An Integrated Assessment of the Continued Spread and Potential Impacts of the Colonial Ascidian, *Didemnum* sp. A, in U.S. Waters

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Cover images: Photo 1: Mussel cage covered with tunicates at Okeover Inlet, Malaspina Peninsula, BC, Photo credit Gordon King (Taylor Shellfish Farms, Inc). Photo 2: Close up of *Didemnum* colony surface at Sandwich Town beach, Mass,. Photo credit: Dann Blackwood (USGS)

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Summary

Didemnum sp. A is a colonial ascidian or "sea squirt" of unknown geographic origin. Colonies of *Didemnum* sp. A were first documented in U.S. waters in 1993 at Damariscotta River, Maine and San Francisco Bay, California. An alarming number of colonies have since been found at several locations in New England and along the West Coast of the contiguous continental United States. Originally believed to be restricted to artificial structures in nearshore habitats, such as ports and marinas, colonies of *Didemnum* sp. A have also been discovered on a gravel-pavement habitat on Georges Bank at depths of 40-65m. The wide distribution of *Didemnum* sp. A, the presence of colonies on an important offshore fishing ground, and the negative economic impacts that other species of noninidigenous ascidians have had on aquaculture operations have raised concerns about the potential impacts of *Didemnum* sp. A. We reviewed the available information on the biology and ecology of *Didemnum* sp. A and potentially closely related species to examine the environmental and socioeconomic factors that may have influenced the introduction, establishment and spread of *Didemnum* sp. A in U.S. waters, the potential impacts of this colonial ascidian on other organisms, aquaculture, and marine fisheries, and the possibility that it will spread to other U.S. waters. In addition, we present and discuss potential management objectives for minimizing the impacts and spread of *Didemnum* sp. A.

Concern over the potential for *Didemnum* sp. A to become invasive stems from ecological traits that it shares with other invasive species, including the ability to overgrow benthic organisms, high reproductive and population growth rates, ability to spread by colony fragmentation, tolerance to a wide range of environmental conditions, apparent scarcity of predators, and the ability to survive in human dominated habitats. At relatively small spatial scales, species of *Didemnum* and other nonindigenous ascidians have been shown to alter the abundance and composition of benthic assemblages. In addition, the Canadian aquaculture industry has reported that heavy infestations of nonindigenous ascidians result in increased handling and processing costs. Offshore fisheries may also suffer where high densities of *Didemnum* sp. A may alter the access of commercially important fish species to critical spawning grounds, prey items, and refugia. Because colonial ascidian larvae remain viable for only 12–24hrs, the introduction and spread of *Didemnum* sp. A across large distances is thought to be predominantly human mediated; hull fouling, aquaculture, and ballast water. Recent studies suggest that colony growth rates decline when temperatures exceed 21 ºC for 7 consecutive days. Similarly, water temperatures above 8 to 10 ºC are necessary for colony growth; however, colonies can survive extended periods of time below this temperature threshold as an unidentified overwintering form.

A qualitative analysis of monthly mean nearshore water temperatures suggest that new colonies of *Didemnum* will continue to be found in the Northeast U.S., California Current, and Gulf of Alaska LMEs. In contrast, water temperatures become less favorable for colony establishment in subarctic, subtropical, and tropical areas to the north and south of *Didemnum*'s current distribution in cool temperate habitats. We recommend that the Aquatic Nuisance Species Task Force serve as the central management authority to coordinate State and Federal management activities. Five objectives for a *Didemnum* sp. A management and control program focusing on preventing the spread of *Didemnum* sp. A to new areas and limiting the impacts of existing populations are discussed. Given the difficulty of eradicating large populations of *Didemnum* sp. A, developing strategies for limiting the access of *Didemnum* sp. A to transport vectors and locating newly established colonies are emphasized.

KEYWORDS: Ascidian, *Didemnum*, integrated assessment, invasive species management, Large Marine Ecosystems, tunicate

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Glossary of Terms

Aquaculture - the cultivation of freshwater or marine plants and animals for food or other purposes.

Ascidian - invertebrate members of the Phylum Chordata (Subphylum Tunicata; Class Ascidiacea) Also referred to as tunicates or ‘sea squirts’.

Ballast water - water used as weight to improve vessel stability.

Dispersal - the spread of a species, population, or individual’s progeny over time.

Invasive species - nonindigenous species whose introduction causes or may cause harm to the economy, environment, or human health.

Large Marine Ecosystem (LME) - large areas of the ocean (i.e., generally 200,000 km^2 or greater) that are characterized by; bathymetry, hydrology, productivity, and trophodynamics

Nonindigenous species - a species that has been intentionally or accidentally transported by human activities into a region where it does not naturally occur. Also commonly referred to as non-native species, exotic species, or introduced species.

Propagule pressure – a composite measure of the number of individuals released into an area to which it is not native. Includes the number of larvae or fragments released during an introduction event and the number of times larvae of fragments are introduced.

Transport vector - The physical means or agent by which a species is transported to a new habitat, such as ballast water and hull fouling.

Introduction

Invasive species are nonindigenous species whose introduction causes or may cause harm to the economy, environment, or human health (Office of Technology Assessment 1993). In the U.S., the effects of invasive species are estimated to cost between $120 - 137 billion per year. This estimate includes the costs of eradication and control efforts, as well as damages to agriculture, fisheries, utilities, and other economic activities (Pimentel et al. 2000, 2005). The environmental impacts of invasive species include the disruption of ecosystem processes, such as natural fire regimes (Lippincott 2000) and nutrient cycling (Vitousek and Walker 1989). They also pose a threat to native plants and animals through predation (Wiles et al. 2003), competition (Thomson 2004), habitat alterations (Botts et al. 1996, Mayer et al. 2001), and hybridization (Echelle and Echelle 1997) (see also, Wilcove et al. 1998, Mack et al. 2000, Simberloff 2005). Furthermore, many of the pressing health issues of the last three decades are the result of introduced pathogens, such as the Human Immunodeficiency Virus (HIV) and the West Nile Virus. Clearly, invasive species have significant socioeconomic and environmental impacts, and preventing or reducing these impacts is an important management priority.

Concern over the number, variety, and effects of nonindigenous species in marine systems has lagged behind that shown for terrestrial and freshwater ecosystems (Carlton 1989, Office of Technology Assessment 1993, Ruiz et al.1997). However, increased monitoring efforts over the past 15 to 20 years have found that nonindigenous species are conspicuous components of marine communities throughout the world, especially in estuaries (Carlton 1989, Cohen and Carlton 1998, Ruiz et al. 1999, Ruiz et al. 2000). In particular, several nonindigenous species of colonial and solitary ascidians, invertebrate members of the Phylum Chordata (Subphylum Tunicata; Class Ascidiacea), have been found across widely separated geographical locations (Whitlatch and Osman 1999, Lambert 2002, Lambert and Lambert 2003, Pederson et al. 2005, Cohen et al. 2005, WGITMO 2005). Most recently, ecologists from Europe (Minchin and Sides 2006, Wijman and Smaal 2006), New Zealand (Coutts 2002), and North America (Bullard et al. 2007a) have reported an alarming increase in the abundance and distribution of either a single species or an unknown number of morphologically similar species of the colonial ascidian genus, *Didemnum* (Family Didemnidae) (Fig. 1).

Figure 1. *Didemnum* colony growing on a rope in San Francisco Bay, CA (Photograph is from Cohen 2005 and credited to Gretchen Lambert, Univ. of Washington).

Although the precise impacts of these newly discovered colonies of *Didemnum* are not known, their relatively sudden appearance and rapid expansion has raised concerns that they may become invasive. This concern stems in part from ecological attributes that this colonial ascidian shares with other invasive species, including 1) the ability to overgrow other species and to carpet bottom substrates, 2) high reproductive and population growth rates, 3) ability to spread by fragmentation, 4) tolerance to a wide range of environmental conditions, 5) apparent scarcity of predators, and 6) the ability to survive in human dominated habitats (Lodge 1993). In addition, the fouling of equipment and stock by other species of introduced ascidians has increased aquaculture maintenance and processing expenses (Cayer et al. 1999, Boothroyd et al. 2002).

It should be noted that although the recently discovered *Didemnum* colonies from Europe, New Zealand and North America appear morphologically similar, their taxonomic relationship and geographic origin remain unresolved issues. The earliest reported colonies were referred to as *Didemnum lahillei* or *D. helgolandicum*. However, these designations were subsequently determined to be incorrect for the recently appearing *Didemnum* populations (Bullard et al. 2007a). Two specimens of these recently discovered colonies have since been described as new species: *Didemnum vestum* Kott, 2004 from Portsmouth Harbor, New Hampshire, and *Didemnum vexillum* Kott, 2002 from Whangamata Harbour, New Zealand. However, a full description of *D. vestum* was not possible because the specimen lacked larvae, a key component for describing new species of *Didemnum.* As a result, *D. vestum* is a questionable designation and remains limited to colonies from its original collection site at Portsmouth Harbor, New Hampshire (G. Lambert, personal communication). Colonies from Europe and North America have not yet been formally compared to *D. vexillum*, and until studies are completed are being referred to as *Didemnum* sp. A (G. Lambert, personal communication). The genus *Didemnum* is a difficult taxonomic group to identify to the species level. There are a large number of undescribed species in addition to the morphologically similar populations discussed here that are currently undergoing a rapid world-wide expansion. Until the taxonomy of these different populations can be clarified, the use of *Didemnum* sp. A is a necessary designation to distinguish these morphologically similar colonies that have recently appeared and are rapidly spreading throughout temperate marine systems from all other unidentified *Didemnum* spp. Genetic sequencing of the *Didemnum* sp. A populations is a research priority that will help determine their geographical origin, their taxonomic identity and the factors underlying their recent expansion. For the purposes of this integrated assessment, we refer to colonies from Portsmouth Harbor, New Hampshire as *Didemnum vestum* (Kott 2004), and provisionally refer to all other morphologically similar colonies on both U.S. coasts as *Didemnum* sp. A (hereafter referred to as *Didemnum*) (Cohen 2005, Bullard et al. 2007a, and G. Lambert personal communication).

In the U.S., colonies of *Didemnum* have been documented in coastal New England from northern Maine to New York, Washington State, and at multiple locations along the California coast. Until recently, species of *Didemnum* were primarily observed attached to artificial structures in harbors and marinas, and were not believed to pose a serious threat to native species occurring on natural habitats (Connell 2001, Lambert 2001). However, the discovery of *Didemnum* on Georges Bank (Valentine et al. 2007b) has raised concerns about the impacts that this ascidian could have on economically important off-shore habitats and the possibility that it will spread to other marine ecosystems. As a result, there is a need to determine the necessity and feasibility of a *Didemnum* management plan. There are several ongoing research activities including: monitoring changes in the distribution and abundance of *Didemnum*; assessing the physical and biological requirements of *Didemnum*; and examining the impact that *Didemnum* may have on native species. In addition, there are efforts to eradicate or control newly-established populations in Puget Sound, WA and Eastport, ME. While these research activities will provide valuable information on the biology and ecology of *Didemnum*, and the feasibility of colony eradication, developing an effective management policy is an interdisciplinary problem that depends as much on input from the social sciences and economics as it does the natural sciences. A formal approach to synthesizing and delivering relevant, independent input to decision making is an Integrated Assessment, a comprehensive analysis of existing economic, natural and social scientific information in the context of policy or management questions (Dowlatabadi et al. 2000).

The present integrated assessment is the first step in a multistage *Didemnum* management process. One of the goals of this assessment is to identify management questions and objectives that will serve as a framework for future planning efforts. The assessment focuses on describing the potential spread and impacts of *Didemnum* in U.S. waters using qualitative analyses based on the environmental conditions within *Didemnum*'s current U.S. distribution and general information on the ecology of *Didemnum* and other ascidians. These analyses consider the potential spread and impacts of *Didemnum* across and within the 10 Large Marine Ecosystems (LMEs) of the U.S. (Fig. 2) LMEs are large areas of the ocean (i.e., generally 200,000 km^2 or greater) that are characterized by; bathymetry, hydrology, productivity, and trophodynamics (Sherman 2000). It is our hope that the results of this assessment will assist in describing the extent and magnitude of the environmental and socioeconomic problems posed by *Didemnum*. Furthermore, we hope to identify information gaps and research needs so that future management efforts can move towards the use of quantitative approaches, such as risk assessments and cost-benefit analyses, at smaller spatial scales. This assessment draws upon peer reviewed journal articles, technical reports, and edited volumes, as well as results from ongoing research activities to address two main questions:

Figure 2. Large Marine Ecosystems of the United States including, clockwise from right to left: 1) Northeast U.S., 2) Southeast U.S., 3) Caribbean, 4) Gulf of Mexico, 5) California Current, 6) Gulf of Alaska, 7) Eastern Bering Sea, 8) Chukchi Sea, 9) Beaufort Sea, and 10) Insular Pacific-Hawaiian (Figure Source: USCOP 2004).

1) To what degree will *Didemnum* continue to spread in U.S. waters within the next 10 years, and
2) What would be the range of impacts of *Didemnum* on different marine ecosystems located within the U.S. and on society's uses of those areas?

Given these questions, the integrated assessment focuses on 1) documenting the *status and trends* of environmental and socioeconomic conditions in the affected regions, 2) describing the potential environmental and socioeconomic *causes and consequences* of those conditions, 3) providing *forecasts* of future conditions with no management action, and 4) providing *guidance* for potential management actions.

Documentation of Status and Trends

***Didemnum* distribution and population abundance**

Colonies of *Didemnum* have been documented in the Northeast US, the California Current, and the southern end of the Gulf of Alaska LME in British Columbia. Colonies were first documented in the U.S. in 1993 at Fort Island Narrows, Damariscotta River, ME and San Francisco Bay, CA. However, there are anecdotal accounts suggesting that colonies may have been present in Maine as early as the 1970's. Additional colonies have since been documented at 54 sites in coastal New England from Eastport, ME to Shinnecock Bay, Long Island, NY (Fig. 2), as well as 57 sites along the west coast of North America from Puget Sound, British Columbia to San Diego, CA (Fig. 3). However, *Didemnum* is not restricted to coastal habitats as colonies have recently been discovered off-shore on Georges Bank and in eastern Long Island Sound. Furthermore, colonies of *Didemnum*, or closely related species, have been documented worldwide at several localities along the coasts of the Netherlands, France, and Ireland (U.S. Geological Survey 2006, Minchin and Sides 2006, Wijman and Smaal 2006).

Within its North American range, *Didemnum* has a highly disjunct distribution. For example, Bullard et al. (2007a) found that colonies of *Didemnum* were present at approximately 50% of the 190 sites surveyed along the east and west coasts of the U.S. from 1998 to 2005. Interestingly, no colonies were observed at five sites

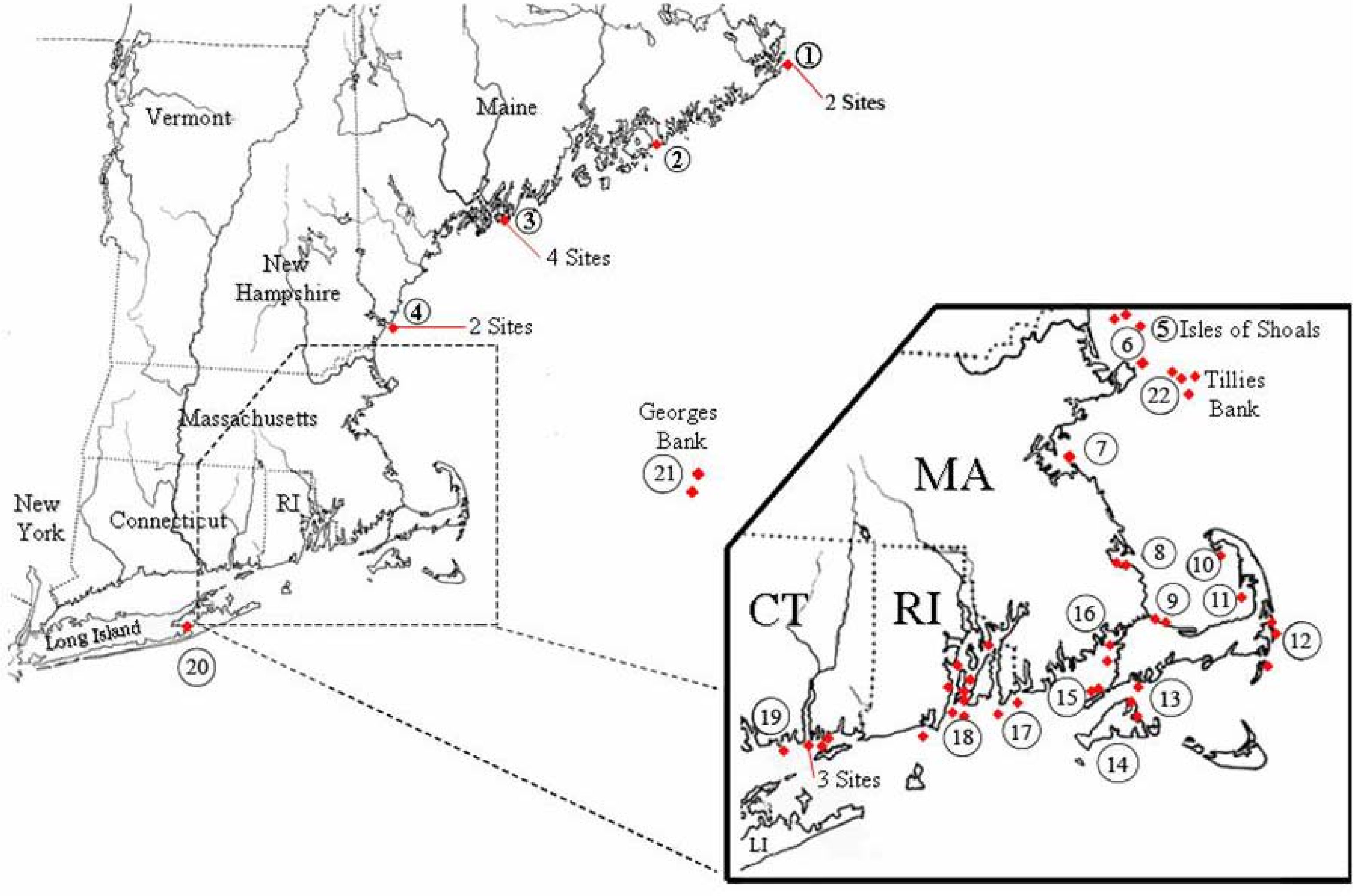

Figure 3. Locations where *Didemnum* colonies have been documented in the Northeast U.S. Large Marine Ecosystem as of November 2006 (modified from U.S. Geological Survey 2006, Bullard et al. 2007a, Osman and Whitlatch 2007). Circled numbers correspond to sites in Tables 1 and 2.

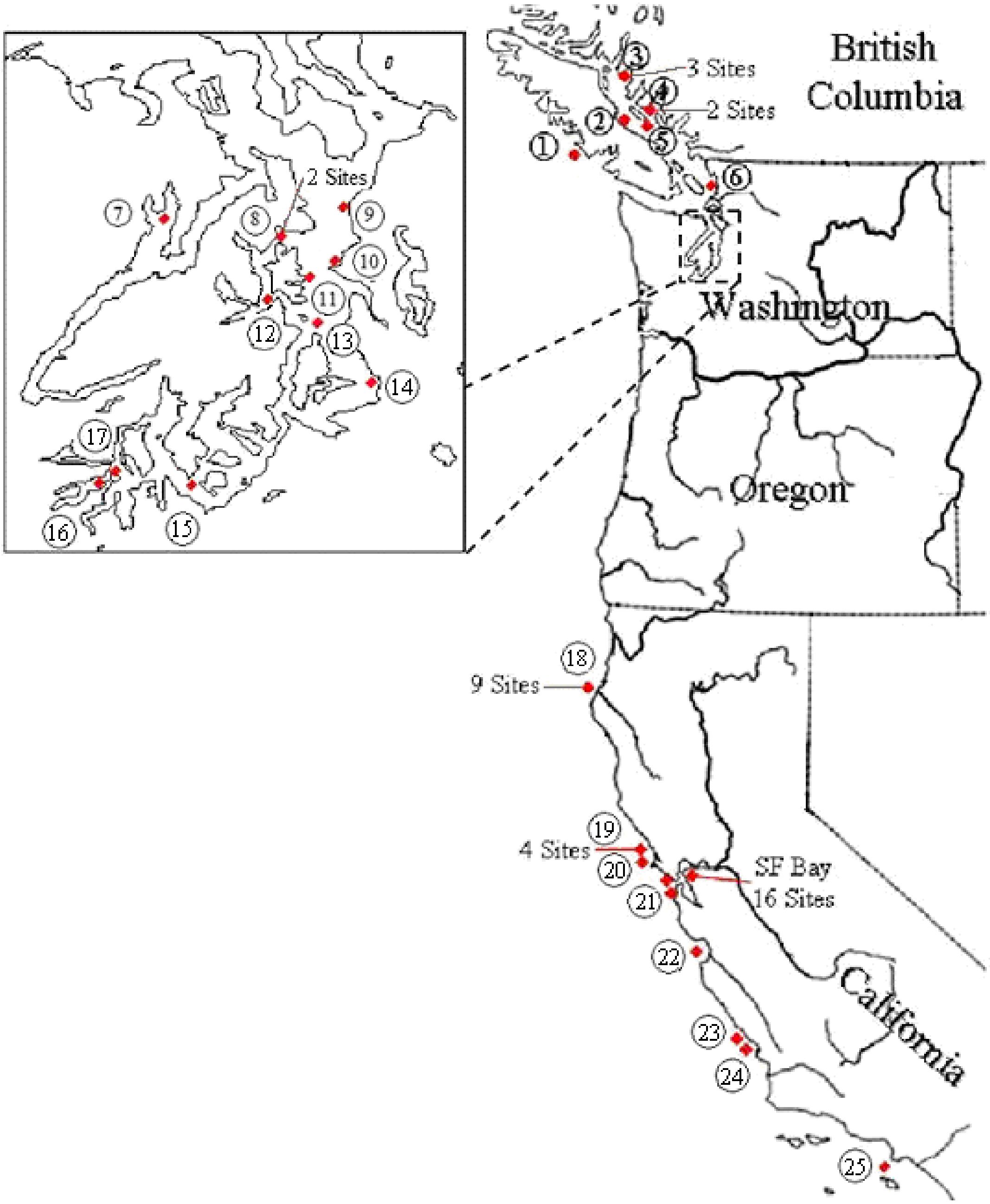

Figure 4. Locations where *Didemnum* colonies have been documented in the Gulf of Alaska and California Current Large Marine Ecosystems as of November 2006 (modified from U.S. Geological Survey 2006 and Bullard et al. 2007a). Circled numbers correspond to sites in Tables 1 and 2.

surveyed along the Oregon coast, eight sites south of Port San Louis, CA, or 14 sites surveyed south of Shinnecock, NY to Virginia Beach, VA. In a separate survey of west coast National Marine Sanctuaries and National Estuarine Research Reserves conducted in 2003 and 2004, colonies of *Didemnum* were found in Elkhorn Slough, Monterey Bay and the Gulf of Farallones, to the north of San Francisco's Golden Gate. The survey did not find colonies at any of the 14 sites surveyed in the Olympic Coast National Marine Sanctuary, WA or the 9 sites surveyed in Kachemak Bay, AK (deRivera et al. 2005). However, updated surveys are needed as new colonies of *Didemnum* continue to be recorded, such as the recent colonies found in San Diego, CA (U.S. Geological Survey 2006), suggesting that it has not reached its maximum distribution or range.

The presence of *Didemnum* on a section of gravel-pavement habitat on the northern edge of Georges Bank, approximately 160 mi. (257 km) east of Cape Cod is currently the larger of only two known occurrences of this colonial ascidian in an off-shore seabed habitat, with the other being an approximately 1.5 square mile (4 km$^{2)}$ area in eastern Long Island Sound (Osman and Whitlatch 2007). However, *Didemnum* may be more common in coastal and off-shore open-water habitats than has been documented, as faunal surveys of these deep-water habitats are logistically difficult to perform and are conducted less frequently relative to the rapid assessment surveys conducted in shallow, near-shore habitats.

While the distribution of *Didemnum* in U.S. waters has been relatively well documented, few studies have estimated the abundance and/or biomass of these populations. However, populations on Georges Bank have generally increased in size and abundance over the past few years, and have been resilient to habitat disturbance due to commercial fishing. Similarly, larval recruitment has increased steadily at two nearshore sites in the Northeast U.S. LME. In the fall of 2003, scientists from the National Oceanographic and Atmospheric Administration, U.S. Geological Survey, and University of Rhode Island discovered colonies of *Didemnum* distributed over an estimated 6 square miles (16 km^2) of ocean bottom on Georges Bank. Subsequent surveys conducted in 2004 and 2005 assessed larger areas of the gravel-pavement habitat, and found colonies distributed over 40 (106 km^2) and 88 square miles (228 km^2), respectively (Fig. 5) (U.S. Geological Survey 2006). In 2003 and 2004 many of the

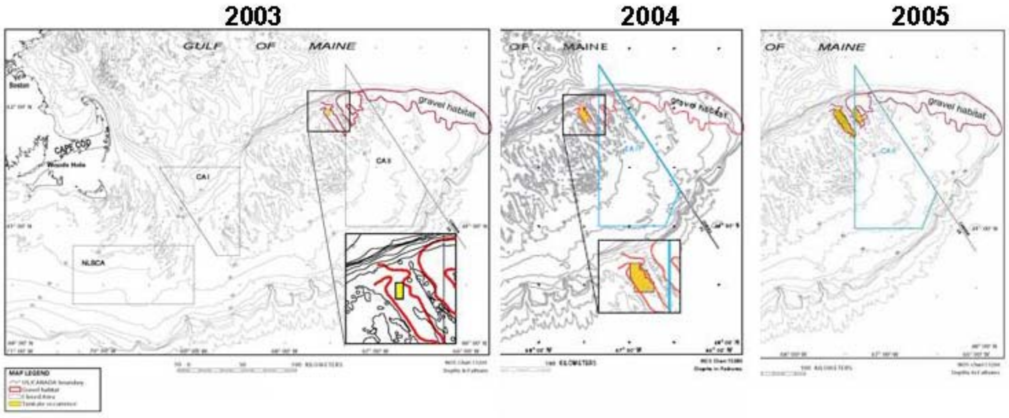

Figure 5. Maps showing size and distribution of *Didemnum* sp. A populations (in yellow) on Georges Bank from 2003 to 2005. Colonies were restricted to semi-isolated outcrop of gravel habitat open to fishing in 2003 and 2004. In 2005, colonies were found on a larger area of gravel habitat including an area closed to fishing (labeled CAII on map) (modified from U.S. Geological Survey 2006).

sampled sites had colonies of *Didemnum* covering 50 to 90 percent of the seabed. Interestingly, the percent cover of *Didemnum* observed in August 2005 was less than 50% at most locations following a period of bottom fishing activity in May-July 2005 (Robert Reid, personal communication). However, this reduction appears to have been short-lived as results from August 2006 showed that the abundance of *Didemnum* had doubled at 75% of the sites surveyed in 2005, and was similar to that observed in 2003 with colonies covering 50 to 75 percent of the seabed. Despite the high percent cover and persistence of *Didemnum* in this 88 square mile section of gravel-pavement habitat, colonies did not appear to be spreading eastward. The 2006 Georges Bank survey also included gravel-pavement habitats located in Canadian waters, and found no evidence of the presence of *Didemnum* (U.S. Geological Survey 2006).

Settlement plate studies in Connecticut and New Hampshire indicate that *Didemnum* recruitment at these sites has increased in recent years. *Didemnum* recruitment at Avery Point and the mouth of the Mystic River in Connecticut was approximately one order of magnitude greater in 2003 relative to 2001 (Osman and Whitlatch 2007). In Portsmouth Harbor, New Hampshire, Dijkstra et al. (2007a) found very little recruitment of *Didemnum* (possibly *D. vestum*) in 2003. However, *Didemnum* was a major component of the fouling community in 2004, and by the spring of 2005 had replaced *Botryllus schlosseri* as the dominant colonial ascidian at the site.

Environmental and socioeconomic impacts of *Didemnum*

Despite the widespread distribution of *Didemnum*, no dramatic environmental impacts, such as species extinctions, have been reported. Similarly, there are currently no data to indicate that it is negatively affecting economic activities, including aquaculture production or fisheries yields. However, results from a small number of studies examining the impacts of *Didemnum* on the composition of benthic and subtidal communities in the Northeast U.S. LME have recently been published (see special feature in J. Exp. Mar. Biol. Ecol., 2007, v. 342 on the Proceedings of the 1st International Invasive Sea Squirt Conference, Robert B. Whitlatch and Stephan G. Bullard, editors). Because these studies span only 1 to 3 years and include only a small subset of the known *Didemnum* populations, we consider these findings to be preliminary. We defer discussion of these studies until the potential environmental and socioeconomic impacts of *Didemnum* are considered.

Description of Potential Causes and Consequences of Status and Trends

Potential causes of *Didemnum* distribution and abundance

The specific factors underlying the relatively sudden appearance of *Didemnum* are not understood. *Didemnum* was first documented on the west and east coasts of the U.S in 1993, suggesting that it has been independently introduced at least twice. It is unclear how many of the subsequent recorded occurrences of *Didemnum* are the result of independent introductions, perhaps of different species, versus how many are due to the spread of *Didemnum* from these initial points of introduction. This section provides a general discussion of the potential factors underlying the introduction, establishment, and spread of *Didemnum.* In particular, we discuss biological and ecological attributes of *Didemnum* that may have contributed to the establishment of viable populations in the U.S. and potential transport vectors and dispersal methods.

General biology, ecology and life history of Didemnum

Didemnum is a colonial ascidian with sessile, filter-feeding adults and motile, non-feeding larvae. Colonies consist of thousands of small individuals or zooids (~1 mm) embedded in a tough outer covering or tunic (Fig. 6), and vary in color from pale pink to yellow or orange (Kott 2004). Colonies range in morphology from long, ropey or beard-like in wave protected environments to low-lying undulating and encrusting mats in habitats with strong currents (Fig. 7).

Figure 6. Close up of *Didemnum* colony surface. Solid, upward arrows point to incurrent siphon openings of 3 zooids. Dashed, downward arrows point to common cloacal openings (Photograph is credited to Gretchen Lambert).

In the U.S., *Didemnum* colonies have been found in water temperatures ranging from -2° to 24 °C (28° to 75 °F), from the low intertidal to depths of 65 m (213 ft) (Table 1), and salinities ranging from 26 to 32 ppt. *Didemnum* colonizes a variety of firm substrates such as rocks, gravel, docks, pilings, and aquaculture equipment, but is unable to establish colonies on mobile sand, mud, or other unstable substrates (Coutts 2002, Valentine et al. 2007b). *Didemnum* can also colonize and overgrow other benthic organisms including sea scallops, mussels, and seaweed (Table 1; Fig. 7), but has no known major predators (Dijkstra et al. 2007a, Valentine et al. 2007b). Furthermore, *Didemnum* appears to be able to tolerate substrate disturbances from fishing gear and ice scour (Valentine 2007a), and excess nitrogen inputs (Carmen et al. 2007).

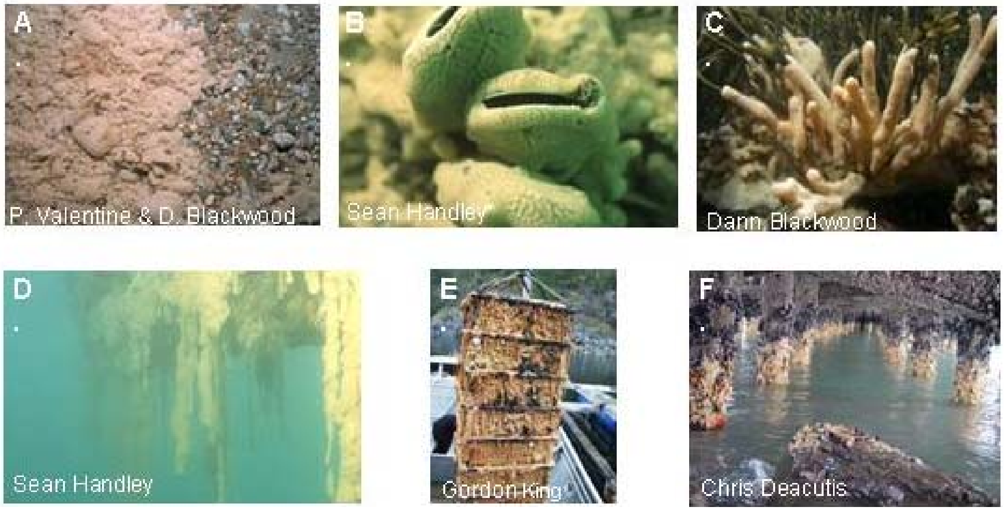

Figure 7. Photographs showing colonies of *Didemnum* sp. A growing on natural and artificial substrates. A) gravel seafloor, B) mussel shells, C) green algae, D) ship hull, E) mussel cage, and F) wood pilings (all photographs are from U.S. Geological Survey 2006, and photograph credits are shown at bottom left of each photograph).

Table 1. Locations and year of *Didemnum* sightings including depth, temperature and substrate types from when specimens were first collected in the Northeast U.S., California Current, and Gulf of Alaska LMEs. LI = lower intertidal; ND = no data (*sources*: U.S. Geological Survey 2006, Bullard et al. 2007a, and Osman and Whitlatch 2007).

LMEs and Sites	Depth (m)	Temp (°C)	Substrate Type	Year
Northeast US				
Nearshore:				
1 Cobscook Bay, ME				2003
Eastport	5.0-6.0	ND	Subtidal rocks, pilings	
2 Schoodic, ME	ND	ND	ND	2003
3 Damariscotta River, ME				1993
Fort Island Narrows	12.0	ND	Shell hash, Gravel	
Thrumcap Island	10.0-15.0	ND	Rocks	
Boothbay	ND	ND	Dock	
Clark's Cove	ND	ND	Dock	
4 Portsmouth Harbor, NH				2001
New Castle	LI – 5.0	ND	Ropes	
Fort Point	5.0	ND	Coast Guard Pier	
4 Piscataqua River, NH				2001
Newington	12.0-15.0	ND	Rock Surface	
5 Isles of Shoals				2003
Duck Island	ND	ND	ND	
6 Rockport, MA				
Sandy Bay	< 5.0	14	Boulder and Cobble	2006
7 Winthrop, MA				
Boston Harbor	1.5-3.0	5	Solitary tunicate	2006
8 Plymouth, MA	ND	ND	Docks	2004
9 Sandwich, MA				1998
Sandwich town beach	LI - 3.4	1	Tidepool rocks, floats	
10 Provincetown, MA				2003
MacMillan Wharf	LI	ND	Metal pilings, Tires, Ropes	
12 Wellfleet Harbor, MA				ND
Mayo Beach	3.3-3.6	ND	Oyster bags	
12 Town Cove, MA				2004
Orleans	LI – 0.1	ND	Floating Dock	
12 Chatham Harbor, MA				2003
Chatham	LI	ND	Wood Pilings, Mussels	
12 Pleasant Bay, MA				ND
Strong Island	2.0-3.0	ND	Rocks	
13 Cotuit Bay, MA				ND
Osterville Grand Island	LI	ND	Channel markers	
14 Martha's Vineyard, MA				2004
Vineyard Haven	LI – 0.5	0	Floats	
Oak Bluffs	LI – 2.0	0	Rope, Steel bulkhead	
15 Woods Hole, MA				2000
Eel Pond	LI-10	2.2, -2.0	Floating Docks, Ropes	
Iselin Dock	18.0-20.0	ND	Steel piling	

Table 1. Locations and year of *Didemnum* sightings continued.

LMEs and Sites	Depth (m)	Temp (°C)	Substrate Type	Year
17 Westport, MA				ND
Tripps Marina	ND	ND		
18 Narragansett Bay, RI				2003
Coasters Harbor Island	ND	ND		
Newport Shipyard	ND	ND		
Schl of Ocean, URI	LI	ND	Pilings, Rocks, Mussels	
Beavertail Point	LI	ND	Rock surface	
19 Groton , CT				2000
Mystic River	ND	ND	Plexiglass plates	
Avery Point	ND	ND	Plexiglass plates	
Pine Island	40.0	2.0-21.0	Plexiglass plates	
Niantic Bay	ND	ND	Plexiglass plates	
19 Groton , CT				2000
Groton Long Point	ND	ND	Plexiglass plates	
Bushy Point	ND	ND	Plexiglass plates	
20 Shinnecock Bay, LI, NY	LI – 0.1	ND	Floating Dock	2004
<u>Offshore & Open water:</u>				
5 Isles of Shoals, NH	ND	ND	Suspended fish cages	2003
21 Georges Bank, NW Atlantic	41.0-47.0	ND	Seafloor gravel, scallops	1998
22 Tillies Bank, MA	ND	ND	Gravel	1996
Gulf of Alaska (Canada)				
<u>Vancouver Island</u>				
1 Meares Island				2005
Tofino - Lemmens Inlet	ND	ND	Oyster Farm	
2 Baynes Sound				2005
Nanoose - Deep Bay	ND	ND	Algae, Oyster Farm	
<u>British Columbia</u>				
3 Pendrell Sound, B.C.				
East Redonda Island	6.0-15.0	ND	Rock wall	
4 Strait of Georgia, B.C.				2005
Jedediah Island	LI -1	ND	Rock surfaces	
5 Okeover Inlet, B.C.				2003
Malaspina Penninsula	12.0-15.0	12.2	Mussel cages	
Lions Rock	~10	12.8	Rock surfaces	
5 Jervis Inlet, B.C.	~6	ND	Rocky seabed	
5 Agamemnon Channel, B.C.				2004
Nelson Island	6.0-12.0	ND	Rock wall	
California Current				
6 Larrabee State Park, WA	ND	ND	ND	2007
7 Dabob Bay, WA	ND	ND	ND	2007
8 Poulsbo, WA				2007

Table 1. Locations and year of *Didemnum* sightings continued.

LMEs and Sites	Depth (m)	Temp (°C)	Substrate Type	Year
13 Dockton Park, WA	ND	ND	ND	2007
14 Des Moines, WA	5.0	ND	crab cage	2004
15 LongBranch Marina, WA	ND	ND	ND	2007
16 Steamboat Isl., WA	ND	ND	ND	2007
17 Totten Inlet, WA	0.5-1.0	ND	mussels, rope	2004
18 Humboldt Bay, CA				2001
Woodley Island	1.0	ND	PVC fouling panels	
19 Bodega Bay, CA	ND	ND	Docks, Rocks	2003
20 Tomales Bay, CA	ND	ND	Docks	2001
21 San Francisco Bay, CA				1993
Sausalito	ND	ND	Rope, Mussels, Bryozoa	
Horseshoe Bay	ND	ND	ND	
Fisherman's Wharf	ND	ND	Rope on floating dock	
22 Monterey Bay, CA				1998
Elkhorn Slough	ND	ND	Docks	
23 Morrow Bay, CA	ND	ND	Docks	2000
24 Port San Luis, CA	ND	ND	Docks	2002
25 San Diego, CA				2007
Mission Bay	0.3	ND	Foam Dock Floats,	

Colonial ascidians, such as *Didemnum,* brood their young before releasing mature larvae that live 12-24 hours. As with most ascidians, newly recruited *Didemnum* larvae probably reach sexual maturity within a few weeks following settlement and metamorphosis (Lambert 2002). At Groton, Connecticut and Bodega Bay, California, recruitment occurs between July and November with a peak in late summer or early fall (Whitlatch and Osman 2007, Bullard et al. 2007a). Furthermore, colonies containing mature larvae have been collected in May at Sausalito, California, in November at Puget Sound and Georges Bank, and as late as December in British Columbia (Valentine et al. 2007b, Bullard et al. 2007a). *Didemnum* can also produce new colonies through asexual budding, and colony fragmentation (Valentine et al 2007b, Bullard et al 2007b).

Prior to settlement, many ascidian larvae become negatively phototactic (Bates 2005, Bingham 1997) and preferentially settle in shaded microhabitats, such as that found on the undersides of rocks, docks, pontoons, and boats. In addition, pontoons and other floating structures experience consistent, mild wave action (Holloway and Connell 2002), and for reasons that are not fully understood, the presence of this ‘swash zone’ appears to favor ascidian establishment (Connell 2000, Glasby 2001).

Environmental tolerances of Didemnum *and interactions with other species*

Tolerance to temperature. Recent studies suggest that colony growth and reproduction occurs at water temperatures between 10 and 25 °C. For example, colonies grown in laboratory aquaria under constant temperature regimes for 1 week grew approximately 1.5 to 2X more rapidly in water temperatures between 15 and 20 °C relative to colonies grown in water temperatures maintained above 21 °C (McCarthy et al. 2007). Similarly, under natural mid- to late summer temperature regimes in eastern Long Island Sound, Osman and Whitlach (2007) found that *Didemnum* colonies were 4 times larger and 3.5 times more abundant (estimated as percent cover) in open-water sites with high water temperatures of 21 °C compared to colonies in the marina sites where summer temperatures exceeded 25 °C. Observations of the seasonal growth cycle of *Didemnum* colonies in a low intertidal pool at Sandwich, MA showed that colony size and morphology varied substantially across seasons (Valentine et al. 2007b). Over a 28 month period, average daily water temperatures at four sites at the Sandwich tide pool fluctuated from -0.6 to 7.3 °C in the winter and from 13.3 to 21 °C in the summer with a minimum temperature of -1.8 °C and a summer maximum of 25.6 °C. As is typical of a number of colonial ascidians, *Didemnum* colonies were reduced to small basal patches during cold winter months (Satoh 1994). Colonies began growing by asexual budding when water temperature reached 8 to 12 °C in the spring, and continued accumulating biomass through the summer. Colonies began to degenerate again when water temperatures declined relatively rapidly to 15 °C in late fall or early winter. In contrast, this cycle of growth and degeneration was not been observed on Georges Bank, which experiences less pronounced daily temperature variations and has more equitable summer and winter water temperatures. Average winter and summer water temperatures on Georges range from 4 to 15 °C, and colonies collected in November 2004 did not exhibit signs of colony degeneration as observed at the Sandwich tide pool (Valentine et al. 2007a).

Tolerance to salinity. In general, ascidians are not able to tolerate salinities below

25‰ (Lambert 2005), and colonies of *Didemnum* in San Francisco Bay have only been found in areas with salinities greater than 26‰ (Cohen 2005). In addition, short-term changes in salinity can have dramatic effects on ascidian populations. For example, *Didemnum* populations in the Damariscotta River, ME were visibly less abundant following severe flooding (L. Harris, personal communication). However, mortality resulting from seasonal changes in salinity is probably rarely, if ever, a major factor controlling the distribution of *Didemnum* over long temporal scales. In a number of Southern California harbors, Lambert and Lambert (2003) found that ascidians in the uppermost 0.5 m of the water column suffered complete mortality following heavy winter rains. However, the ascidians removed by the decrease in salinity were quickly replaced by recruits from adults living below the affected area after the rains had subsided.

<u>Tolerance to environmental stress and disturbance.</u> The ability of *Didemnum* to establish a self-sustaining population may also depend on the frequency and intensity of environmental disturbances. Communities exposed to novel disturbances (e.g., human activities) are predicted to be more vulnerable to species invasions (Mack et al. 2000). Specifically, native species unable to acclimate or adapt to the novel disturbance may be replaced by pre-adapted, nonindigenous species. Ports, harbors, and marinas are obviously heavily impacted by human activities. In particular, water quality in most of the world's estuaries is being degraded due to increasing nitrogen inputs, which can stimulate algal blooms and bacterial production (Galloway et al. 2003, Howarth et al. 2000). *Didemnum* appears to be able to tolerate moderate levels of nitrogen pollution. A recent survey of ascidian diversity in 17 southern Massachusetts coastal habitats only found *Didemnum* colonies at 7 of the 10 sites where water quality was ranked as 'fair' based on nitrogen concentration, water clarity, and the frequency of algal blooms. In contrast, colonies were not found at sites where water quality was considered 'good' or 'poor' (Carman et al. 2007).

In general, ascidians are efficient filter feeders that can take advantage of the higher bacterial levels associated with high nitrogen inputs. For example, the colonial ascidian, *Trididemnum solidum,* was historically a rare component of coral reef communities in the Caribbean. However, as concentrations of suspended bacteria increased with coastal development, colonies of *T. solidum* tripled in biomass, overgrowing and killing corals (Bak et al. 1996, 1998).

In addition to water quality degradation associated with high nutrient concentrations, many of the larger U.S. estuaries face pollution and sediment contamination from heavy metals. While heavy metals are lethal to most benthic organisms, many adult ascidians are able to tolerate and even accumulate heavy metals, including arsenic, cadmium, chromium, cobalt, copper, iron, lead, mercury, selenium, tin (as tributyltin), and zinc (Papadopoulou and Kanias 1977, Philp et al. 2003).

Physical disturbances rather than water quality degradation may have played an important role in the establishment of *Didemnum* on off-shore habitats, such as Georges Bank. The destruction of benthic habitats by dredging and trawling is a major concern in many off-shore fishing grounds (Collie et al. 2000, Fogarty and Murawski 1998, Hermsen et al. 2003). Physically disturbing benthic habitats reduces the abundance of native species and increases the availability of bare space that could potentially be colonized by *Didemnum*. Furthermore, physical disturbances may increase colony fragmentation resulting in greater local dispersal and colony growth (Stoner 1989).

Changing temperature regimes due to global warming may have provided *Didemnum* with an advantage over native species for securing settlement sites. For example, Stachowicz et al. (2002) suggested that the successful invasions of three nonindigenous species of ascidians in New England may be due to the increase in mean winter temperatures observed over the past 25 years. Specifically, they found that nonindigenous ascidians recruited earlier than native species following warm winters, giving them an advantage in securing primary space. It is currently unclear if the temperature changes documented by Stachowicz et al. (2002) have influenced the success of *Didemnum*, as additional information concerning the effects of water temperature on the reproduction and growth rates of *Didemnum* are needed before this relationship can be established.

Effects of competition. Because *Didemnum* is a sessile organism, its abundance is limited in part by the availability of hard substrates. However, *Didemnum* exhibits a number of characteristics that allow it to successfully compete with other benthic organisms for limited space. In addition to early maturation, rapid colony growth due to asexual budding, and the ability to overgrow other benthic organisms, *Didemnum* may prevent other benthic organisms from settling and growing on colony surfaces by sequestering acidic and organic allelopathic compounds in their tunics (Bryan et al. 2003, Joullie et al. 2003). For example, Dijkstra et al. (2007b) found that significantly fewer individuals and species settled on the surface of *Didemnum* (possibly *D. vestum*) compared to benthic species that do not synthesize allelopathic chemical compounds, such as solitary ascidians, bryozoans, hydroids, and bivalves.

Effects of predators. Although a variety of macro- and micro-predators consume recruits of the nonindigenous ascidians *Botrylloides* and *Diplosoma* (Osman and Whitlatch 1995), there have been no experimental studies examining the effect of predators on the abundance or distribution of *Didemnum.* However, Valentine et al. (2007a) observed the periwinkle, *Littorina littorea*, grazing on senescing colonies at Sandwich, MA. Similarly, chitons, sea urchins and seastars have been observed feeding on colonies of *D. vexillum* in New Zealand. Predators may actively avoid *Didemnum*, because it is a low quality resource or unpalatable. For example, researchers at the National Marine Fisheries Service have found that colonies of *Didemnum* consist largely of water and considerable cellulose (indigestible by most marine species), and offer little nutritional value to potential consumers (Valentine et al. 2007b). In addition, Pisut and Pawlik (2002) found that *Didemnum candidum* and *Didemnum vanderhosti* tunics had a surface pH less than 3, which served as an effective deterrent to bluehead wrasse predation.

Potential methods of introduction and spread

The introduction and spread of *Didemnum* is thought to be predominantly the result of three transport vectors; hull fouling, aquaculture, and ballast water. Historically, hull fouling has been the primary vector for the transport and introduction of nonindigenous species in marine habitats (Carlton 1989). Although the use of modern anti-fouling paints has greatly reduced the transfer of nonindigenous species via hull fouling, recent studies from Hawaii (Godwin 2003), California (Takata et al. 2006), Germany (Gollasch 2002) and New Zealand (Coutts and Taylor 2004) indicate that fouling remains an important and overlooked transport vector for sessile marine organisms (see also, Godwin et al. 2004). For example, a total of 84 species (30 native and 44 nonindigenous) were found attached to the hulls of 7 overseas cargo barges and a floating dry dock in Oahu, Hawaii (Godwin 2003). More specifically, *Didemnum vexillium* (Kott 2002) was introduced to the South Island of New Zealand on the hull of a barge. The barge is believed to have been infested while moored at the Tauranga bridge marina located on the North Island before traveling to Picton on the South Island (Coutts 2002). Species of *Didemnum* can be introduced as larvae spawned from adult colonies in the fouling community, or through colony fragmentation. Indeed, colony fragments as small as 5 cm^2 collected from the Sandwich, MA tidepool were 15 times larger after 30 days (Valentine et al. 2007a). Furthermore, fragments of *Didemnum* colonies from fishing boats or gear may have resulted in the introduction and/or spread of *Didemnum* on Georges Bank (Robert Reid and Page Valentine, personal communications).

The transport of aquaculture equipment and shellfish stock between facilities can inadvertently transfer nonindigenous species, and may have played a role in the introduction of *Didemnum* to the Northeastern U.S. The species first appeared at Fort Island Narrows, ME 11 years after oyster culture was introduced to the area. As a result, Dijkstra et al. (2007a) suggested that *Didemnum* may have been introduced to New England as a fouling organism on seed stock of the European flat oyster, *Ostrea edulis* and/or the Japanese oyster, *Crassostrea gigas*.

The threat of species introductions due to ballast water dumping has received a great deal of attention (Carlton and Geller 1993, Cangelosi 1999, Lavoie et al. 1999). However, in the case of *Didemnum,* it is likely the least important of the three transport mechanisms. Colonial ascidians produce lecithotropic larvae that have a planktonic stage lasting from 12 to 24 hours (Lambert 1968; Olson 1985; Svane and Young 1989). As a result the likelihood of *Didemnum* larvae surviving long distance transport in ballast water is low. Indeed, Carlton and Geller (1993) found that ascidian larvae were rare in ballast water samples collected from 159 cargo ships that had traveled from Japan to Oregon. However, the likelihood that larvae supplied from colonies growing in ballast tanks or that colony fragments could be transported in ballast water has not been examined and may pose transport risks (Drake et al. 2005). Similarly, the transport of larvae in ballast water during intercoastal voyages lasting less than 24 hours may have played a role in the spread of *Didemnum* following its initial introduction and establishment.

As with its introduction, the spread of *Didemnum* is most likely human mediated. Because of the short lifespan of *Didemnum* larvae and their tendency to settle near adults, the spread of *Didemnum* due to larval dispersal is likely to be primarily local. However, ports, harbors, and marinas have probably served as important corridors for the regional spread of *Didemnum* due to ballast water and hull fouling. For example, *Didemnum vexillum* was spread to different bays on the South Island of New Zealand as a fouling organism on barges ad recreational vessels (Coutts and Sinner 2004). Similarly, Wasson et al. (2001) documented colonies of *Didemnum* in Elkhorn Slough, a coastal wetland with recreational boat traffic and aquaculture facilities, but no international shipping. The role of recreational boats and aquaculture operations in spreading nonindigenous organisms beyond large ports to smaller bays, including marine reserves, is a growing concern (Wyatt 2005, Floerl and Inglis 2005).

As previously discussed, colony fragmentation due to fishing gear may have been important in generating the high abundance of *Didemnum* on Georges Bank. In addition, colony fragments caught in fishing gear may be spread to other areas when gear used in locations where *Didemnum* is present is pulled up and reset in areas outside of the areas infested by *Didemnum*. Similarly, scallop fisherman may promote the spread of *Didemnum* due to the practice of shucking scallops at sea. In particular, discarded scallop shells colonized by *Didemnum* could generate new colonies (Page Valentine, personal communication).

Although the spread of *Didemnum* has primarily been attributed to human mediated processes, other dispersal mechanisms need to be investigated. For example, larvae and colony fragments could be passively transported to new areas by water currents, or released by colonies that have colonized the carapaces of crustaceans or other mobile, hard shelled organisms. The importance of these dispersal mechanisms and the spatial scales over which they may operate are unknown, but deserve further attention.

Potential environmental and economic consequences of *Didemnum*

A potential consequence of the establishment of *Didemnum* could be a decrease in the abundance of native species and an increasing risk of local extinctions. There are numerous anecdotal accounts of *Didemnum* and *Didemnum vexillum* overgrowing and smothering benthic organisms, including scallops and mussels on Georges Bank and in New Zealand, respectively (Coutts 2002, U.S. Geological Survey 2006, Valentine et al. 2007b). However, it is unclear if overgrowth and smothering by these species of *Didemnum* is a significant source of mortality in benthic communities. At relatively small spatial scales, species of *Didemnum* and other nonindigenous ascidians have been shown to alter the abundance and composition of benthic assemblages (Oren and Benayahu 1997, Osman and Whitlatch 1995). For example, Oren and Benayahu (1997) found that *Didemnum granulatum* and an unidentified species of *Didemnum* overgrew and excluded benthic organisms on 50 x 50 cm experimental reefs in the Red Sea. In contrast, surveys of benthic assemblages on Georges Bank have not shown a strong effect of *Didemnum* on the diversity and abundance of benthic organisms.

Despite the high percent cover of *Didemnum* colonies observed on Georges Bank, the diversity and abundance of benthic organisms prior to the discovery of *Didemnum* did not differ significantly from surveys conducted after *Didemnum* was discovered, although subtle differences in community structure were present. Specifically, two polychaete species were more abundant in areas with dense *Didemnum* than in areas where it is scarcer (Valentine et al. 2007b). Although the impact of *Didemnum* on mortality rates of benthic organisms is equivocal, *Didemnum* may still adversely impact native fauna by limiting larval recruitment. In particular, *Didemnum* may restrict larval recruitment directly and indirectly by dominating hard substrates and chemically preventing larvae from settling on colony surfaces (Dijkstra et al. 2007b). While small-scale experiments can provide a mechanistic understanding of the direct and indirect interactions (e.g., overgrowth or recruitment limitation) between *Didemnum* and benthic organisms, understanding the effects of *Didemnum* on the abundance and diversity of benthic organisms will require additional studies conducted at larger spatial and temporal scales. However, even without changing the abundance of benthic and infaunal organisms, *Didemnum* could negatively impact consumers at higher trophic levels in several ways.

In areas where colonies of *Didemnum* cover significant portions of the benthos, consumers may be unable to reach prey items. Specialized predators or those with limited mobility would be particularly vulnerable to food limitation as their access to prey items decreases. Alternatively, the abundance of generalist predators capable of switching to *Didemnum* could increase, putting additional predation pressure on native prey species. Similarly, mobile predators could move to other locations, increasing predation pressure in these areas.

In addition to the potential negative impacts of *Didemnum* on species diversity, there are also concerns that *Didemnum* will have negative economic impacts on commercially important aquaculture operations and fisheries. Heavy infestations of nonindigenous ascidians in Canadian aquaculture operations have increased handling and processing costs (Cayer et al. 1999, Boothroyd et al. 2002). Lines and cages weighed down by ascidians require cleaning before they can be retrieved, and ascidians need to be removed from shells before they are marketable (Neil MacNair, personal communication). *Didemnum* will likely have similar impacts as it is capable of achieving high population densities and overgrows shellfish.

Studies examining the impacts of ascidians on shellfish mortality (Dalby and Young 1993, Mazouni et al. 2001) and meat yields (Petersen et al. 1997) have not found a consistent relationship between ascidian infestations and shellfish survivorship or production. For example, Dalby and Young (1993) found that overgrowth by ascidians had variable effects on the survivorship and size of the oyster, *Ostrea equistris*. Specifically, they found that allowing ascidians to colonize oyster cultures could decrease or enhance oyster survivorship, and either had no effect or actually increased oyster size relative to ascidian exclusion treatments. Other studies suggest that bivalves and ascidians are capable of coexisting because they partition food resources (Petersen et al. 1997, Lesser et al. 1992, Mazouni et al. 2001). Feeding trials conducted by Lesser et al. (1992) showed that blue mussels, *Mytilus edulis,* had significantly higher feeding rates on particles ranging in size from 3 to >16 μm compared to the ascidians, *Botryllus schlosseri* and *Ciona intestinalis.* In contrast, colonial ascidians have been shown to retain particles smaller than 2 μm; whereas, bivalves are less efficient at filtering these smaller items (Petersen et al. 1997). While differences in resource utilization may limit competition for food, interference competition may still be an important factor. Colonies of *Didemnum* have been observed overgrowing mussels, scallops, and oysters to the point where the ability of these bivalves to open their shells and feed is compromised (U.S. Geological Survey 2006).

Finfish fisheries on Georges Bank may also be negatively impacted by *Didemnum*. The gravel habitat where *Didemnum* is currently found serves as an important nursery for juvenile cod and haddock (Collie et al. 2000), as well as a critical spawning ground for Atlantic herring (Smith and Mores 1993). The high water column and benthic productivity of this habitat provides young fish with an abundant food supply (Townsend and Thomas 2002). In addition, the structural complexity of the gravel matrix likely provides juvenile fishes with crevices and holes for avoiding predators (Gotceitas and Brown 1993). High densities of *Didemnum* may dramatically alter the availability of food and refugia for these commercially important fish species. Before there was intensive bottom trawling and dredging in this area, the gravel bottom probably supported more emergent epifauna (e. g., hydrozoans, bryozoans) than it presently does. This epifauna may increase the forage and shelter value of the gravel pavement for some species and life stages. Recovery of the emergent epifauna could be more difficult in areas with dense colonies of *Didemnum*. In addition, the allelopathic chemicals that prevent benthic organisms from overgrowing *Didemnum* may be harmful to the eggs of fish that rely on the gravel pavement for spawning sites, such as the Atlantic herring (Dijkstra et al. 2007b). Atlantic cod, haddock, and Atlantic herring had a dockside value of over 55 million dollars in 2004, and accounted for approximately 24% of the total dockside value of fish landings in New England (National Marine Fisheries Service 2005). *Didemnum* has the potential therefore to negatively impact the abundance or distribution of these economically important fish species.

Forecasts of Future Conditions with No Management Action

At present, there is insufficient information to accurately predict the future distribution and impacts of *Didemnum* in U.S. waters. In particular, the northern and southern limits of *Didemnum's* range, the factors controlling its abundance, and the relative importance of potential transport vectors remain to be fully examined. In addition, studies documenting the effects of *Didemnum* on native species and economic activities have not been completed. However, new populations of *Didemnum*, or closely related species, continue to be found in the U.S. and worldwide (U.S. Geological Survey 2006, Minchin and Sides 2006), suggesting that the spread of *Didemnum* has not subsided. Furthermore, the studies and examples discussed in the proceeding sections indicate that it is conceivable that *Didemnum* will negatively impact native species, aquaculture, and fisheries. Although the lack of information prevents the use of quantitative analyses to predict the spread and impacts of *Didemnum*, general predictions about the potential spread of *Didemnum* can be made using existing information on the tolerance of *Didemnum* to water temperature and temperature regimes within its current range.

1. New colonies of *Didemnum* will continue to be found in the U.S. Northeast, California Current, and Gulf of Alaska LME's.

2. The ability of *Didemnum* to establish colonies in the Southeast U.S., Caribbean, Gulf of Mexico, and Insular-Pacific Hawaiian LMEs is limited due to high summer water temperatures.

3. The ability of *Didemnum* to establish colonies in the East Bering Sea, Chuckchi Sea, and Beaufort Sea LMEs is limited due to the short duration of the growing season.

Most studies that have examined the ecological factors controlling the distribution and abundance of *Didemnum* have focused on water temperature. Results of these field observations and experimental studies suggest that colony growth rates decline when temperatures exceed 21 ºC for 7 consecutive days (McCarthy et al. 2007). However, colonies can survive in habitats where daily temperatures fluctuate between ~15 and 26 ºC (Valentine et al. 2007b). Similarly, water temperatures above 8 to 10 ºC are necessary for colony growth; however, colonies can survive extended periods of time below this temperature threshold as an unidentified overwintering form (Valentine 2007b). We investigated the potential spread of *Didemnum* by examining mean monthly water temperatures and monthly maximum and minimum temperatures at multiple locations within LMEs of the U.S. Areas where mean monthly temperatures were above 25 ºC and minimum temperatures were greater than 21 ºC for consecutive months were considered potentially unsuitable habitats for supporting large populations of *Didemnum.* Temperature data for locations where *Didemnum* colonies currently exist were included to provide insights into the number of months colonies are capable of overwintering. The data used for this analysis were collected from NOAA's National Oceanographic Service tides and currents meteorological stations (http://tidesandcurrents.noaa.gov). These stations are located onshore in ports or other nearshore habitats, and record subtidal water temperatures every hour.

Although we expect new colonies of *Didemnum* to continue to be found in the Northeast U.S., California Current, and Gulf of Alaska LMEs, the spread of *Didemnum* beyond its current range in the Northeast U.S. and Gulf of Alaska may be limited. Colonies are currently restricted to the northern half of the Northeast U.S. and the southernmost region of the Gulf of Alaska (Figs. 3 and 4). In the Northeast U.S., average monthly temperatures above 25 ºC and temperatures ranging from 21 ºC up to the low 30's begin just south of Long Island at Sandy Hook, NJ (Table 2). Similarly, the ability of *Didemnum* to extend its range northward in the Gulf of Alaska may decrease as the length of the growing season shortens. Seasonal temperatures in Ketchikan, AK are similar to those in Eastport, ME, the coldest location where *Didemnum* colonies have been documented. However, the 6 months with temperatures above 9 ºC in Ketchikan is reduced to 4 months in Anchorage and areas to the west, which may prevent the establishment of *Didemnum* or limit it to small colonies (Table 2). In contrast to the Northeast U.S. and Gulf of Alaska, nearshore water temperatures suitable for the growth of *Didemnum* occur throughout the U.S. portion of the California Current LME. As a result, areas currently free of *Didemnum*, such as the coasts of Oregon and Washington may be susceptible to the establishment of *Didemnum*.

Water temperatures become less suitable for *Didemnum* establishment as you move away from the temperate systems where *Didemnum* is currently found towards subarctic LMEs in the north and subtropical and tropical LMEs in the south and western pacific. The East Bering Sea, Chukchi Sea, and Beaufort Sea LMEs are remote, high latitude systems with extended periods of sea ice cover and water temperatures below 9 ºC for 7 to 12 months of the year (Table 2). Mean monthly water temperatures in the Southeast US, Insular Pacific-Hawaiian, Gulf of Mexico, and Caribbean LMEs are above 25 ºC for 4 to 12 months of the year (Table 2). These temperature regimes should hinder the ability of *Didemnum* to establish large, viable populations at nearshore sites in these LMEs.

We emphasize that these forecasts are tentative and subject to a number of data limitations and other uncertainties. For example, our assessment is not applicable for predicting the spread of *Didemnum* in off-shore habitats in the Northeast U.S, the northern part of the Southeast U.S., and the Gulf of Alaska LMEs. Because water temperatures in deeper habitats are colder and less seasonally variable, *Didemnum*'s distribution limit in off-shore habitats in the Northeast U.S. may extend further south than suggested by our analysis of nearshore water temperatures. For example, mean water

Table 2. 2006 monthly mean and maximum-minimum water temperatures from multiple nearshore locations within six Large Marine Ecosystems for 2006. Mean monthly averages 25 ºC or above and minimum temperatures above 21 ºC are highlighted in yellow. Mean monthly averages below 9 ºC are highlighted in blue. Numbered locations correspond to Figures 3 and 4.

LME and Buoy Location	Month											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Northeast U.S.												
1 Eastport ME	5.4 (4.1, 6.7)	4.1 (2.4, 5.2)	3.4 (2.2, 4.7)	4.9 (3.9, 6.3)	6.9 (5.5, 8.6)	8.7 (7.2, 10.0)	10.6 (8.5, 12.9)	12.3 (11.1, 13.4)	12.8 (12.0, 13.7)	12.6 (11.2, 13.9)	11.0 (9.8, 13.9)	10.2 (8.1, 13.7)
2 Bar Harbor ME	3.8 (0.5, 4.7)	2.1 (-0.9, 3.9)	2.0 (0.3, 4.0)	5.2 (3.1, 7.9)	9.0 (6.5, 11.6)	12.2 (9.9, 15.7)	14.5 (6.2, 18.7)	14.1 (11.4, 17.8)	13.7 (12.5, 15.7)	12.2 (9.9, 13.5)	10.0 (8.3, 11.1)	7.0 (3.3, 9.8)
Portland ME	4.3 (2.9, 5.4)	3.4 (1.5, 4.3)	3.2 (1.8, 5.4)	6.6 (4.7, 8.4)	9.1 (7.5, 12.7)	12.9 (11.0, 15.2)	14.2 (11.2, 18.2)	15.1 (13.3, 19.8)	15.2 (13.2, 17.2)	12.6 (-0.1, 14.1)	9.6 (7.7, 10.6)	8.0 (5.8, 9.1)
7 Boston Harbor MA	4.3 (3.3, 5.9)	3.6 (2.0, 5.2)	3.2 (1.5, 6.1)	8.1 (5.2, 11)	11.0 (9.3, 14.7)	14.8 (11.3, 18.5)	15.8 (12.6, 19.4)	17.9 (15.3, 23.4)	18.0 (15.1, 19.9)	13.6 (10.4, 16)	10.3 (9.1, 12.1)	8.6 (6.7, 17.3)
15 Woods Hole MA	3.9 (3.1, 5.5)	3.0 (0.5, 5.1)	2.6 (0.1, 6.1)	7.8 (5.0, 10.6)	11.9 (9.6, 15.2)	17.2 (14.7, 20.6)	21.6 (19.2, 24.0)	22.3 (19.9, 25.5)	19.4 (17.7, 20.8)	15.6 (11.0, 18.8)	11.5 (10.1, 13.4)	8.0 (5.6, 12.0)
18 Newport RI	5.4 (2.4, 7.4)	4.5 (1.0, 6.7)	4.7 (2.1, 7.4)	8.1 (5.6, 11.0)	11.1 (8.9,16.7)	15.8 (12.0, 21.0)	20.4 (16.0, 26.5)	20.7 (17.8, 27.2)	18.7 (17.2, 20.5)	15.6 (10.0, 18.8)	12.4 (10.4, 13.9)	9.0 (5.2, 12.4)
19 New London CT	5.0 (2.4, 7.2)	4.6 (1.9, 6.5)	5.1 (2.0, 8.4)	8.8 (5.8, 12.0)	12.0 (8.8, 16.2)	16.4 (12.4, 22.0)	20.2 (17.1, 24.7)	21.2 (19.0, 24.1)	19.8 (18.6, 20.9)	16.5 (10.8, 19.5)	12.2 (8.1, 14.8)	ND
Kings Point NY	4.1 (0.9, 6.1)	3.4 (-1.3, 6.1)	4.3 (-0.7, 9.0)	9.6 (6.8, 19.3)	14.7 (12.1, 24.4)	18.5 (16.1, 24.5)	20.6 (17.6, 25.5)	22.6 (16.3, 26.0)	20.2 (7.5, 25.9)	17.8 (11.3, 22.0)	14.0 (10.4, 14.2)	9.6 (3.0, 15.9)
Sandy Hook NJ	5.2 (2.9, 8.5)	4.0 (2.0, 6.7)	6.1 (1.4, 12.8)	11.8 (8.6, 16.7)	15.9 (13.0, 20.0)	20.2 (17.6, 23.3)	23.7 (18.0, 30.0)	25.0 (21.6, 31.5)	20.8 (16.7, 23.6)	15.5 (9.6, 19.0)	11.6 (8.5, 12.9)	7.7 (4.0, 12.6)
Cape May NJ	6.6 (4.3, 12.5)	7.3 (3.7, 15.6)	8.1 (3.6, 12.0)	14.6 (11.2, 18.7)	18.1 (15.2, 21.7)	21.9 (18.8, 34.0)	22.9 (18.5, 27.3)	25.0 (21.8, 27.2)	22.1 (20.3, 24.2)	17.0 (10.0, 22.2)	13.1 (10.2, 16.4)	9.1 (2.9, 13.7)
Lewes DE	6.0 (3.2, 7.6)	5.4 (2.7, 7.2)	6.5 (3.5, 9.3)	11.6 (8.5, 15.3)	16.3 (12.0, 20.6)	20.2 (18.0, 24.2)	21.5 (16.8, 26.1)	23.4 (20.7, 27.8)	21.9 (19.7, 23.8)	17.1 (12.2, 21.2)	12.5 (9.5, 15.3)	9.2 (5.3, 13.4)

Table 2. 2006 monthly mean and maximum-minimum water temperatures continued.

LME and Buoy Location	**Month**											
	JAN	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**
Northeast U.S.												
Baltimore MD	5.0 (3.4, 6.8)	4.8 (3.3, 6.4)	6.6 (4.3, 11.4)	13.1 (9.7, 19.3)	17.6 (14.6, 24.2)	23.3 (18.9, 26.2)	27.5 (25.0, 29.2)	27.3 (25.4, 29.4)	23.2 (20.6, 26.2)	18.4 (12.4, 22.1)	12.3 (7.9, 14.3)	8.6 (6.7, 11.5)
Solomons Island MD	6.5 (5.6, 12.0)	5.0 (4.2, 6.4)	7.0 (3.9, 10.5)	12.8 (8.8, 17.1)	18.2 (15.1, 22.9)	23.3 (20.5, 29.2)	27.5 (25.0, 30.8)	28.5 (26.4, 31.5)	24.0 (22.3, 26.7)	19.2 (13.3, 23.8)	12.5 (9.8, 15.4)	8.8 (6.5, 12.2)
Southeast U.S.												
Cape Hatteras NC	8.5 (6.0, 11.4)	7.7 (5.8, 9.4)	8.1 (5.8, 12.2)	11.8 (7.9, 16.9)	15.3 (11.6, 21.6)	18.1 (11.8, 24.6)	19.6 (12.7, 26.9)	23.6 (17.9, 28.9)	23.4 (21.6, 31.6)	19.8 (15.0, 22.8)	15.4 (12.0, 29.3)	11.9 (9.8, 14.6)
Beaufort NC	11.5 (9.0, 13.4)	10.4 (8.5, 17.3)	12.7 (10.1, 17.1)	19.1 (16.0, 22.1)	21.4 (15.8, 27.7)	25.8 (20.1, 28.7)	28.3 (25.1, 30.6)	28.6 (25.4, 30.5)	25.2 (22.7, 28.7)	21.4 (15.8, 25.5)	15.9 (11.6, 19.7)	13.6 (8.8, 17.2)
Charleston SC	14.2 (13, 15.4)	13.8 (12, 15.2)	16.1 (13, 18.9)	21.3 (17.1, 24.4)	24.0 (21.5, 28.4)	27.9 (27.0, 30.9)	30.6 (28.4, 32.2)	31.1 (29.2, 32.9)	28.9 (27.1, 31.2)	24.8 (19.1, 30.0)	16.5 (13.1, 20.2)	15.6 (12.0, 20.2)
Fernandina Beach FL	14.4 (12.5, 16.8)	13.8 (10.3, 16.6)	16.6 (13.4, 20.1)	20.6 (17.5, 24.2)	23.8 (19.8, 27.6)	27.5 (25.7, 29.8)	29.3 (27.0, 31.0)	30.0 (27.1, 31.0)	27.8 (26.2, 29.9)	23.5 (19.6, 27.3)	17.6 (12.9, 21.8)	15.5 (11.9, 19.1)
Trident Pier FL	18.5 (15.8, 21.8)	19.3 (16.4, 21.9)	21.9 (19.7, 24.2)	25.2 (22.8, 28.6)	27.3 (24.2, 30.0)	30.2 (28.0, 31.9)	30.9 (26.7, 32.8)	27.9 (21.4, 30.7)	31.1 (28.5, 31.6)	28.6 (25.9, 30.4)	24.2 (20.8, 27.0)	23.2 (20.7, 25.5)
Virginia Key FL	21.2 (18.8, 23.5)	21.5 (17.3, 25.2)	23.2 (21.2, 26.0)	25.6 (22.4, 28.9)	27.1 (23.8, 29.6)	29.4 (27.6, 31.6)	29.7 (28.2, 31.4)	30.2 (27.9, 32.3)	25.6 (22.0, 31.8)	ND	ND	20.2 (14.6, 23.5)
Caribbean												
Key West FL	21.4 (18.7, 24.1)	21.3 (17.4, 24.7)	23.5 (20.8, 27.4)	26.3 (22.7, 29.5)	28.2 (25.9, 30.7)	30.0 (27.7, 31.3)	30.1 (28.9, 31.3)	30.7 (29.0, 32.3)	30.5 (28.7, 31.8)	28.1 (25.0, 30.0)	24.4 (20.2, 26.5)	23.9 (21.7, 25.7)
Gulf of Mexico												
Naples FL	19.0 (16.3, 22.8)	18.5 (14.8, 22.7)	22.1 (19.0, 26.4)	25.2 (21.1, 29.5)	28.2 (25.3, 28.3)	30.0 (27.6 ,32.0)	30.2 (28.3, 32.4)	31.2 (28.9, 32.8)	30.7 (29.2, 33.1)	27.3 (23.0, 30.6)	22.1 (17.1, 25.5)	21.0 (18.2, 23.4)
St. Petersburg FL	17.6 (16.2, 20.3)	17.2 (14.4, 20.7)	20.8 (18.6, 23.5)	23.4 (21.1, 26.6)	26.0 (23.1, 28.9)	29.0 (26.5, 31.2)	30.5 (28.6, 32.5)	31.1 (29.4, 33.4)	29.7 (28.6, 31)	26.1 (22.1, 30.4)	21.2 (17.2, 22.8)	19.7 (17.3, 21.7)

Table 2. 2006 monthly mean and maximum-minimum water temperatures continued.

LME and Buoy Location	Month											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Gulf of Mexico												
Pensacola FL	17.5 (14.4, 19.4)	17.9 (14.3, 19.9)	22.3 (19.4, 26.1)	26.9 (23.1, 30.6)	28.0 (25.3, 35.8)	29.9 (27.0, 32.4)	31.9 (30.2, 33.3)	32.1 (30.8, 34.2)	28.7 (26.7, 31.7)	25.7 (20.7, 29.9)	19.2 (14.3, 23.3)	16.4 (12.1, 23.0)
Pilot Station East, SW Pass LA	12.3 (10.4, 15.0)	10.8 (9.9, 12.2)	14.2 (10.0, 18.5)	18.6 (13.4, 22.7)	ND	ND	29.7 (28.6, 31.1)	30.5 (29.5, 32.1)	28.6 (23.7, 35.7)	27.5 (20.0, 27.7)	20.5 (13.5, 20.6)	11.5 (10.4, 13,7)
Galveston Bay TX	14.9 (12.4, 17.3)	14.2 (10.1, 17.4)	18.3 (13.5, 21.5)	23.2 (19.6, 26.4)	25.7 (23.5, 28.0)	28.4 (25.2, 30.7)	29.7 (27.6, 32.6)	30.9 (29.2, 32.9)	28.7 (24.9, 31.7)	24.7 (18.8, 28.7)	19.2 (14.0, 23.3)	14.0 (8.9, 16.9)
Corpus Cristi TX	17.8 (15.6, 20.2)	16.8 (13.4, 19.2)	20.2 (16.1, 23.4)	24.5 (21.7, 27.7)	26.8 (21.8, 29.0)	28.8 (26.5, 31.0)	30.1 (28.2, 32.4)	30.5 (28.7, 32.3)	28.7 (26.4, 31.9)	26.4 (21.7, 29.3)	22.3 (19.0, 25.5)	16.9 (13.1, 19.7)
Port Isabel TX	18.1 (15.5, 20.4)	17.5 (13.6, 20.2)	20.8 (16.5, 25.5)	24.7 (22.2, 27.0)	26.8 (25.1, 28.6)	29.7 (26.3, 33.3)	30.2 (27.7, 46.7)	30.0 (27.3, 38.5)	29.6 (27.1, 37.5)	27.9 (23.1, 30.9)	23.4 (19.4, 27.0)	19.1 (14.7, 23.5)
Beuforet Sea												
Red Dog Dock AK	-1.7 (-1.8, -1.7)	-1.7 (-1.8, -1.5)	-1.7 (-1.8, -1.7)	-1.8 (-1.8, -1.7)	-1.7 (-1.8, -0.4)	-0.7 (-1.7, 7.5)	7.3 (-1.0, 15.0)	8.6 (6.8, 10.4)	8.9 (6.6, 12.7)	4.4 (0.0, 8.3)	0.7 (-1.6, 3.5)	-1.7 (-1.8, -1.5)
East Bering Sea												
Nome AK	-5.3 (-10.6, -1.1)	-3.1 (-11.6, 0.0)	-1.4 (-8.4, 0.0)	-2.4 (-8.4, 0.0)	0.5 (-1.8, 5.9)	6.7 (0.9, 12.4)	11.3 (5.7, 15.0)	11.4 (6.0, 14.3)	9.0 (5.3, 12.3)	4.6 (-1.5, 7.5)	0.4 (-5.2, 2.9)	-1.5 (-9.2, -0.2)
Unalaska AK	3.3 (1.2, 4.5)	2.9 (0.6, 4.3)	3.7 (3.1, 4.4)	4.1 (3.1, 5.1)	6.0 (3.4, 11.6)	8.9 (7.5, 11.3)	8.9 (6.8, 11.1)	9.8 (9, 11.1)	8.7 (7.9, 10.1)	7.0 (5.2, 8.4)	4.9 (3.4, 6.8)	4.0 (1.7, 5.5)
Adak Island AK	7.3 (6.4, 8.0)	6.9 (6.0, 7.3)	7.2 (6.6, 7.6)	7.4 (7.0, 8.6)	8.1 (7.3, 9.5)	8.6 (7.7, 9.8)	9.3 (8.5, 10.7)	10.5 (9.4, 12.8)	11.0 (9.5, 13.4)	10.0 (9.4, 10.6)	9.4 (8.9, 9.9)	8.6 (7.5, 9.2)
Gulf of Alaska												
Sand Point AK	3.9 (2.0, 6.3)	2.9 (1.6, 3.7)	3.0 (2.1, 3.9)	3.2 (0.5, 3.9)	4.3 (3.0, 7.9)	6.9 (4.3, 8.6)	9.8 (6.7, 11.2)	10.7 (9.7, 11.4)	10.6 (9.8, 12.7)	9.8 (8.5, 10.4)	6.8 (4.9, 8.6)	4.7 (3.2, 6.1)
Kodiak AK	4.2 (0.4, 5.4)	1.9 (0.4, 3.0)	1.9 (0.5, 3.6)	4.4 (3.2, 5.7)	6.7 (4.9, 10.3)	9.9 (8.5, 11.2)	10.8 (9.6, 11.8)	11.9 (10.8, 14.1)	11.8 (10.3, 12.9)	8.9 (6.0, 10.5)	3.2 (0.0, 8.0)	2.2 (0.5, 3.8)

Table 2. 2006 monthly mean and maximum-minimum water temperatures continued.

LME and Buoy Location	Month											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Gulf of Alaska												
Anchorage AK	-1.0 (-1.1, 2.4)	-1.1 (-0.2, 2.9)	-1.1 (-1.2, -0.2)	0.5 (-0.9, 2.8)	6.4 (2.3, 11.8)	11.9 (10.6,13.5)	13.9 (12.4,15.5)	13.0 (9.8, 14.6)	11.4 (9.4, 12.5)	7.4 (3.5, 10.2)	0.3 (-.9, 4.4)	-0.9 (-1.0, -0.8)
Seward AK	5.6 (3.5, 6.9)	3.9 (2.8, 5.3)	4.6 (3.7, 5.7)	4.6 (3.9, 6.0)	6.2 (4.7, 11.4)	9.7 (5.3, 13.5)	12.1 (10.4, 14.8)	13.4 (10.0, 14.6)	12.2 (8.6, 13.2)	9.8 (5.1, 11.7)	7.4 (4.0, 10.6)	5.1 (3.3, 6.8)
Valdez AK	5.2 (3.4, 6.6)	4.3 (2.1, 6.1)	5.4 (3.9, 6.2)	5.6 (4.2, 6.6)	7.4 (5.4, 11.4)	9.4 (6.9, 12.8)	10.6 (7.2, 12.1)	10.8 (4.4, 11.9)	11.2 (8.8, 11.8)	10.2 (4.5, 11.3)	6.1 (2.9, 9.9)	4.7 (3.3, 5.7)
Yakutat AK	6.3 (5.3, 7.0)	5.2 (4.3, 5.4)	4.2 (2.3, 4.9)	5.3 (4.2, 6.7)	6.9 (5.6, 11.1)	11.7 (9.0, 13.6)	12.4 (8.6, 15.3)	12.6 (8.1, 14.0)	11.8 (11.2, 12.9)	10.4 (7.7, 11.7)	7.2 (4.5, 10.3)	4.8 (4.3, 5.2)
Juneau AK (2004)	3.8 (1.0, 5.3)	3.2 (1.2, 3.9)	3.8 (3.3, 4.3)	5.1 (3.7, 7.8)	7.4 (4.8, 12.5)	ND	ND	ND	ND	ND	ND	ND
Sitka AK (2005)	6.4 (5.6, 7.4)	5.4 (4.5, 6.0)	6.0 (5.6, 6.4)	6.9 (5.8, 9.9)	10.0 (7.1, 12.7)	11.6 (9.2, 14.4)	14.0 (12.5, 15.8)	15.0 (12.5, 18.2)	ND	ND	ND	ND
Ketchikan AK	7.7 (6.7, 8.4)	7.2 (6.3, 8.0)	7.4 (7.1, 7.9)	8.1 (7.6, 8.6)	9.3 (7.9, 11.5)	13.1 (10.9, 14.9)	14.6 (11.8, 17.0)	14.0 (12.0, 15.8)	12.7 (11.4, 19.1)	10.8 (9.6, 12.2)	7.9 (5.7, 9.9)	6.8 (5.7, 7.4)
California Current												
Cherry Point WA	8.4 (7.9, 8.7)	8.0 (7.2, 8.5)	8.0 (7.3, 8.8)	9.0 (8.3, 10.8)	10.5 (9.0, 12.7)	11.5 (9.7, 15.9)	13.6 (10.8, 16.5)	ND	ND	10.7 (9.8, 11.2)	9.3 (7.5, 10.4)	8.3 (7.1, 8.8)
6 Seattle, WA	9.2 (8.4, 9.8)	8.6 (8.3, 9.2)	8.6 (8.2, 9.0)	9.0 (8.6, 10.2)	10.2 (9.3, 11.6)	11.8 (10.2, 14.0)	12.8 (11.9, 14.8)	13.4 (12.7, 14.9)	13.3 (12.9, 14.4)	12.6 (11.8, 13.3)	11.2 (8.4, 12.0)	9.9 (8.8, 10.3)
Neah Bay WA	9.4 (8.7, 10.1)	8.1 (6.1, 9.5)	8.3 (7.3, 9.5)	9.9 (8.8, 11.6)	10.9 (9.2, 12.8)	12.6 (10.3, 15.4)	11.6 (10.0, 14.4)	11.5 (9.5, 14.6)	10.8 (9.1, 12.6)	9.8 (8.5, 11.2)	9.3 (6.6, 10.2)	8.3 (6.9, 9.2)
Astoria OR	7.0 (6.3, 7.8)	5.8 (3.9, 7.8)	6.7 (4.8, 9.0)	10.4 (8.5, 15.4)	14.3 (11.6, 16.8)	17.0 (14.9, 20.0)	20.2 (15.4, 23.0)	20.0 (15.2, 22.7)	18.2 (14.9, 20.8)	15.2 (12.0, 18.3)	10.6 (7.1, 13.3)	6.6 (5.3, 8.0)
Charleston OR	11.0 (9.4, 12.2)	10.1 (7.7, 11.8)	9.8 (8.2, 12.0)	11.3 (10.0, 13.8)	11.6 (8.5, 15.5)	13.0 (8.8, 16.6	11.9 (9.3, 15.5)	11.0 (8.9, 19.7)	11.1 (9.3, 14.1)	10.8 (9.2, 12.8)	11.2 (8.5, 13.3)	10.5 (8.4, 12.0)

Table 2. 2006 monthly mean and maximum-minimum water temperatures continued.

LME and Buoy Location	Month											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
California Current												
7 North Spit CA	11.4 (9.9, 12.7)	10.4 (9.3, 12.2	10.2 (8.8, 11.5)	11.3 (9.3, 13.3)	11.3 (8.5, 15.4)	12.6 (9.6, 16.0)	12.4 (10.0, 16.2)	12.8 (10.6, 16.5)	12.2 (10.3, 15.9)	12.0 (10.7, 13.9)	12.2 (10.2, 14.0)	11.0 (9.4, 12.2)
Arena Cove CA	12.0 (11.0, 12.9)	10.8 (8.4, 12.4)	10.8 (8.9, 12.1)	11.2 (9.1, 13.7)	9.9 (8.1, 13.4)	10.2 (8.3, 14.6)	10.4 (8.3, 16.6)	11.5 (9.6, 13.7)	11.3 (10.0, 12.9)	13.0 (11.3, 14.5)	12.6 (10.1, 15.1)	11.5 (10.3, 12.8)
10 San Franscico CA	11.9 (11.1, 13.7)	12.0 (11.3, 14.1)	11.6 (10.8, 13.2)	12.5 (11.7, 14.2)	13.6 (12.6, 15.6)	13.6 (12.5, 16.3)	14.5 (13.0, 17.8)	16.4 (15.8, 17.8)	15.2 (14.2, 17.4)	15.1 (14.3, 16.4)	14.2 912.3, 15.10	11.7 (10.7, 12.9)
11 Monterey CA	12.5 (11.1, 13.5)	11.9 (11.0, 12.8)	12.3 (11.6, 13.0)	13.5 (11.6, 15.1)	14.0 (11.0, 16.2)	14.0 (10.2, 16.3)	14.3 (11.8, 17.3)	16.3 (13.1, 17.8)	14.7 (13.2, 16.7)	15.2 (13.6, 16.3)	14.1 (12.5, 15.9)	12.8 (11.6, 14.0)
13 Port San Luis CA	12.4 (11.3, 13.9)	11.9 (11.2, 13.1)	11.5 (10.1, 12.8)	12.4 (9.3, 14.4)	12.7 (10.4, 14.8)	13.3 (11.2, 16.1)	14.8 (11.8, 18.2)	15.0 (13.1, 18.8)	14.6 (12.6, 16.4)	15.2 (14.6, 16.4)	13.8 (11.8, 16.1)	12.9 (11.3, 14.3)
Los Angeles CA	14.8 (13.5, 15.9)	14.8 (13.8, 15.8)	13.2 (11.9, 15.1)	13.5 (12.2, 14.8)	16.5 (14.3, 18.5)	17.6 (13.4, 20.1)	18.6 (13.8, 21.7)	17.6 (15.3, 20.6)	18.5 (15.9, 20.2)	17.5 (15.8, 18.7)	18.0 (17.3, 18.4)	16.3 (15.1, 17.5)
14 San Diego CA	15.2 (14.6, 16.0)	15.6 (14.7, 16.6)	15.5 (14.6, 16.5)	17.2 (15.9, 20.7)	18.8 (16.7, 21.6)	21.2 (18.1, 23.1)	23.1 (17.5, 24.9)	22.2 (19.2, 25.5)	21.2 (18.9, 23.2)	19.7 (17.7, 21.4)	19.0 (17.8, 19.9)	15.9 (14.6, 18.0)
Insular Pacific-Hawaiian												
Nawilliwil HI	25.1 (22.8, 29.8)	24.6 (22.1, 26.2)	24.4 (22.0, 26.4)	25.0 (23.1, 26.9)	25.6 (24.5, 27.4)	26.5 (25.7, 28.1)	27.3 (26.0, 29.3)	28.0 (26.3, 29.5)	28.2 (26.5, 29.8)	27.7 (26.2, 29.0)	27.2 (25.9, 28.9)	26.0 (24.5, 27.4)
Honolulu HI	26.4 (22.1, 32.6)	26.3 (25.3, 27.4)	26.5 (25.2, 27.3)	26.4 (23.9, 27.8)	27.2 (26.5, 28.0)	27.5 (27.5, 28.8)	ND	29.2 (28.4, 30.2)	28.9 (28.4, 29.4)	28.9 (27.9, 30.3)	28.7 (27.9, 26.9)	27.0 (26.4, 28.2)
Kahului HI	24.2 (23.2, 26.0)	24.0 (22.1, 25.1)	24.2 (23.4, 25.3)	23.8 (22.4, 25.1)	25.1 (24.0, 26.4)	25.8 (24.8, 27.0)	26.2 (25.1, 27.6)	26.8 (25.7, 28.0)	26.7 (25.5, 31.9)	27.0 (25.5, 28.4)	26.6 (25.7, 27.6)	24.9 (23.8, 26.2)
Hilo HI	24.3 (21.4, 25.9)	23.5 (20.8, 24.2)	23.3 (20.1, 24.1)	23.7 (22.3, 24.7)	24.0 (20.6, 25.3)	25.2 (23.3, 26.4)	25.7 (23.8, 26.7)	25.8 (24.2, 27.0)	26.5 (24.7, 27.4)	26.0 (24.2, 26.9)	25.7 (24.6, 26.5)	25.0 (23.6, 26.0)

temperatures from September through November 2000 were approximately 12 ºC at four 40 m deep off-shore sites located between Delaware Bay and Chesapeake Bay (Rasmussen et al.2004). In contrast, autumn nearshore temperatures at Lewes, DE and Solomons Island, MD ranged from 21.9 to 12.5 ºC and 24.9 to 12.5 ºC, respectively (Table 2). Similarly, colder off-shore temperatures in the Gulf of Alaska may constrain the northward expansion of *Didemnum* more than predicted based on nearshore temperature data. In addition, our current understanding of the relationship between water temperature and the growth of *Didemnum* does not allow us to distinguish between areas where *Didemnum* will be restricted to small colonies of little concern versus areas where *Didemnum* colonies will dominate benthic substrates and could potentially create serious problems. Finally, the predicted range expansion of *Didemnum* is based on current temperature regimes. An increase in water temperatures due to global warming could result in a northward extension of temperatures suitable for the establishment of *Didemnum*. Despite these uncertainties, the temperature regimes in the subarctic, subtropical, and tropical LMEs are sufficiently unfavorable to the establishment of large colonies of *Didemnum*, that future forecasting efforts should focus on the predicting the spread of *Didemnum* at smaller spatial scales at the edges of and within its current distribution.

Our ability to predict the likelihood of *Didemnum* spreading to a specific area will benefit from analyses that incorporate local environmental factors in addition to water temperature, such as salinity, depth, hard substrate availability. For example, much of the shoreline in the southern section of the Northeast U.S. is located within the Chesapeake Bay where surface salinities can be as low as 18 ‰. Similarly the ability of *Didemnum* to colonize off-shore habitats in the southern Northeast U.S. may be limited due to the predominance of mobile substrates unsuitable for *Didemnum* establishment. Future analyses should also distinguish between sites where environmental conditions are favorable to *Didemnum* establishment versus sites where *Didemnum* is likely to be introduced. The possibility that a nonindigenous species will be introduced into a new habitat depends on the number of individuals released during an introduction event and the number of times a species is introduced, which taken together are referred to as propagule pressure (Carlton 1996). The number of individuals released during a *Didemnum* introduction should vary with the size and condition of the colony, the presence of larvae, and the possibility of colony fragmentation. However, it is currently not possible to estimate the number of individuals or fragments that may be released during an introduction event, or how this variable may differ between types of transport vectors. Although the number of times *Didemnum* may be introduced into a habitat may be approximated by examining the vessel traffic statistics and the number of aquaculture facilities, details on vessel traffic patterns and aquaculture shipments are needed before we can confidently estimate propagule pressure for specific locations.

Provision of Guidance for Potential Management Actions

Devising a management strategy for *Didemnum* is clearly a national issue. However, the methods and resources required to control *Didemnum* will differ between industries, states, and other private and public stakeholders. Coordinating these independent needs requires a central management authority. Coordinating policy development will prevent confusion over variable but overlapping industry and state management policies, minimize gaps in management activities, and clarify regulatory responsibilities. In the U.S., nonindigenous aquatic species management and coordination is conducted by the Aquatic Nuisance Species Task Force (ANSTF). The ANSTF is an intergovernmental committee established by Congress under the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (Act, 6 USC. 4701-4741), and later amended by the National Invasive Species Act of 1996. The ANSTF is charged with developing and implementing a national strategic plan for preventing the introduction and dispersal of nonindigenous aquatic species, and coordinating the development of management plans for specific species considered to be of national significance.

The ANSTF also addresses regional and state nonindigenous aquatic species issues and has created six regional panels made up of federal, state, industry, public, and environmental stakeholders. Furthermore, states that submit State Management Plans are eligible for funding to implement these plans following ANSTF approval. Although the ANSTF does not have any regulatory authority, it can serve as an advisory body in the coordination of state and federal regulations. The ANSTF has the institutional framework, access to financial resources, and personnel network to address the tasks and activities needed to develop a cohesive *Didemnum* management plan. Below, we outline a *Didemnum* management and control program that focuses on preventing the spread of *Didemnum* to new areas and limiting the impacts of existing populations. Specifically, we outline a management plan that addresses the following objectives:

1. Continue conducting research on the environmental and socioeconomic impacts of *Didemnum* sp. A.
2. Continue conducting research on the ecology and control methods of *Didemnum* to better understand and manage the risks and threats associated with this nonindigenous species.
3. Prevent the spread of *Didemnum* to new areas.
4. Develop monitoring programs to detect changes in the abundance of existing populations of *Didemnum* as well as the establishment of new colonies outside of its current distribution.
5. Eradicate pioneering colonies, and determine the feasibility of eradicating or reducing the abundance of established colonies.
6. Educate the public, private industry, and policy makers about potential environmental and economic costs of *Didemnum*, and how their actions can reduce the risks and impacts of this nonindigenous species.

For each objective, we describe potential management actions and provide technical guidance where feasible. It is our hope that this management outline will facilitate the development of formal management plans that identify funding sources and state and federal agency responsibilities.

Objective 1. Continue conducting research on the environmental and socioeconomic impacts of *Didemnum* sp. A.

Not all nonindigenous species become invasive and eradicating or controlling invasive species is costly. For example, the *Didemnum vexillum* eradication and control program attempted in the South Island of New Zealand cost approximately $120,000 (Pannell and Coutts 2007). Although there are a number of viable reasons to eradicate and control nonindigenous species, including aesthetics and biotic homogenization, management decisions are often based on cost-benefit analyses. The effort and expense of eradication or control efforts are compared to the likelihood of success and the environmental or economic conditions that will be maintained. Complete eradication of all U.S. populations of *Didemnum* is unlikely, particularly colonies in offshore habitats. Rather, long-term management and control efforts and funding will be needed. Although *Didemnum's* wide distribution, ability to overgrow other benthic organisms, ability to spread rapidly, and occurrence in aquaculture facilities and on Georges Bank are causes for concern that *Didemnum* will have negative environmental and economic impacts, there is currently little quantitative data on the magnitude of these potential impacts. Understanding the impacts of *Didemnum* on native and economically important species will help justify the need for any management actions and long-term funding.

Objective 2: Continue conducting research on the ecology and control methods of *Didemnum* to better understand and manage the risks and threats associated with this nonindigenous species.

As with any effort to control a newly discovered nonindigenous species, the management strategies outlined here contain a number of uncertainties. However, preliminary speculation about the effects of *Didemnum* on native species, aquaculture, and marine fisheries, as well as the continued discovery of new colonies, indicate that the problem may worsen without management intervention. As a result, an adaptive management approach is needed, in which control efforts are viewed as hypotheses to be tested, monitored, and modified as information limitations are identified and addressed. Some preliminary research priorities and information needs highlighted throughout this integrated assessment are described below:

1. Determine the taxonomic identity and geographic origin of *Didemnum*.

The forecasts and management recommendations presented in this integrated assessment are based on the assumption that colonies on the East and West coasts of the U.S. and in nearshore and offshore habitats are the same species. It is possible that they are different species with unique environmental tolerances that would require unique management plans. Similarly, we have focused on minimizing the spread of *Didemnum* from populations from sources in U.S. waters. However, there is a need to determine where and how these populations arrived so that additional introductions from sources outside of U.S. waters can be prevented.

2. Quantify the environmental tolerances and preferences of *Didemnum*.

Our ability to identify locations that have the greatest risk of being colonized by *Didemnum* is currently limited by our incomplete understanding of the environmental tolerances and preferences of this nonindigenous ascidian. In addition to continuing research on the effects of water temperature on colony growth and survival, information on other environmental variables known to be important to ascidians would be useful. For example, a better understanding of the range of salinities tolerable to *Didemnum* would determine if open ocean ballast exchange is an effective vector control method.

3. Create and maintain databases on transport vectors in addition to ballast water.

With the exception of ballast water, we have very little information on the role of hull fouling, aquaculture and other potential transport vectors in spreading *Didemnum* as well as other nonindigenous species. Information housed in the National Ballast Information Clearinghouse provided a valuable resource for identifying ballast water management practices and vessel traffic patterns, and ultimately in developing a BWM plan. A similar program is needed for hull fouling, aquaculture, and other potential transport vectors. This will help identify methods for minimizing the risk of spreading *Didemnum*, and allow managers to quickly identify transport vectors for other nonindigenous species.

Objective 3: Limit the spread of *Didemnum* to new areas.

Limiting the spread of *Didemnum* will require controlling the transport vectors that contribute to its dispersal: vessel fouling, aquaculture, commercial fishing, and ballast water. Programs developed to control these transport vectors need to include voluntary or mandatory practices that minimize the risk of spreading *Didemnum* as well as mechanisms for regulatory agencies to monitor compliance and identify individual, high-risk vectors that require inspection or other management actions. Current U.S. vector management policies and regulations focus predominantly on preventing the unintentional introduction and dispersal of nonindigenous aquatic species in ballast water. Specifically, in 2004 the U.S. Coast Guard began requiring mandatory ballast water management practices, record keeping requirements, and noncompliance penalties for all vessels equipped with ballast tanks entering and operating in U.S. waters (Appendix I). However, some states (e.g., Hawaii and California), and other countries (e.g., New Zealand) are considering programs to control the introduction of nonindigenous species due to hull fouling, aquaculture, and fisheries (Coutts 2005, Floerl et al. 2005, Godwin 2005, Takata et al. 2006). Similarly, the aquaculture industry has been actively developing methods for controlling the abundance of fouling organisms on equipment and stock, including a number of nonindigenous tunicate species. Below, we discuss the potential effectiveness of existing and pending management policies and industry practices to minimize the spread of *Didemnum*, and where possible make suggestions for improvements.

Ballast water: Vessels equipped with ballast tanks can potentially spread *Didemnum* by transporting colony fragments or larvae in their ballast water. An additional concern is the ability of *Didemnum* to colonize ballast tanks, as these vessels could serve as continual sources of colony fragments and larvae. The most effective way of minimizing the risk of spreading *Didemnum* through ballast water is to prevent colony fragments and larvae from entering ballast tanks. Currently, this means that ballast water uptake needs to be conducted in areas free of *Didemnum*. However, methods for filtering out or separating organisms from ballast water are being developed and may be available in the future.

Federal regulations do stipulate that vessel operators should avoid taking ballast water in areas with known infestations of harmful nonindigenous aquatic species and in shallow water where propellers can disturb the bottom. Although these guidelines will reduce the risk of colony fragments and larvae from entering ballast tanks, *prohibiting* vessels from taking ballast water in areas with large populations of *Didemnum*, or when colonies are reproducing would be an improvement over existing guidelines Vessel operators need to be strongly encouraged to not take ballast water in areas with known populations of *Didemnum* until methods for removing or destroying colonies and larvae from ballast water can be verified.

The most widely used method for removing or destroying organisms taken up in ballast water is to conduct an open ocean ballast water exchange. Exchanging ballast water in the open ocean reduces the density of coastal organisms in ballast tanks and replaces them with oceanic organisms that should have a lower probability of surviving in nearshore waters with lower salinities. Although some states (i.e., Oregon, Washington and California) require vessels involved in intercoastal shipping, to conduct ballast water exchanges, the BWM program only requires vessels arriving from outside of the U.S. Exclusive Economic Zone to conduct ballast water exchanges. As a result, current ballast water exchange requirements will have little impact on reducing the spread of *Didemnum* in U.S. waters. Furthermore, ballast water exchange may not be an effective treatment as colonies attached to tank walls would not be flushed out. However, ballast water treatment is the subject of extensive research, and several technologies and methodologies for removing or destroying organisms taken up in ballast water are being tested (e.g., sterilization with ultraviolet light, ozone, heat, or biocides).

Because there are no proven methods for preventing the spread of *Didemnum* in ballast water, there needs to be a mechanism for identifying high risk vessels. The national BWM program requires all vessels to submit ballast operation reports, which include information on the port of departure, location of ballast water operations, as well the use of any ballast water treatment methods. Currently these reports are required to be sent to the National Ballast Information Clearinghouse (National Ballast Information Clearinghouse 2004). If these reports were required to be sent to the vessels' arrival port prior to departure, they could be used by the coast guard or port authority to identify vessels that have taken ballast water in high risk areas and are potentially transporting *Didemnum* in their ballast water.

<u>Vessel fouling:</u> As with ballast water, controlling the spread of *Didemnum* due to hull fouling will largely depend on developing a process for identifying high risk vessels. At present, there are no programs or regulations that focus specifically on preventing the introduction of nonindigenous species due to vessel fouling. The BWM program requires that anchors and chains be rinsed at their place of origin and that fouling organisms be removed from hulls, piping, and tanks on a regular basis (Appendix I). However, what constitutes a “regular” basis is not defined. In addition, the focus on vessels with ballast tanks misses other types of potential fouling vectors, such as recreational vessels. Hawaii is developing a hull fouling management strategy, which includes a “risk matrix” for identifying high risk vessels and possible actions to be taken before the vessel enters state waters. Although this management strategy provides a good framework for managing hull fouling, a number of important details remain to be worked out. For example the “’risk matrix” has not yet been formally adopted as the criteria for determining a vessels “risk” have not been finalized, such as what hull maintenance standards should be adhered to, or how long a vessel can remain in port before it is considered a fouling risk (Godwin 2005).

Although there are a variety of antifouling coatings that can prevent a great deal of vessel fouling when properly maintained, maintenance schedules are dictated by economic concerns (e.g., fuel efficiency) rather than preventing the introduction and spread of nonindigenous species. Furthermore, recent studies have found that even with antifouling coatings, organisms can still attach to vessel areas where antifouling coatings do not adhere well or are difficult to apply, such as along weld seams, sea chest intakes, rudder posts, and where wood blocks are placed while the vessel is in dry dock (Dodgson and Coutts 2002, Minchin and Gollasch 2003, Godwin 2003).

Despite the emphasis on preventing the introduction of nonindigenous species in ballast water, a growing number of studies indicate that vessel fouling remains an important mechanism by which nonindigenous species are introduced into new areas (e.g., Eldredge and Carlton 2002, Godwin 2005, Takata et al. 2006). In addition, the importance of hull fouling as a transport vector is likely to increase in the future, as the use of antifouling paints containing tributyltin (TBT) become restricted due to their toxicity (EPA 2003, see also IMO 2001). Overall, there is a need to collect information on how hull maintenance patterns and fouling levels vary so that a fouling management plan can be developed.

Until specific guidelines can be established, management should focus on identifying high risk vessels. One option is to expand the ballast water reporting forms required by the U.S. Coast Guard to include information relevant to hull fouling, such as estimated speed during voyage, time since last antifouling coating application or cleaning, length of stay at port of departure, and hull was visually inspected for fouling organisms prior to departure. For example, researchers in Hawaii found that slow moving vessels or those that have had a long inactive period were more likely to harbor fouling organisms relative to faster commercial vessels with short residency times (Godwin et al. 2004).

Aquaculture: Aqua-culturists employ a variety of techniques to control fouling organisms on equipment and shellfish stock, including low concentration metal-based antifouling paints, manual cleaning, chemical treatments (e.g., vinegar, chlorine), hot water baths, exposure to air, and exposure to benthic predators (Goldburg et al. 2001). Studies need to be conducted to determine which of these techniques is most effective for controlling *Didemnum*. There is also a need to provide preshipment and arrival inspectors with the training necessary to identify *Didemnum*.

Commercial Fishing: Controlling the spread of *Didemnum* through commercial fishing activities could be done by closing areas with known populations or requiring vessels to clean all equipment after fishing in an area known to support *Didemnum*. Similarly, it will be necessary to restrict the disposal of potentially infected materials such as scallop shells.

Objective 4: Develop monitoring programs to detect changes in the abundance of existing populations of *Didemnum* as well as the establishment of new colonies outside of its current distribution.

A monitoring program would greatly compliment efforts to control the spread of *Didemnum*. Tracking changes in the size of existing colonies of *Didemnum* as well as the abundance of larvae would help identify locations and seasons where vector control efforts should be closely observed. Monitoring in areas where colonies of *Didemnum* have not yet appeared but are at risk of an introduction would provide a way of measuring the success of vector control programs. Furthermore, early detection greatly increases the likelihood of eradicating newly established populations (see objective 3). Developing a monitoring program requires identifying monitoring sites and developing a network of university and agency researchers who can assist in establishing standardized monitoring protocols and data collection.

Although any of the locations with existing populations of *Didemnum* could be used to track changes in colony and larval abundance (Table 1, Figs. 3 and 4), we recommend monitoring existing populations near areas where there are substantial amounts of vector activity, such as aquaculture facilities, ports, and marinas. In addition, we suggest that sites should span a range of geographical locations and environmental conditions. Coordinating with researchers with ongoing research programs such as the surveys being conducted on Georges Bank by scientists from NOAA, USGS and the University of Rhode Island could reduce the time and expense needed to train personnel. Such collaboration would also take advantage of existing long-term data sets that may provide insights into the effects of *Didemnum* on native species.

Efforts to detect new populations of *Didemnum* should be done in areas that are vulnerable to colonization. Although we have already discussed the general vulnerability of different LMEs, specific locations could be identified using port statistics, vessel traffic patterns, aquaculture equipment sharing practices or stock shipping patterns. Areas along the edges of the current distribution of *Didemnum*, particularly at the boundary of the Northeast and Southeast U.S. LMEs, should also be monitored.

Contact information for University and Agency researchers that contributed to this integrated assessment, have research programs involving tunicates, and should be contacted about developing a monitoring program are provided below:

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Objective 5: Eradicate pioneering colonies, and where feasible, eradicate or reduce the abundance of established colonies.

Although a monitoring program can help locate newly established colonies, resource managers need to develop a *Didemnum* rapid response plan, which includes identifying proven and cost-effective eradication methods. It may also be desirable to reduce the abundance of *Didemnum* along the edge of its range as part of a larger prevention program. There have been few systematic attempts to eradicate or control colonies of *Didemnum*, or colonies of potentially closely related species that have also recently appeared in temperate, marine systems world-wide. The results of these attempts have been variable, but demonstrate that small colonies can be eradicated by smothering or bleach.

In 2003, the Cawthron Institute attempted to eradicate colonies of *Didemnum vexillum* from benthic substrates, dock pilings, and boat hulls in Shakespeare Bay, New Zealand (Sinner and Coutts 2003). The different eradication methods employed include smothering with dredge material, filter fabric, and plastic, as well as manual removal and treating boat hulls with a dilute bleach solution (Sinner and Coutts 2003). Follow-up surveys conducted in 2004 showed that smothering with dredge material killed 100% of the colonies occupying an approximately 3200 m^2 area of relatively homogeneous seabed substrate. In contrast, the use of filter fabric to smother colonies in spatially complex habitats with large boulders was ineffective. Wrapping pilings with 50 μm polypropylene plastic smothered the underlying colonies; however, the surface of the plastic wrap was colonized by recruits from other areas. Similarly, colonies of *Didemnun vexillum* quickly reoccupied a boat hull after researchers manually removed approximately 80-90% of the colonies. However, the use of bleach proved to be effective in killing colonies on boat hulls. Bleach was applied by securing the edges of a tarp the barge and then poring two 20 liter buckets of granulated chlorine mixed with freshwater between the plastic and the barge. All fouling material, including *D. vexillum*, was killed within 48 hours.

In addition to the various efforts to eradicate *Didemnum vexillum* from Shakespeare Bay, New Zealand, there has been two small scale attempts to eradicate colonies of *Didemnum* in the U.S. In 2005, volunteer divers also used bleach to eradicate a small colony from the hull of a submerged boat in the Edmonds Underwater Park in Puget Sound. Divers secured a tarp over the 4 m^2 colony and added 4 chlorine tablets to the approximately 32 gallons of entrapped water. The chlorine treatment appears to have been effective, as new colonies were not found in follow-up surveys.

In spring 2007, Dr. Larry Harris from the University of New Hampshire began a pilot attempt to eradicate or control *Didemnum* by manually removing colonies from a commercial wharf in Eastport, ME. Follow up surveys have shown that while manual removal succeeded in reducing size of the colonies, difficult to remove fragments in crevices were beginning to regenerate.

These examples emphasize the need for a rapid response program. Eradication of large established colonies is probably not feasible, or desirable given current eradication methods. For example bleach and smothering are effective, but have negative effects on other native species. While complete mortality of all benthic organisms may be acceptable at small spatial scales, the environmental cost will eventually outweigh the benefit of eradicating *Didemnum.*

Objective 6: Educate the public, private industry and policy makers about potential environmental and economic costs of *Didemnum*, and how their actions can reduce the risks and impacts of this nonindigenous species.

Minimizing the spread and impact of *Didemnum* will require public and private stakeholders participation in the management process. Human activities play an important role in spreading *Didemnum*, and preventing further introductions will require changing individual behaviors and industry practices. However, identifying which behaviors pose the greatest risk of spreading *Didemnum*, and how these behaviors need to be modified to limit the risk of further spread, depends in part on stakeholder provided information. For example, the BWM program is based on ballast water reports submitted by the shipping industry as part of the National Ballast Information Clearinghouse database jointly overseen by the Smithsonian Environmental Research Center and the U.S. Coast Guard (National Ballast Information Clearinghouse 2004). Similar databases are needed to determine how different hull and equipment cleaning practices and schedules or vessel travel patterns influence the relative risks posed by hull fouling and aquaculture, respectively (on aquaculture, this would need to be done on state by state basis, unless in federal waters). Devising a management strategy also requires that policy makers and government agencies adequately fund and support management activities. The willingness of resource users and policy makers to contribute to management efforts will be greatly improved provided that they understand the scope of the threat posed by *Didemnum*.

A number of invasive species websites already contain information on *Didemnum*, including sites maintained by the U.S. Geological Survey (woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/), the San Francisco Estuary Institute (www.exoticsguide.org/), and the Invasive Species Specialist Group (http://www.issg.org/database/welcome/). Similarly, a number of web-based articles and press releases are available, which describe local concerns regarding *Didemnum* (e.g., www.pnwscuba.com/critterwatchers/invasive.htm). These are excellent resources for stakeholders that are already aware of *Didemnum*, and interested in actively learning more about this nonindigenous ascidian. There are also a number of national and state programs dedicated to invasive species education in general that need to incorporate information on *Didemnum*. For example, the ANSTF's Stop Aquatic Hitchhikers! campaign's focus on freshwater systems could be expanded to include marine systems. Other activities that may be useful include making information available at industry and trade show and with state boating license registration packages.

Conclusions

Although a large number of *Didemnum* colonies have only recently been discovered in U.S. waters, this nonindigenous colonial ascidian may conceivably have negative impacts on native species, aquaculture, and marine fisheries. As a result, management agencies, policy makers, industry members, and the general public need to recognize *Didemnum* as an invasive species. Raising the awareness of these different stakeholder groups will require developing and supporting education and outreach programs, and should provide stakeholders with sufficient information to work collaboratively to identify actions and resources needed to limit the spread and impacts of *Didemnum*. While providing all stakeholders with the opportunity to contribute to the development of *Didemnum* management plan will greatly improve the long-term management of this nonindigenous species, such a process will take time. However, the rate at which new colonies are being discovered and the lack of proven methods for eradicating well-established colonies indicates that an interim plan is necessary.

Current management actions should focus on limiting the spread of *Didemnum,* via hull fouling and the movement of aquaculture stock and equipment. In addition, increasing monitoring efforts are needed to rapidly locate and eradicate newly colonies in areas likely to be invaded. Given our current understanding of the biology and ecology of *Didemnum,* areas most susceptible to invasion are those with growing season of 6 months or longer and where mean monthly water temperatures are below 25 ºC. Levels of vector activity could be determined through vessel traffic patterns, aquaculture shipment patterns, and fishing activity. Areas at the edges of and within *Didemnum*'s current distribution should be monitoring priorities, followed by the northern end of the Southeast U.S. LME. As an important precaution, we recommend monitoring the southern end of the Southeast U.S. LME. Recent evidence suggests that hull fouling is a larger problem than currently believed.

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Literature Cited

Bak, R.P.M., Lambrechts, D.Y.M., Joenje, M., Nieuwland, G., VanVeghel, M.L.J., 1996. Long-term changes on coral reefs in booming populations of a competitive colonial ascidian. Mar. Ecol. Prog. Ser. 133, 303-306.

Bak, R.P.M., Joenje, M., de Jong, I., Labrechts, D.Y.M., and G. Nieuwland. 1998. Bacterial suspension feeding by coral reef benthic organisms. Mar. Ecol. Prog. Ser. 175: 285-288.

Bates, W.R. 2005. Environmental factors affecting reproduction and development in Ascidians and other protochordates. Can. J. Zoo. 83: 51-61.

Bingham B.L. 1997. Light cycles and gametogenesis in three temperature ascidian species. Invertebrate Biology 116: 61-70.

Boothroyd, F.A., MacNair, N.G., Landry, T., Locke, A. and T J. Davidson. 2002. Dealing with an aquatic invader: the clubbed tunicate (Styela clava) in Prince Edward Island waters. In: Aquaculture Canada 2002. Charlottetown, PEI.

Botts, P.S., Patterson, B.A, and D.W. Schloesser. 1996. Zebra mussel effects on benthic invertebrates: physical or biotic. J. North Am. Benthol. Soc. 15: 179-184.

Bryan, P.J., McClintock, J.G., Slattery, M. and D.P. Rittschof. 2003. A comparative study of the non-acidic chemically mediated antifoulant properties of three sympatric species of ascidians associated with seagrass habitats. Biofouling 19: 235-245.

Bullard, S.G., Lambert, G., Carman, M.R., Byrnes, J., Whitlatch, R.B., Ruiz, G.M., Miller, R.J., Harris, L. G., Valentine, P.C., Collie, J.S., Pederson, J., McNaught, D.C., Cohen, A.N., Asch, R.G., Dijkstra, J., and 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. J. Exp. Mar. Biol. Ecol. 342: 99-108.

Bullard, S.G., Sedlack, B., Reinhardt, J.F., Litty, C., Gareau, K. and R.B. Whitlatch. 2007b. Fragmentation of colonial ascidians: differences in reattachment capability among species. J. Exp. Mar. Biol. Ecol. 342: 166-168.

Cangelosi, A. 1999. Ballast water management: developments in policy and technology. In Marine Bioinvasions: Proceedings of the first National Conference. J. Pederson, editor. MIT Sea Grant College Program. Cambridge, MA.

Carlton, J.T. 1989. Man's role in changing the face of the ocean: Biological invasions and implications for conservation of near-shore environments. Conservation Biology 3: 265-263.

Carlton, J.T. 1996. Pattern, process, and prediction in marine invasion ecology. Biol. Conserv. 78: 97–106.

Carlton, J.T. and Geller, J. 1993. Ecological roulette: The global transport of nonindigenous marine organisms. Science 261: 78-82.

Carman, M.R., Bullard, S.G., and J.P. Donnelly. 2007. Water quality, nitrogen pollution, and ascidian diversity in coastal waters of southern Massachusetts, USA. J. Exp. Mar. Biol. Ecol. 342: 175-178.

Cayer, D., MacNeil, M., and A.G. Bagnall. 1999. Tunicate fouling in Nova Scotia aquaculture: a new development. J. Shellfish Res. 18:327,

Cohen, A.N. 2005. Guide to the Exotic Species of San Francisco Bay. San Francisco Estuary Institute, Oakland, CA. www.exoticsguide.org.

Cohen A.N., Harris L.H., Bingham, B.L., Carlton J.T., Chapman, J.W. Lambert C.C., Lambert G., Ljubenkov, J.C., Murray, S.N., Rao, L.C., Reardon, K. and E. Schwindt. 2005. Rapid assessment survey for exotic organisms in southern California bays and harbors, and abundance in port and non-port areas. Biological Invasions 7: 995-1002.

Cohen, A.N. and J.T. Carlton. 1998. Accelerating invasion rate in a highly invaded estuary. Science 279: 555-558.

Collie, J.S., Hall, S.J., Kaiser, M.J. and I.R. Poiner. 2000. A quantitiative analysis of fishing impacts on shelf-sea benthos. J. Anim. Ecol. 69:785-798.

Connell, S.D. 2000. Floating pontoons create novel habitats for subtidal epibiota. J. Exp. Mar. Biol. Ecol. 247: 183-194.

Connell, S.D. 2001. Urban structures as marine habitats: an experimental comparison of the composition and abundance of subtidal epibiota among pilings, pontoons and rocky reefs. Mar. Env. Res. 52: 115-125.

Coutts, A.D.M., 2002. A biosecurity investigation of a barge in the Marlborough Sounds. Cawthron Institute, Nelson, New Zealand, Report 744, 59 pp.

Coutts, A.D.M., and J. Sinner. 2004. An updated benefit-cost analysis of management options for Didemnum vexillum in Queen Charlotte Sound (New Zealand): Cawthron Institute Report 925, 29 p.

Coutts, A.D.M. and M.D. Taylor. 2004. A preliminary investigation of biosecurity risks associated with biofouling on merchant vessels in New Zealand. New Zealand J. Mar. Freshwater Res. 38: 215-229.

Coutts, A.D.M. 2005. Managing marine biosecurity in New Zealand: from a research provider's perspective. In Hull fouling as a mechanism for marine invasive species introductions. Proceedings of a Workshop on Current Issues and Potential Management Strategies. February 12-13, 2004. Honolulu, Hawaii. L.S. Godwin, editor. pp. 36-41.

Dalby, J.E. and C.M. Young. 1993. Variable effects of ascidian competitors on oysters in a Florida epifaunal community. J. Exp. Mar. Biol. Ecol. 167: 47-57.

deRivera, C. E., Ruiz, G. M., Crooks, J. A., Wasson, K., Lonhart, S. I., Fofonoff, P., Steves, B. P., Rumrill, S. S., Brancato, M. S., Pegau, W. S., Bulthuis, D. A., Preisler, R. K., Schoch, G. C., Bowlby, E., DeVogelaere, A., Crawford, M. K., Gittings, S. R., Hines, A. H., Takata, L., Larson, K., Huber, T., Leyman, A. M., Collinetti, E., Pasco, T.; Shull, S., Anderson, M., and S. Powell. 2005. Broad-scale nonindigenous species monitoring along the west coast in National Marine Sanctuaries and National Estuarine Research Reserves. National Fish and Wildlife Foundation.

Dijkstra, J., Harris, L.G., and E. Westermen. 2007a. Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. J. Exp. Mar. Biol. Ecol. 342: 61-68.

Dijkstra, J., Harris, L.G. and Sherman, H. 2007b. The role of colonial ascidians in altering biodiversity of a marine fouling community. J. Exp. Mar. Biol. Ecol. 342:169-171.

Dodgson, T. and A.D.M. Coutts. 2002. In Hull fouling as a mechanism for marine invasive species introductions. Proceedings of a Workshop on Current Issues and Potential Management Strategies. February 12-13, 2004. Honolulu, Hawaii. L.S. Godwin, editor. pp. 22-28.

Dowlatabadi, H, Rotmans, J., and P. Martins. 2000. Integrated assessment: a new international journal. Integrated Assessment 1:1-2.

Drake, L.A., Meyer, A.E., Forsberg, R.L., Baier, R.E., Doblin, M.A., Heinemann, S., Johnson, W.P., Koch, M., Rublee, P.A. and F.C. Dobbs. 2005. Potential invasion of microorganisms and pathogens via 'interior hull fouling': biofilms inside ballast water tanks. Biological Invasions 7: 969-982.

Echelle, A.A. and A.F. Echelle. 1997. Genetic introgression of endemic taxa by non-natives: A case study with Leon Springs pupfish and sheepshead minnow. Cons. Bio. 11: 153-161.

Eldredge, L.G. and J.T. Carlton. 2002. Hawaiian marine bioinvasions: A preliminary assessment. Pacific Science. 56: 211-212.

United States Environmental Protection Agency. 2003. Ambient aquatic life water quality criteria for trybutyltin (TBT). EPA-822-R-03-031.

Floerl, O. and G.J. Inglis. 2005. Potential for the introduction and spread of marine pests by private yachts. In Hull fouling as a mechanism for marine invasive species introductions. Proceedings of a Workshop on Current Issues and Potential Management Strategies. February 12-13, 2004. Honolulu, Hawaii. L.S. Godwin, editor. pp. 22-28.

Floerl, O., Inglis, G.J., Gust, N., Hayden, B., Fitridge, I., and G. Fenwick. 2005. Current research in marine biosecurity undertaken by the National Institute of Water and Atmospheric Research, New Zealand. In Hull fouling as a mechanism for marine invasive species introductions. Proceedings of a Workshop on Current Issues and Potential Management Strategies. February 12-13, 2004. Honolulu, Hawaii. L.S. Godwin, editor. pp. 42-46.

Fogarty, MJ and SA Murawski. 1998. Large-scale disturbance and the structure of marine system: fishery impacts on Georges Bank. Ecol Appl 8:6–22

Galloway, J.N., Aber, J.D., Erisman, J.W. Seitzinger, S.P., Howarth, R.W., Cowling, E.B., and J.B. Cosby. 2003. The nitrogen cascade. Bioscience 53: 341-356.

Glasby, T.M. 2001. Development of sessile marine assemblages on fixed versus moving substrate. Mar. Ecol. Prog. Ser. 215: 37-47.

Godwin, L.S. 2003. Hull fouling of maritime vessels as a pathway for marine species invasion to the Hawaiian Islands. Biofouling 19: 123-131.

Godwin, L.S. 2005. Development of an initial framework for the management of hull fouling as a marine invasive species transport mechanism. In Hull fouling as a mechanism for marine invasive species introductions. Proceedings of a Workshop on Current Issues and Potential Management Strategies. February 12-13, 2004. Honolulu, Hawaii. L.S. Godwin, editor. pp. 47-54.

Godwin, L.S., Eldredge, L.G., and K. Gaut. 2004. The assessment of hull fouling as a mechanism for the introduction and dispersal of marine alien species in the main Hawaiian Island. Final report submitted to the Hawai'I Coral Reef Initiative Research Program. Bishop Museum Technical Report 28.

Goldburg, R.J., Elliott, M.S., and R.L. Naylor. 2001. Marine aquaculture in the United States: environmental impacts and policy options. Pew Oceans Commission, Arlington, Virgina.

Gollasch, S. 2002. The importance of ship hull fouling as a vector of species introductions in into the North Sea. Biofouling 18: 105-121.

Gotceitas, V. and J.A. Brown. 1993. Substrate selection by juvenile Atlantic cod (Gadus morhua): effects of predation risk. Oecologia 93: 31-37.

Hermsen, JM, Collie, J.S, and P.C. Valentine. 2003 Mobile fishing gear reduces benthic megafaunal production on Georges Bank. Mar. Ecol. Prog. Ser. 260, 97–108

Holloway, M.G. and S.D. Connell. 2002. Why do floating structures create novel habitats for subtidal epibiota? Mar. Ecol. Prog. Ser. 235: 43-52.

Howarth, R.W., Anderson, D., Church, T., Greening, H., Hopkinson, C., Huber, W., Marcus, N., Naiman, R., Segerson, K., Sharpley, A. and W. Wiseman. 2000. Clean Coastal Waters – Understanding and Reducing the Effects of Nutrient Pollution. National Academy of Sciences. Washington, D.C.

International Maritime Organization. 2001. International convention on the control of harmful antifouling systems on ships, 2001.

Joullie, M.M., Leonard, M.S., Portonovo, P., Liang, B., Ding, X., La Clair, J.J., 2003. Chemical defense in ascidians of the Didemnidae family. Bioconj. Chemi. 14, 30-37.

Kott, P. 2002. A complex didemnid ascidian from Whangamata, New Zealand. J. Mar. Biol. Ass. U. K. 82, 625-628.

Kott, P. 2004. A new species of Didemnum (Ascidiacea, Tunicata) from the Atlantic coast of North America. Zootaxa 732:1–10.

Lambert, G. 1968. The general ecology and growth of a solitary ascidian, Corell willmeriania. Biol. Bull. (Woods Hole) 135: 296-307.

Lambert, G. 2001. A global overview of ascidian introduction and their possible impact on the endemic fauna. In The Biology of Ascidians. H. Sawada, H. Yodosawa, and C. C. Lambert, editors. Springer-Verlag, Tokyo. pp. 249-257.

Lambert, G. 2002. Nonindigenous ascidians in tropical waters. Pac. Sci. 56: 291-298.

Lambert, G. 2005. The ecology and natural history of protochordates. Can. J. Zool. 83:34-50

Lambert, C.C., Lambert, G., 2003. Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Mar. Ecol. Prog. Ser. 259, 145-161.

Lavoie, D.M, Smith L.D. and G.M. Ruiz. 1999. The potential for intracoastal transfer of non-indigenous species in the ballast water of ships. Estuarine Coastal and Shelf Science 48: 551-564.

Lesser, M. P., Shumway, S.E., Cucci, T., and J. Smith. 1992. Impact of fouling organisms on mussel rope culture: interspecific competition for food among suspension-feeding invertebrates. J. Exp. Mar. Biol. Ecol. 165:91–102.

Lippincott, C.L. 2000. Effects of Imperata cylindrica (L.) Beauv. (cogongrass) invasion on fire regime in Florida sandhill (USA). Natural Areas Journal 22: 120-129.

Lodge, D.M. 1993. Biological invasions: Lessons for ecology. TREE 8: 133-137.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., and F.A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequence and control. Ecological Applications 10: 689-710.

Mayer, C.M., Rudstam, L.G., Mills, E.L., Cardiff, S.G. and C.A. Bloom. 2001. Zebra mussels (Dreissena polymorpha), habitat alteration, and yellow perch (Perca flavescens) foraging: system-wide effects and behavioural mechanisms. Can. J. Fish. Aquat. Sci. 58: 2459-2467.

Mazouni, N., Gaertner, J.C., and J.M. Deslous-Paoli. 2001. Composition of biofouling communities on suspended oyster cultures: an in situ study of their interactions with the water column. Mar. Ecol. Prog. Ser. 214: 93-102.

McCarthy, A., Osman, R.W. and R.B. Whitlatch. 2007. Effects of temperature on growth rates of colonial ascidians: a comparison of Didemnum sp. to Botryllus schlosseri and Botrylloides violaceus. J. Exp. Mar. Biol. Ecol. 342: 172-174.

Minchin, D. and E. Sides. 2006. Appearance of a cryptogenic tunicate, a Didemnum sp. fouling marina pontoons and leisure craft in Ireland. Aquatic Invasions 1:143-147.

Minchin, D. and S. Gollasch. 2003. Fouling and ships' hulls: how changing circumstances and spawning events may result in the spread of exotic species. Biofouling 19: 111-122.

National Ballast Information Clearinghouse 2004. NBIC Online Database. Electronic publication, Smithsonian Environmental Research Center & United States Coast Guard. Available from http://invasions.si.edu/nbic/search.html; searched 17-May-2007.

National Marine Fisheries Service, Office of Science and Technology. 2005. Fisheries of the United States, 2004.

Olson, R.R. 1985. The consequences of short-distance larval dispersal in a sessile marine invertebrate. Ecology 66: 30-39.

Oren, U. and Y. Benayahu. 1998. Didemnid ascidians: rapid colonizers of artificial reefs in Eilat (Red Sea). Bull. Mar. Sci. 63: 199-206.

Osman, R.W., Whitlatch, R.B., 1995. Influence of resident adults on larval settlement: a comparison to settlement. J. Exp. Mar. Biol. Ecol. 190: 169-198.

Osman, R.W. and R.B. Whitlach. 2007. Variation in the ability of Didemnum sp. to invade established communities. J. Exp. Mar. Biol. Ecol. 342: 40-53.

Papadopoulou, C., and Kanias, G.D. 1977. Tunicate species as marine pollution indicators. Mar. Pollut. Bull. 8: 229–231

Pederson, J., Bullock, R., Carlton, J., Dijkstra, J., Dobroski, N., Dyrynda, P., Fisher, R., Harris, L., Niels, H., Lambert, G., Lazo-Wasem, E., Mathieson, A., Miglietta, M. P., Smith, J., Smith J. and M. Tyrrell. 2005. Marine invaders in the Northeast: Rapid assessment survey of non-native and native marine species of floating dock communities, August 2003. MIT Sea Grant College Program.

Peterson, J.K., Schou, O. and P. Thor. 1997. In situ growth of the ascidian Ciona intestinalis (L.) and the blue mussel Mytilus edulis in an eelgrass meadow. J. Exp. Mar. Biol. Ecol. 218: 1-11.

Philp, R.B., Leung, F.Y., and C. Bradley. 2003. A comparison of the metal content of some benthic species from coastal waters of the Florida panhandle using high-resolution inductively coupled plasma mass spectrometry (ICP–MS) analysis. Arch. Environ. Contam. Toxicol. 44: 218–223.

Pimentel, D., Loch, L., Zuniga, R. and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience 50: 53-65.

Pimentel, D., Zuniga, R., and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52: 273-288.

Pisut, D.P. and J.R. Pawlik. 2002. Anti-predatory chemical defenses of ascidians: secondary metabolites or inorganic acids? J. Exp. Mar. Biol. Ecol. 270: 203-214.

Ruiz, G.M., Carlton, J.T., Grosholz, E.D., and A.H. Hines. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. American Zoologist 37: 621-632.

Ruiz, G.M., Fofonoff, P.W., Hines, A.H. and E.D. Grosholz. 1999. Non-indigenous species as stressors in estuarine and marine communities: assessing invasion impacts and interactions. Limnol. Oceanogr. 44: 950-972.

Ruiz, G.M., Fofonoff, P.W., Carlton, J.T., Wonham, M.J., and A.H. Hines. 2000. Invasion of coastal marine communities in North America: Apparent patterns, processes, and biases. Annu. Rev. Ecol. Syst. 31: 481-531.

Satoh, N. 1994. Developmental biology of ascidians. Cambridge University Press, Cambridge, UK.

Schmitz, D.C. and D. Simberloff. 1997. Biological invasions: a growing threat. Issues in Science and Technology 13: 33-40.

Sherman, K. 2000. Why regional coastal monitoring for assessment of ecosystem health? Ecosystem Health 6: 205-216.

Simberloff, D. 2005. The politics of assessing risk for biological invasions: the USA as a case study. TREE 20: 216-222.

Sinner, J. and Coutts, A.D.M., 2003, Benefit-cost analysis of management options for Didemnum vexillum in Shakespeare Bay (New Zealand): Cawthron Institute Report 924, 29 p.

Smith, W. G. and W. W. Morse. 1993. Larval distribution patterns: early signals for the collapse/recovery of Atlantic herring, Clupea harengus, in the Georges Bank area. Fish. Bull., U.S. 91:338-347.

Stachowicz, J.J., Terwin, J.R., Whitlatch, R.B., and R.W. Osman. 2002. Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. PNAS 99: 15497-15500.

Stoner, D. S. 1989. Fragmentation a mechanism for the stimulation of genet growth rates in an encrusting colonial ascidian. Bull. Mar. Sci. 45:277-287.

Svane, I. and C.M. Young. 1989. The ecology and behavior of ascidian larvae. Oceanogr. Mar. Biol. Rev. 27:45-90.

Takata, L., Falkner, M., and S. Gilmore. 2006. Commercial vessel fouling in California: analysis, evaluation, and recommendations to reduce nonindigenous species release from the non-ballast water vector. California State Lands Commission, Marine Facilities Division. pp. 81.

Thomson, D. 2004. Competitive interactions between the invasive European honey bee and native bumble bees. Ecology 85: 458-470.

Townsend, DW and MA Thomas. 2002. Springtime nutrient and phytoplankton dynamics on Georges Bank. Marine Ecology Progress Series 228, 57–74.

U.S. Congress, Office of Technology Assessment. 1993. Harmful nonindigenous species in the United States, OTA-F-565. US Government Printing Office. Washington, D.C., USA.

U.S. Geological Survey. 2006. USGS National Geologic Studies of Benthic Habitats, Northeastern United States, Marine Invasive Species, Didemnum sp., a colonial tunicate: ascidian; sea squirt http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnun/index.htm.

Valentine, P.C., Carman, M.R., Blackwood, D.S., and E.J. Heffron. 2007a. Ecological observations of the colonial tunicate Didemnum sp. in a New England tide pool habitat. J. Exp. Mar. Biol. Ecol. 342: 109-121.

Valentine, P.C., Collie, J.S., Reid, R.N., Asch, R.G., Guida, V.G and D.S. Blackwood 2007b. The occurrence of the colonial ascidian Didemnum sp. on Georges Bank gravel habitat – ecological observations and potential effects on groundfish and scallop fisheries. J. Exp. Mar. Biol. Ecol. 342: 179-181.

Vitousek, P.M. and L.R. Walker. 1989. Biological invasion by Myrica faya in Hawai'i: plant demography, nitrogen fixation, ecosystem effects. Ecological Monographs 59: 247-265.

Wasson, K., Zabin, C.J., Bedinger, L., Diaz, M.C. and J.S. Pearse. 2001. Biological invasions of estuaries without international shipping: the importance of intraregional transport. Biological Conservation 102: 143-153.

Wijman, J.W.M and A.C. Smaal. 2006. Risk analysis of mussel transfer. Institute for Marine Resources and Ecosystem Studies. Ijmuden, The Netherlands. Report No. CO44/06 103 pp.

Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A. and E. Losos. 1998. Quantifying threats to imperiled species in the United States. BioScience 48: 607-615.

Wiles, G.J., Bart, J., Beck R.E. and C.F. Aguon. 2003. Impacts of the brown tree snake: patterns of decline and species persistence in Guam's avifauna. Cons. Bio. 17: 1350-1360.

Whitlatch, R.B. and R.W. Osman. 1999. Geographical distributions of organism-habitat associations of shallow-water introduced marine fauna in New England. In Marine

Bioinvasions: Proceedings of the First National Conference. J. Pederson, editor. MIT Sea Grant College Program. Cambridge, MA.

Working Group on Introduction and Transfers of Marine Organisms (WGITMO). 2005. Report of the Working Group on Introduction and Transfers of Marine Organisms. ICES Advisory Committee on the Marine Environment, ICES CM 2005/ACME:05 Ref. E.

Wyatt, A.S., Hewitt, C.L., Walker, D.I. and T.J. Ward. 2005. Marine introductions in the Shark Bay World Heritage Property, Western Australia: a preliminary assessment. Diversity Distrib. 11: 33-44.

Appendix I. United States National Ballast Water Program regulations.

A. Mandatory practices for all vessels with ballast tanks operating in all U.S. waters.

1. Avoid ballast operations in or near marine sanctuaries, reserves, parks, or coral reefs.
2. Avoid ballast water uptake:
    a. Where infestation, harmful organisms and pathogens are located.
    b. Near sewage outfalls.
    c. Near dredging operations.
    d. Where tidal flushing is poor or when a tidal stream is known to be more turbid.
    e. In darkness when organisms may rise up in the water column.
    f. In shallow water or where propellers may stir up the sediment.
    g. Areas with pods of whales, convergence zones and boundaries of major currents
3. Regularly clean ballast tanks to remove sediment.
4. Only discharge minimal amounts of ballast water in coastal and internal waters.
5. Rinse anchors and anchor chains during retrieval to remove organisms and sediments at their place of origin.
6. Remove fouling organisms from hull, piping, and tanks on a regular basis and dispose of any removed substances in accordance with local, state and federal regulations.
7. Maintain a vessel specific ballast water management plan.
8. Train vessel personnel in ballast water and sediment management and treatment procedures.
9. Submit Ballast Water Reporting Forms.
10. Ballast water records must be kept on board the vessel for a minimum of two years.

B. Additional Mandatory Practices for all vessels transiting to U.S. waters with ballast water that was taken on within 200 nautical miles of any coast after operating beyond the U.S. Exclusive Economic Zone.

1. Conduct one of the following:
    a. conduct mid-ocean ballast water exchange prior to entering U.S. waters;
    b. retain the ballast water on board while in U.S. water; or
    c. use a Coast Guard approved alternative environmentally sound method to treat the ballast water .

C. Penalties for failing to comply with the Mandatory BWM Requirements.

1. Maximum fine of $27,500 per day
2. Willful violations = Class C felony

D. Exemptions

1. Crude oil tankers engaged in coastwise trade;
2. Vessels of the Department of defense, Coast Guard, or any of the Armed Services as defined within 33 USC 1322 (a) and (n);
3. Vessels that operate exclusively within one Captain of the Port Zone (COTPZ)

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