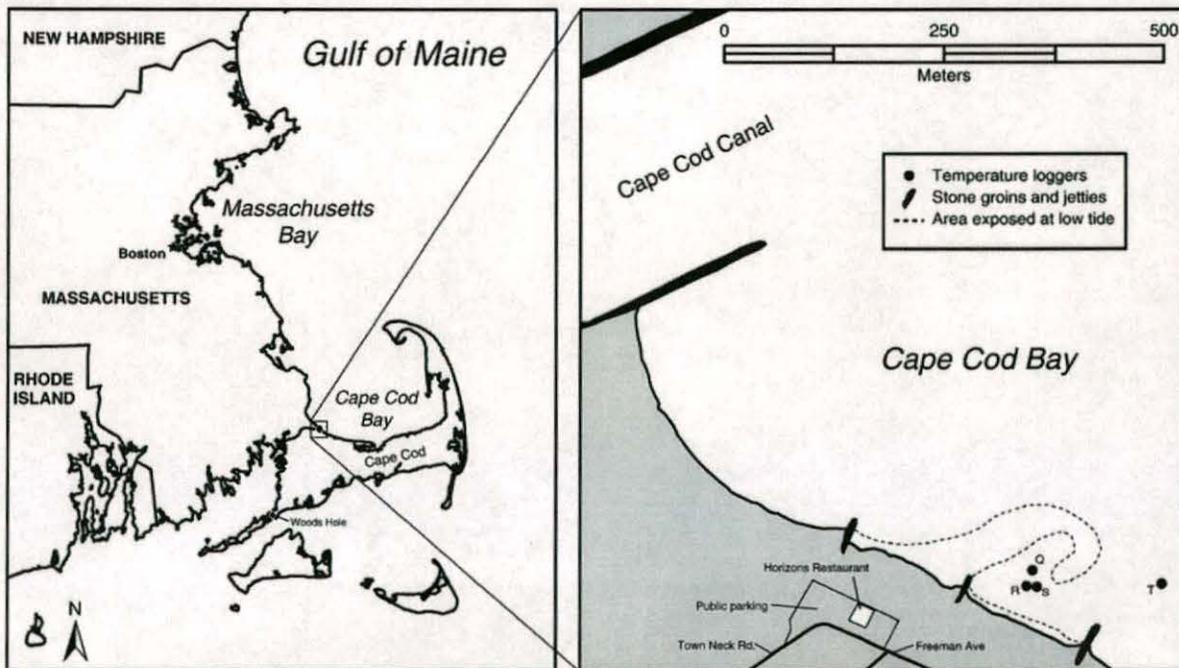

Fig. 1. Location maps. Study area is the largest and lowest of three tide pools located at a Sandwich town beach on the south side of the east entrance of the Cape Cod Canal, Massachusetts. The other two pools are too small to delineate. The tide pools are bounded by a gravel bar and a beach at low tide (dashed line). Principal tide pool (41°46.2′ N, 70°29.4′W) is approximately 50 × 75 m (3750 m²) in size. Temperature logger sites are at Q, R, and S in the tide pool and at T in the channel that connects to Cape Cod Bay at low tide. Site T (0.63 m at low tide) is the location of the experiment to test the viability of transplanted fragments of living *Didemnum* sp. colonies.

Photographs of *Didemnum* from North America, Europe, and New Zealand are presented on a web site dedicated to these organisms (U.S. Geological Survey, 2006).

New England tide pools experience climates that are far harsher than those of deeper subtidal habitats. Tide pool species are subject to air exposure, to daily changes in water depth and temperature, and to a broad annual temperature range. Tide pools are more accessible for study than sites in deeper water, and their environments are in many respects analogous to coastal aquaculture settings.

The ecology of *Didemnum* sp. is poorly known. The Sandwich tide pool on Cape Cod serves as a living laboratory for studying some of its ecological characteristics, including (1) temperature tolerance, (2) seasonal growth cycle, (3) effects of exposure to air (emersion), (4) predation, (5) overgrowth on non-living substrates and on other species, and (6) viability of colony fragments to re-attach and grow. Our observations serve as a basis for further detailed studies of these topics and of sexual reproduction. The purpose of this paper is to document some of the principal ecological characteristics of *Didemnum* sp. and to utilize this information to determine its potential to spread along the east coasts of the U.S. and Canada, to show that fragmentation of the species' colonies will promote its spread in both coastal and offshore habitats, and to develop a basis for managing the species in shellfish aquacultures.

2. Materials and methods

2.1. Sandwich tide pool

The Sandwich tide pool (41°46.2′ N, 70°29.4′W) is located at a town beach just south of the east entrance of the Cape Cod Canal and faces eastward onto Cape Cod Bay (Fig. 1). We have made 34 visits to the pool from December 2003 to February 2006. The shore is protected from storm activity by stone groins that extend seaward from the canal entrance and the beach, but it is vulnerable at high tide to waves from the northeast and east that are generated in Cape Cod Bay by winds that have a maximum fetch of 44 km.

The study area is a complex of several tide pools. At low tide, they are almost completely surrounded by a bar and a beach composed of clean pebbles and cobbles that support no attached epifauna or epiflora due to the disturbance of breaking waves. A satellite image that shows the study area and gravel bar at mid tide can be viewed by conducting a Google map search for 23 Freeman Avenue, Sandwich, MA. The mean tidal range

is 2.4 m with a maximum range of 3.3 m. At low tide, a small upper pool and a somewhat larger middle pool are separated from each other and from a large lower pool by gravel berms. *Didemnum* sp. does not occur in either the upper or middle pools. The principal study area is the large lower pool that measures approximately 50×75 m (3759 m^2) at low tide. It is protected from storm waves at low tide by the gravel bar composed of pebbles and cobbles that is exposed for 6 h twice a day (Fig. 1). During this period, it is possible to walk on the gravel bar around the margin of the tide pool, and all parts of the study area are easily accessible by foot. At low tide, the tide pool remains connected by a narrow channel on its southeast side to Cape Cod Bay, and water continues to flow from the pool to the sea at the lowest tide. At Sandwich, we have conducted our studies on *Didemnum* sp. during several hours before and after low tide in the large lower tide pool and in the channel (Fig. 1, site T) that connects the pool to the sea.

The Sandwich tide pools and channel are floored by gravel (pebbles, cobbles, and boulders) and sand, with the gravel predominating in the pools and sand predominating in the channel. In the tide pools, sand occurs in small patches only. Rippled sand in the tide pool and the channel is moved by storm wave and tidal currents. Patches of eelgrass (*Zostera marina* Linnaeus) grow in sandy substrates. The large cobbles and small boulders provide substrate for attached flora and fauna, including red and green seaweeds, calcareous algae, an encrusting sponge, bryozoa, mussels, barnacles, and the colonial ascidians *Botrylloides violaceus* Oka, *Botryllus schlosseri* Pallas and *Styela clava* Herdman. *Didemnum* sp. is the dominant tunicate in the tide pool.

2.2. Observational and experimental methods

2.2.1. Digital photography

Digital handheld photography in situ was the principal method used to document the visual observations of *Didemnum* sp., including its growth cycle, its overgrowth of other species and non-living substrates, its role as a prey species, and its susceptibility to emersion. Gridded photographic images were used to measure changes in colony size during an experiment to test the survivability and growth of colony fragments.

2.2.2. Water temperature

Early in this study we recorded water temperatures in the tide pool using a hand-held thermometer. This proved to be unsatisfactory, and starting in late July 2004, we deployed three temperature loggers (Q, R, S) in the tide pool; and in September 2004, we placed an

Table 1
Water temperatures at the Sandwich tide pool

Site	Water depth, low tide; m, approx.	January 2005, 2006 daily temperatures; min °C/max °C	July–August 2004, 2005 daily temperatures; min °C/max °C
Q	0.48	−1.7 to 4.8/0.2 to 8.7	9.3 to 18.6/15.3 to 23.5
R	0.13	−1.2 to 5.0/0.3 to 12.4	11.3 to 18.3/17.1 to 25.6
S	0.16	−1.8 to 4.8/0.2 to 10.7	11.2 to 18.2/16.9 to 24.9
T	0.63	−1.0 to 4.6/0.4 to 7.7	9.9 to 14.9/15.2 to 23.0

Low and high values of daily temperature minima and maxima at four sites (Fig. 1) for the coldest (January) and warmest (July–August) periods of the year.
Record length for site Q is July 2004 to February 2006 (Fig. 2); for sites R and S, July 2004 to August 2005; for site T, September 2004 to June 2005.

additional logger (T) in the channel that connects the tide pool to Cape Cod Bay at low tide (Fig. 1). The loggers were in place for various periods of time during the study (Table 1). A continuous record from site Q for the entire study period extends from July 2004 to February 2006. The loggers (3 cm in diameter, 1.7 cm high) recorded temperature at five-minute intervals with an accuracy of 0.2 °C (StowAway TidbiT, Onset Computer Corporation, Bourne, MA). They were wrapped in nylon material to protect them from damage by sediment movement and wave action and were

attached to cords tied around the bases of boulders at depths ranging from 0.13 to 0.63 m at low tide. Temperature data were downloaded at the site or in the lab and analyzed to determine daily maximums, minimums, and ranges.

2.2.3. Emersion

The effects of emersion on *Didemnum* sp. were observed on cobbles and small boulders where the colonies were naturally exposed to air during low tide. In addition, we moved boulders so that parts of the attached colonies were exposed to air for 2 to 3 h at low tide; and we inspected them after 30 days. Controlled experiments are needed to determine the combinations of air temperature and minimum exposure time that are required to weaken and kill colonies of *Didemnum* sp.

2.2.4. Colony fragments

Experiments were conducted to observe the viability of colony fragments in order to determine if the transport of fragments is a possible mechanism for spreading this species. On July 20, 2004 we cut pieces (6 to 10 cm^2) from the edges and interiors of healthy colonies and placed them in circular clear plastic containers (10 cm diameter, 4.3 cm deep) whose sides and plastic screw-on tops had been perforated to provide water circulation.
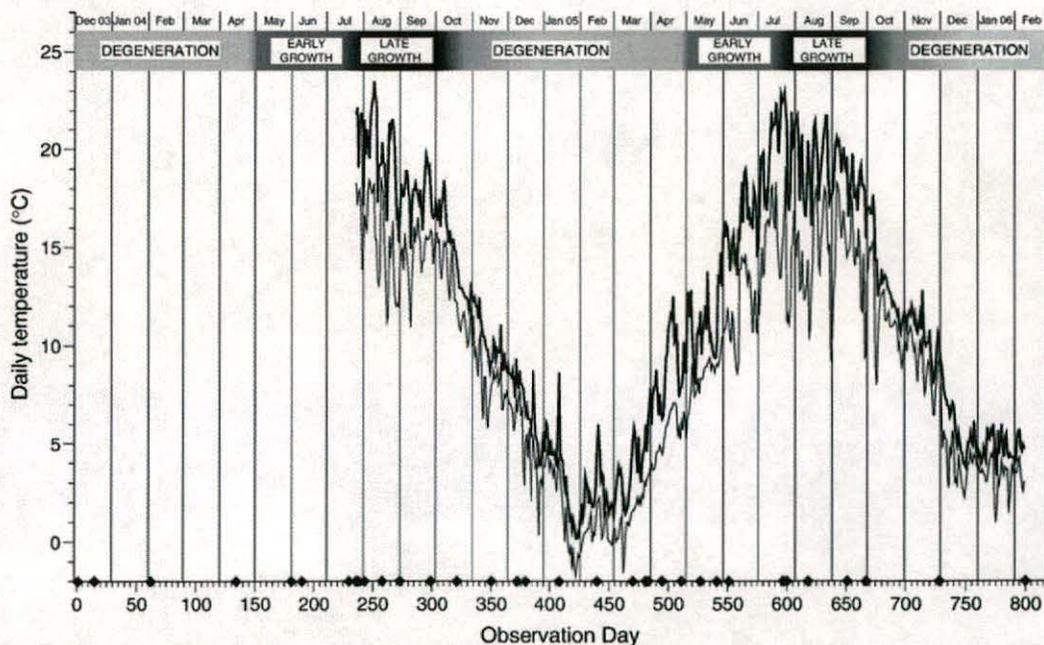

Fig. 2. Seasonal growth cycle of *Didemnum* sp. and graphs of the daily maximum and minimum water temperatures at Site Q (Fig. 1) in the Sandwich tide pool. This temperature pattern is representative of patterns observed at sites R, S, and T. High and low values of daily maximums and minimums are from temperature data recorded at 5-min intervals. Seasonal growth cycle includes periods of early growth, late growth, and degeneration identified as a result of 34 visits (solid diamonds) to the study area. For some colonies, degeneration begins in October and correlates with the start of a seasonal decline in water temperature. See text for details of the seasonal growth cycle.

Fig. 3. Underside of small boulder with remnants of colonies (irregular patches of light color) from previous year that have survived the winter and predation by periwinkles *Littorina littorea* (see Fig. 8). Boulder placed upside down and photographed in situ; image DSCF1111; tide pool at Sandwich, MA; June 8, 2005. Scale bar 5 cm.

This experiment was only partially successful due to loss of containers and to the infiltration of fine-grained sediment, which smothered the colonies.

Two weeks later on August 2, we repeated the experiment using improved techniques. Six fragments of *Didemnum* sp. (5 to 9 cm²) were excised from the margins (3 samples) and the interiors (3 samples) of healthy colonies growing on cobbles and boulders and placed in containers identical to the ones used in the first experiment. Container walls were perforated to allow water circulation, and the open tops were covered with plastic mesh (0.4×0.4 cm) from onion bags that was attached with plastic bands. The plastic mesh was allowed to extend out from the container top to provide additional substrate for colony growth. The containers were attached upright onto flat oval cobbles (10×15 cm) by plastic-coated wire. They were deployed on a gravelly coarse sand seabed (site T, 0.63 m at low tide) in eelgrass adjacent to a large boulder in the channel leading seaward from the tide pool (Fig. 1).

The containers were inspected at varying time intervals until June 2005. Attachment and initial growth of the colony fragments were documented with digital photography after 15 days, on August 17, 2004 and after 30 days, on September 1, 2004. The areas occupied by the colonies were estimated from gridded photographic images and the known dimensions of the containers. Colony growth, the effects of sedimentation, predation by the periwinkle *L. littorea* (Linnaeus), and seasonal degeneration of the colonies were documented on subsequent visits.

Changes in colony size were not analyzed after the first 30 days because storms in September, 2004 disturbed the

containers and filled them with coarse sand. Other storm effects included extensive damage to the eelgrass bed and deep scouring around the boulder at the site. Two of the six containers were lost during this storm period. The remaining four containers were partly buried in gravelly coarse sand, but the colonies survived. On December 16, the containers were moved approximately 25 m east of the original site to a more protected location and placed on pebbles and cobbles between several large boulders. A month later, on January 14, 2005, the containers were still in place, but the next visit on February 15 revealed that a major winter storm had moved two of the containers and removed the other two containers altogether. As of June 30, 2005, the surviving two containers were still in place. Although the containers were subjected to strong currents during storms, we never found them upside down, probably due to the weight of the attached heavy flat cobbles on which they sat.

3. Results

3.1. Temperature tolerance

In general, tide pools experience a wider range of water temperatures on a daily basis than deeper subtidal sites because of the effects of insolation and air temperature on shallow water, especially at low tide. Maximum water temperatures occur at low tide on hot summer days; and minimum temperatures occur at low tide on cold winter days or nights when sea ice can be present floating or grounded in the shallow waters of the tide pool.

Fig. 4. *Didemnum* sp. colony with open zooids showing oral tentacles (small flaps of tissue around the opening of the oral siphons); colony is regenerating from a remnant that survived the previous winter. Photograph in situ from surface of boulder shown in Fig. 3; image DSCF1101; tide pool at Sandwich, MA; June 8, 2005. Scale bar 5 mm.

Fig. 5. *Didemnum* sp. colony growing on bottom and sides of a small boulder. Smooth and warty colony surfaces present; cloacal canals visible; warty protuberances developing at cloacal apertures. Boulder placed upside down and photographed in situ; image DSC0179; tide pool at Sandwich, MA; July 20, 2004. Scale bar 10 cm.

The four temperature loggers in the study area provided a continuous record of water temperatures at 5-minute intervals. Temperature plots show the same patterns at the four sites, and slight temperature differences are attributed to variations in water depths at the sites during low tide. Site Q has the longest temperature record and is considered to be most representative of the part of the tide pool where *Didemnum* sp. lives. Sites R and S are located on the very shallow (at low tide) edge of the tide pool where the species is less abundant. Site T is located in the channel outside the tide pool, where the species also occurs. The lowest daily temperature minima at the four sites for January of 2004 and 2005 varied from -1.0 to -1.8 °C; and highest daily maxima for July–August of 2005 and 2006 varied from 23.0 to 25.6 °C (Table 1). Daily temperature changes at the sites over this period (late July 2004 to early February 2006) varied from <1 to >11 °C.

Based on our observations, *Didemnum* sp. in the Sandwich tide pool tolerates temperatures that range from <-1 to >24 °C (Figs. 1, 2). In a recent paper, Bullard et al. (2006-this volume) report that in New England, the species can tolerate subtidal temperatures of -2 to >24 °C. It is likely that temperature regimes are a major factor governing the seasonal growth cycle of the species in shallow subtidal habitats like the Sandwich tide pool.

3.2. Seasonal growth cycle

Didemnum sp. exhibits a seasonal growth cycle in the study area that may be characteristic of this species in very shallow subtidal habitats where both daily and seasonal temperatures vary appreciably (Fig. 2, Table 1). At Sandwich, minimum daily water temperatures of -1 °C and lower occur in the latter part of January; maximum daily temperatures of 24 °C and higher occur in July–August. Seasonal temperature extremes may affect the survival and geographic distribution of the species, and seasonal temperature trends possibly control the stages of its growth cycle.

At Sandwich, from May into July, the early growth phase of the seasonal cycle is initiated by small colonies of *Didemnum* sp. that develop from the remnants (Figs. 3, 4) of colonies that have survived the winter and predation by periwinkles (*L. littorea*). The colonies have relatively smooth surfaces and grow asexually by budding. The surfaces of expanding colony margins are smooth, but the surfaces of colony interiors gradually develop wart-like protuberances located at cloacal apertures. In June and July, the colonies enlarge and thicken, interior surfaces are covered with well-developed warty protuberances, and growing margins are relatively thin and smooth (Fig. 5). Colony color is variable and ranges from pink to pale yellow to pale orange. During the late growth phase, colonies grow rapidly from July to September, covering both living and non-living substrates; and growth can continue into December (Figs. 6, 7). In October, a decrease in both water temperature and in the magnitude of daily temperature fluctuations (Fig. 2) coincides with the beginning of a gradual decline in health for some colonies, although many continue to grow for several months. It is probable that early signs of degeneration

Fig. 6. *Didemnum* sp. colony on small boulder and spreading onto pebble and cobble seabed; at or near end of seasonal growth period of colony. Surface mostly warty with little smooth area; edges of colony are thickened. Photographed in situ; image DSCF1566; tide pool at Sandwich, MA; November 17, 2004. Scale bar 10 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 7. *Didemnum* sp. colony on small boulder; at or near end of seasonal growth period of colony. Surface with warty protuberances located at cloacal apertures; dark areas at tops of warts are interpreted to be clumps of fecal pellets in cloacal cavities below cloacal apertures. Photographed in situ; image DSCN1858; tide pool at Sandwich, MA; December 4, 2003. Scale bar 4 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

observed in October occur in those colonies that are partly exposed to air during very low tide. We observed that parts of colonies that naturally grow up the sides of rocks can survive exposure to air at low tide for short (as yet unmeasured) periods of time, probably not exceeding 2 h. By the end of April, the degenerated remnants of most colonies have disappeared, and in May colonies begin to regenerate. Larvae were present in colonies collected in August 2003 (G. Lambert, personal communication, 2005), and we surmise that sexual reproduction occurs during the July–September period, in which the tide pool experiences the warmest water temperatures of the year (Fig. 2). Studies will be conducted to better identify the species' temperature requirements for sexual reproduction.

We have observed the degeneration of colonies in other subtidal settings on Cape Cod that include pier pilings and floating docks. Indicators of a slowing of colony growth and the onset of degeneration include: thickening of colony margins (Fig. 6) and slowing of lateral growth; peeling of colonies from substrates (Fig. 8); open zooids associated with the appearance of brown areas (Fig. 9) or spots (Figs. 8, 10, 11) that represent clumps of fecal pellets that have accumulated in the cloacal canals and cavities of a colony; scavenging of colony tissues by periwinkles which produces subcircular scour depressions in the colony surface (Fig. 8); appearance of an orange/brown film (possible fecal material) on parts of the colony surface (Fig. 8); and an amorphous colony surface in which zooid features have lost definition

(Fig. 12). Colonies exhibit color shadings that range from white to pink to orange. During this phase of the growth cycle, the parts of colonies that are exposed to air at low tide become desiccated and are scavenged by periwinkles, leaving thin chalky white films that are composed of didemnid spicules, tests of benthic foraminifera, sand grains, and organic debris. Colonies of the ascidian *B. violaceus* are able to overgrow the edges of *Didemnum* sp. only during the period of colony degeneration. By March and April, remnants of *Didemnum* colonies remain that have survived cold winter temperatures and predation by periwinkles.

3.3. Colonization of substrates and predation

The species colonizes all substrate types in the tide pool, except moving sand, and is vulnerable only to smothering by sediment. *Didemnum* sp. spreads by asexual reproduction and overgrows: Irish moss, *Chondrus crispus* Stackhouse; knotted wrack, *Ascophyllum nodosum* (L.) LeJolis; rock weed, *Fucus evanescens* (C. Agardh); eelgrass, *Z. marina*; calcareous algae, *Corallina officinalis* Linnaeus; encrusting sponge, *Halichrondria* sp.; mussel, *Mytilus edulis* Linnaeus; other colonial tunicates, *B. violaceus* and *B. schlosseri*; barnacles; and attached anemones. In other shallow water settings around Cape Cod, we have observed *Didemnum* sp. growing on rubber, plastic, metal, rope, and wooden docks (U.S. Geological Survey, 2006). We have not
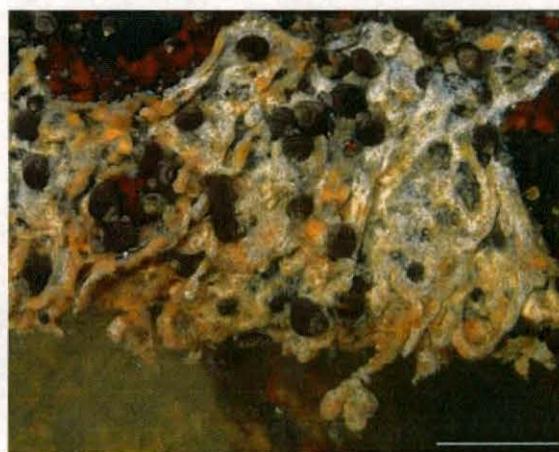

Fig. 8. *Didemnum* sp. colony on side of boulder. Periwinkles (*Littorina littorea*) grazing colony surface. Colony is degenerating and peeling away from boulder substrate. *Didemnum* sp. fecal pellet clumps (small dark areas), snail scour marks (subcircular depressions), and yellow/brown film visible. Photographed in situ; image DSCF0104; tide pool at Sandwich, MA; March 17, 2005. Scale bar 4 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
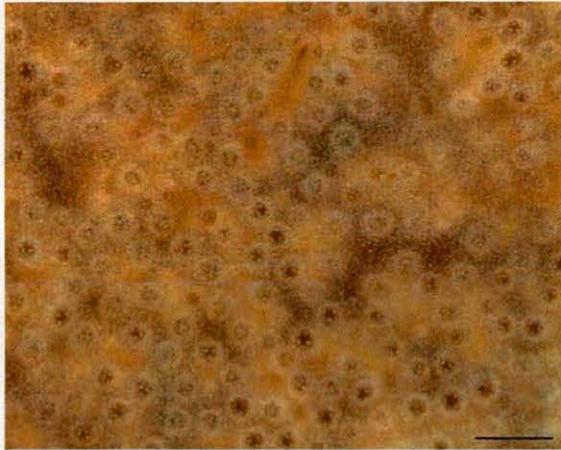
Fig. 9. *Didemnum* sp. colony surface showing degenerating open zooids with oral tentacles visible. Dark areas are clumps of fecal pellets in cloacal canals and cloacal cavities of colony. Photographed in situ; image DSCF2073; tide pool at Sandwich, MA; January 14, 2005. Scale bar 1 mm.

observed a man-made substance that repels the species; but we have no information on the effects of anti-fouling paints. The surfaces of healthy tunicate colonies are very clean to the naked eye, and to our knowledge no organisms attach to it. We have not looked for microscopic organisms such as bacteria and fungi. Predation on degenerating colonies by periwinkles removes tissue within colonies and at their margins and results in a characteristic pattern of subcircular holes that expose the substrate below the tunicates (Fig. 8). Scavenging by periwinkles is not complete and leaves irregular patches of colony tissue that survive to regenerate in May and June.

3.4. Emersion

We have observed that *Didemnum* sp. colonies during their period of rapid growth (July–September) can survive short periods of emersion at low tide. However, colonies die when exposed to air for an extended period of time, and the time required will vary with seasonal climate at a particular site. In an uncontrolled experiment in late October 2004, we turned over several large boulders to expose large well-developed colonies to air for 2 to 3 h at low tide. We inspected the boulders 30 days later in November and observed that the parts of the colonies that were exposed to air were desiccated and that periwinkles were grazing on them and had removed much of the tissue. The parts of the colonies below the low tide level were unaffected. Later, in January 2005, we observed the same phenomenon on an undisturbed boulder.

3.5. Viability of colony fragments

The tide pool provides a relatively controlled setting in which to observe the fate of groups of zooids that have been excised from colonies. We are interested in this phenomenon because the transport of living colony fragments is a potential method for spreading *Didemnum* sp. In an experiment, six containers containing colony fragments were emplaced on July 20, 2004. When we inspected them 6 days later on July 26, we found that three containers were missing. In two of the three remaining, the colonies had been partly covered by fine sediment, and the colony fragments were smaller than their original size. In one container, the colony fragment had attached, and 2 days later on July 28, it was growing up the side of the container and developing lobes. This experiment had to be terminated because the containers were not properly weighted, and too many were lost. However, these observations suggest a vulnerability of *Didemnum* sp. to smothering by fine-grained sediment.

A second experiment, using improved methods began on August 2, 2004. Colony fragments taken from the interiors and edges of healthy colonies were placed in six containers similar to those used in the first experiment and photographed (Fig. 13). This time the containers were held onto flat cobbles by wire, and the open container tops were covered with plastic mesh from onion bags. They were placed on a relatively stable substrate of gravelly coarse sand to minimize the effects of fine sediment accumulation in the containers. Fifteen days later, on August 17, the containers were inspected

Fig. 10. Clumps of fecal pellets (dark areas) lodged in cloacal canals and cloacal cavities near surface of a degenerating *Didemnum* sp. colony. Fresh specimen retrieved from below frozen surface of sea water; image DSC0273; Eel Pond, Woods Hole, MA; January 15, 2004. Scale bar 1 cm.

Fig. 11. Clumps of fecal pellets (dark areas) lodged in cloacal canals and cloacal cavities of a degenerating *Didemnum* sp. colony. Very small dark spots are individual pellets. Views of colony section (upper part of image) and of colony surface (lower). Preserved specimen; image DSC0316; Eel Pond, Woods Hole, MA; collected January 15, 2004. Scale grid 5 mm.

and re-photographed. All six colony fragments (3 from colony interiors and 3 from colony edges) had attached and were actively growing on the bottoms and sides of the containers (Fig. 14). On September 1, 30 days after the start of the experiment, the containers were inspected again, and the colonies showed further growth. Analysis of the photographs showed that in the first 15 days the colonies had enlarged from 6 to 11 times by asexual budding; and after 30 days, they had enlarged a total of from 11 to 19 times (Table 2). It is evident that fragments of *Didemnum* sp., when separated from growing colonies, can successfully survive and grow and can serve to spread this species.

We continued to inspect and photograph the containers throughout the summer, fall, winter, and spring, but we did not conduct further analyses of growth because of disturbance by storms and the onset of the seasonal decline in colony health. However, the *Didemnum* sp. colonies survived and grew even though storm currents transported coarse sandy sediment that filled the containers. The plastic mesh that allowed sediment to enter the containers also provided a substrate for colony growth. By the time the accumulated sediment had smothered the part of a colony attached to the inside of a container, the colony had grown up its inside walls, out through holes in the walls, onto the mesh covering the tops, and onto the excess mesh that extended away from the containers. As of March 17, 2005, the *Didemnum* sp. colonies had degenerated and were being preyed upon by common periwinkles (Fig. 12). On June 8, 2005, remnants of these colonies had begun to regenerate. Of

the six containers with colony fragments, four have been lost to storms, but on June 8 two remained, 310 days after the start of the experiment.

4. Discussion

The tendencies of *Didemnum* sp. to attach to firm substrates, to rapidly overgrow other attached species, to tolerate a wide temperature range, to be free from predation, and to spread by colony fragmentation combine to make it a potential threat to benthic marine habitats and aquaculture.

4.1. Overgrowth, predation, and colony fragmentation

Didemnum sp. is very aggressive and can colonize and overgrow attached green and red seaweed, erect and encrusting calcareous algae, eelgrass, encrusting sponge, barnacles, attached anemones, and the colonial ascidians *B. violaceus*, and *B. schlosseri*. We have observed it overgrowing mussels, *M. edulis*, and oysters, *Crassostrea virginica* (Gmelin), at various locations on Cape Cod, and sea scallops, *Placopecten magellanicus* (Gmelin), on Georges Bank. It likely envelops other bivalves. In shallow water settings around Cape Cod, we have observed it growing on various man-made materials. The species cannot easily grow on mobile substrates such as rippled sand and cannot survive smothering by fine- or coarse-grained sediment, although on Georges Bank colonies
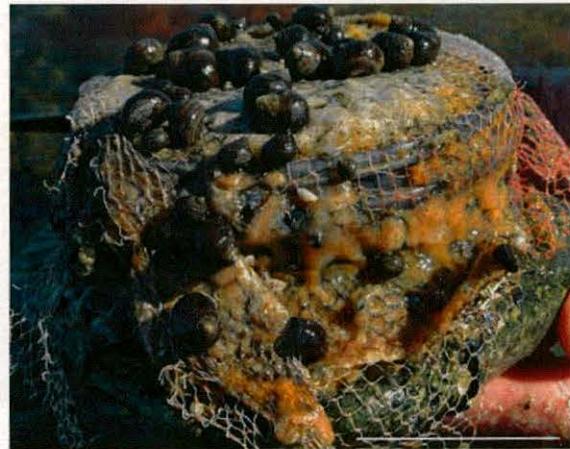

Fig. 12. *Didemnum* sp. colony (grown from fragment) 227 days after being excised from colony interior on Aug. 2, 2004; growing on onion bag mesh over mouth of container no. 11, which is filled with sediment. Colony degenerating; surface amorphous, scavenged by periwinkles (*Littorina littorea*), and showing *Didemnum* sp. fecal pellet clumps (small dark areas). Photographed and replaced on seabed; image DSCF0099; tide pool at Sandwich, MA; March 17, 2005. Scale bar 5 cm.

Fig. 13. Fragment excised from edge of *Didemnum* colony; on bottom of plastic container no. 7; compare with Fig. 14 and Table 2. Photographed and placed on seabed at site T (Fig. 1); image DSC0044; tide pool at Sandwich, MA; August 2, 2004. Scale bar 2 cm.

attached to gravel have been observed to survive partial covering by sand.

Actively growing colonial ascidians commonly are resistant to predation, to lateral overgrowth, and to colonization (fouling) by settlement and growth of the larvae of other organisms. For example, in a study of the anti-predatory chemical defenses of colonial ascidians, Pisut and Pawlik (2002) showed that *Didemnum candidum* from Wrightsville Beach, NC displayed high acidity (pH 1) in both surface and internal tissues and that organic compounds and acid in the tissues deterred predation by a fish, the bluehead wrasse, *Thalassoma bifasciatum*. A study of the anti-fouling defenses of a colonial didemnid *Polysyncraton lacazei* from the Mediterranean coast of France showed that the species contained organic compounds that deterred bacteria, fungi, algae, and larvae (Wahl and Banaigs, 1991). Healthy and growing colonies of *Didemnum* sp. at Sandwich have no known predators, and we have not observed other organisms growing on them. However, colonies undergoing seasonal degeneration in the October–April time period, or weakened by emersion and desiccation at any time, are vulnerable to scavenging by the common periwinkle, *L. littorea*, and to overgrowth by the colonial tunicate *B. violaceus*. These observations suggest that *Didemnum* sp. possesses attributes that deter predation and fouling similar to those reported for other ascidians.

It is well known that fragments of ascidian colonies are capable of re-attachment and enlargement by asexual budding. Stoner (1989) describes fragmentation experiments with the didemnid species *Displosoma similis*, which showed not only that colony fragments survived

and grew by asexual budding but that their growth rate increased. We have shown that fragments of *Didemnum* sp., both from interiors and edges of colonies, readily attach to firm substrates and grow vigorously by asexual budding. The transport of living colony fragments is a reliable method for spreading the species geographically.

4.2. Seasonal growth cycle in shallow and deep subtidal habitats

In the shallow subtidal tide pool setting at Sandwich, *Didemnum* sp. exhibits a seasonal cycle of growth and degeneration. We expect the timing of phases of the cycle to shift slightly in response to annual variations in temperature; for example, January 2005 was 3 to 4 °C colder than January 2006 (Fig. 2). Colony initiation and growth by asexual budding begins in May when the water temperature ranges from 8 to 12 °C and continues into December. We speculate that sexual reproduction at Sandwich occurs when waters are warmest and the colonies are growing rapidly from July through September. Larvae have been observed in colonies from the tide pool and from floating docks from near the western end of the Cape Cod Canal in early August 2003 (G. Lambert, personal communication, 2005). The beginning of some colonies' decline in October may be controlled by a relatively rapid decline in water temperature that begins at approximately 15 °C (Fig. 2). The species survives as an unidentified overwintering form in small remnants of colonies on the bases of cobbles and boulders, and can

Fig. 14. *Didemnum* sp. colony (from fragment) 15 days after being excised from edge of colony; growing on bottom and side of plastic container no. 7. Surface mostly smooth; cloacal canals visible; compare with Fig. 13 and Table 2. Photographed and replaced on seabed at site T (Fig. 1); image DSCF0193; tide pool at Sandwich, MA; August 17; 2004. Scale bar 2 cm.

Table 2
Growth of *Didemnum* sp. colony fragments 15 and 30 days after excision from the edges and interiors of healthy colonies and placed in containers on the seabed at site T in Sandwich on August 2, 2004 (Fig. 1).

Container no.; sample location in colony	Colony size; Aug 2, 0 days; cm²	Colony size; Aug 17, 15 days; cm²	Colony size; Sep 1 30 days; cm²	Total colony size increase after 15 days/ 30 days
7; edge	7	45	103	6.4×/14.7×
8; edge	7	47	81	6.7×/11.6×
9; interior	8	57	94	7.1×/11.8×
10; edge	5	33	77	6.6×/15.4×
11; interior	9	69	144	7.7×/16.0×
12; interior	8	90	152	11.2×/19.0×

Colony sizes estimated from gridded digital photographs using known dimensions of the containers.

tolerate water temperatures as low as −1.8 °C. This seasonal cycle resembles the behavior of *Didemnum* sp. in the Netherlands. In the Oosterschelde and Grevelingen, beginning in May–June and continuing through the summer and fall, *Didemnum* sp. colonies grow and cover large areas of the seabed. In November–January, whenever water temperatures reach approximately 4 °C, most colonies degenerate (A. Gittenberger, personal communication, 2005).

The degenerative phase of the seasonal growth cycle of *Didemnum* sp. can be compared with the processes of regression, dedifferentiation, resorption, hibernation, overwintering, or resting in ascidians, as described by other authors. Berrill (1951) describes regression as a phenomenon typical of colonial ascidian life cycles that involves the disintegration and reorganization of zooid tissues into "compact masses" followed by their reconstitution (or regeneration) into new zooids. Regression (also called dedifferentiation or resorption) is triggered by adverse conditions such as starvation and does not necessarily lead to colony death; in the genus *Clavelina* and the species *Diazona violacea*, regression commences after sexual breeding has been completed (Berrill, 1951); and regression in *Clavelina* can be initiated by unfavorable levels of oxygen, temperature, and food availability (Berrill, 1950). At Laguna Veneta, Italy, Burighel et al. (1976) describe winter hibernation of the colonial ascidian *Botrylloides leachi* as a period in which zooids regress, filtering zooids are absent, and the colony resembles a homogeneous mass often covered by diatoms and detritus. The authors conclude that hibernation occurs in response to unfavorable environmental conditions and imply that decreasing water temperature gives rise to regression and that subsequent increasing temperature leads to the formation of filtering zooids. Of further interest, they report the presence of "large quantities of food residues" in

the branchial chambers or guts of regressing adult zooids. A recent study of colonies of *Didemnum albidum* from northern Norway describes their overwintering (resting) stages (August to March) as being characterized by: non-feeding zooids; degeneration of zooid thoraces; and the formation of a thick, shiny cuticle that is fouled with other organisms and is susceptible to lateral overgrowth, unlike the clean surfaces of feeding colonies (Marks, 1993, 1996; and personal communication, 2005).

In a previous section, we described the characteristics of the degeneration process that leads to the disintegration and disappearance of *Didemnum* sp. colonies in the October–April time period at Sandwich. In common with the descriptions of the above authors, we have observed compact masses of zooid tissues that reconstitute themselves, but we have not observed them to be covered by detritus or by a thick cuticle fouled with other organisms. In keeping with our observations of clumps of fecal pellets in degenerating colonies, Burighel et al. (1976) report the presence of food residues in regressing zooids. At Sandwich, the clumping of fecal pellets in *Didemnum* sp. signals a loss of the colonies' ability to evacuate their cloacal canals and cavities and may be a reliable indicator of the early stages of degeneration.

The seasonal growth cycle documented in the *Didemnum* sp. colonies in the Sandwich tide pool most likely does not occur in the colonies in offshore deep subtidal (40–65 m) habitats of Georges Bank. Analyses of fortnightly mean bottom temperatures on Georges Bank indicate that the study area experiences annual temperatures ranging from a minimum of 4–5 °C to a maximum of 14–15 °C (Mountain and Holzwarth, 1989), and daily temperature fluctuations are minimal, if present at all. The annual range of water temperatures on the bank is similar to temperatures that occur at Sandwich in October–December, during the time when some colonies begin to degenerate but others continue to grow (Fig. 2). We have not observed signs of degeneration on the bank. A sample collected in November 2004 did not display clusters of fecal pellets, a characteristic of degenerating colonies at Sandwich. A sample collected in November 2003 had larvae (G. Lambert, personal communication, 2005). The Georges Bank colonies survive and reproduce in a temperature range (4–5 to 14–15 °C) that is well within the range tolerated by the colonies at Sandwich. We speculate that the daily temperature variations and the higher and lower seasonal temperatures of the tide pool may play a role in causing the seasonal growth cycle observed at Sandwich that might not occur on Georges Bank where daily and seasonal temperatures vary slowly within a relatively narrow range.

4.3. Tolerance of bivalves and Didemnum sp. to emersion

Numerous studies have shown that bivalves adapted to intertidal and subtidal habitats can be very resistant to the effects of emersion. Genera with species that exhibit high tolerance to drying in air include: *Aulacomya* (Kennedy, 1976); *Cerastoderma* (Boyden, 1972; Hummel et al., 1988); *Crassostrea* (Stanley and Sellers, 1988); *Macoma* (Hummel et al., 1988); *Mytilus* (Hummel et al., 1988; Kennedy, 1976; Myrand et al., 2002); and *Perna* (Kennedy, 1976).

Depending on conditions, bivalve species, including some species that now are raised in aquacultures, can survive for many hours to days exposed to air. These studies show that the length of time a species can withstand desiccation depends on air temperature, relative humidity, and size of individual animals, with air temperature being the most influential factor. Survival time increases with an increase in relative humidity and a decrease in air temperature. Intuitively, we know that soft-bodied ascidians are more vulnerable to drying in air than are bivalves that can seal their shells to protect their soft parts (Fig. 15). However, as shellfish and didemnids occur together in aquacultures, it is important for management purposes to quantify and compare the tolerances of both ascidian and bivalve species to water temperature climates and to drying in air.

The only study known to us that tested methods of controlling a colonial ascidian species that was fouling bivalves is by Katayama and Ikeda (1987). The authors conducted a series of experiments at the Fisheries Experiment Station (Okayama Prefecture) on the Japan Inland Sea. Their goal was to determine the tolerance of *Didemnum moseleyi* (a relative of *D.* sp.) growing on a living cultured oyster substrate (presumably *Crassostrea gigas*) to drying in air and to immersion in both fresh water and heated salt water. Katayama and Ikeda showed that *D. moseleyi* was susceptible to all treatments, but that the cultured oyster was unaffected. The results of their study provide an important basis for developing strategies to manage colonial ascidians in aquaculture.

4.4. Potential distribution and effects of Didemnum sp.

The colonial ascidian *Didemnum* sp. requires only firm substrates, food resources, and suitable water temperatures to flourish in coastal and offshore waters. As it tolerates water temperatures of −1.8 °C, it has the potential to extend its range northward from New England into both shallow and deep subtidal habitats in Canada. At present, it occurs near the US–Canada boundary at
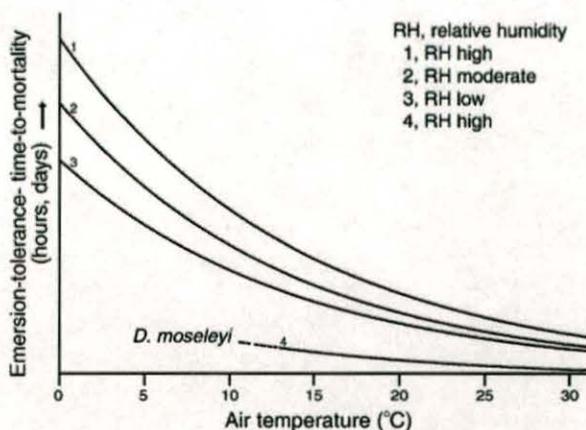

Fig. 15. Graphs showing the general relationship of air temperature and relative humidity to the emersion-tolerance-time-to-mortality of a theoretical mussel (or other cultured bivalve species) and of a theoretical colonial didemnid ascidian. Tolerance to drying increases as temperature decreases and as relative humidity (RH) increases. Lines 1, 2, 3 represent conditions of high, moderate, and low RH, respectively. No values are shown on the emersion tolerance time axis as they vary by bivalve species from tens of hours to days. Adapted from McMahon et al. (1993, Fig. 5) who experimentally determined the effects of air temperature and relative humidity on the emersion tolerance of the zebra mussel *Dreissena polymorpha*. Line 4 represents colonial didemnid ascidians at presumed high relative humidity, which are much less tolerant to emersion than bivalves; based on data for *Didemnum moseleyi* in Katayama and Ikeda (1987, Table 1).

Eastport, ME and on Georges Bank (U.S. Geological Survey, 2006). South of Long Island, we speculate it cannot extend into shallow subtidal habitats that experience water temperatures of 28–30 °C, but it should be able to survive in deeper settings where maximum temperatures are lower. The tunicate's propensity to rapidly overgrow other organisms and inorganic substrates makes it a potential threat to kill or at least slow the growth of aquaculture species such as mussels, clams, oysters, and scallops. It is known to foul shellfish and facilities such as structures, ropes, bags, cages, and fish pens (U.S. Geological Survey, 2006). *Didemnum* sp. is also a threat to alter benthic habitats by overgrowing the seabed and benthic fauna and flora, by becoming a barrier between benthic species and prey that live in the seabed, and by being an unsuitable substrate for larval settlement, thus diminishing available habitat. In both intertidal and subtidal habitats, the spread of the species can be slowed by preventing the fragmentation of colonies by human activities.

Acknowledgements

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