Risk Assessment for the Compound Tunicate *Didemnum vexillum in* Oregon's Marine Waters

June 2014

Prepared for the

Oregon Invasive Species Council

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Summary: The compound ascidian *Didemnum vexillum* (*aka* tunicate or sea squirt) is native to Japan, and the biogeographic range of this species is rapidly increasing at many locations around the world. The specific vectors for introduction of *D. vexillum* into new marine and estuarine waters are largely unknown, but international shipping and maritime commerce, local boat traffic, and the transport and handling of commercial mariculture products and materials are thought to be likely sources for new introductions. In Oregon, colonies of *D. vexillum* were first observed within the Umpqua Triangle in 2010 (Figure 1), and they have since been documented within the Charleston Boat Basin (2010). Like other didemnid tunicates, *D. vexillum* possess chemical defenses and acidic substances in the surrounding gelatinous tunic, and the benthic colonies can tolerate a wide range of environmental temperatures, salinities, sediment loads, and nutrient levels in marine and estuarine waters. Colonies of *D. vexillum* reproduce by fragmentation, and by production of short-lived lecithotrophic tadpole larvae that swim in the water column for less than 24 hrs before settlement, metamorphosis, and attachment to the bottom. Few known predators successfully prey upon the benthic colonies, and the tunicates are capable of rapid growth. In some locations colonies of *D. vexillum* have become the dominant spatial competitor in benthic habitats where they spread out to overgrow other native species such as mussels, scallops, and other sessile species. Previous efforts have been made in other locations to remove the tunicates from the undersides of docks and pilings by the application of wraps and immersion in noxious fluids (*e.g.*, acetic acid, bleach, freshwater, brine, hypoxic seawater), and these control efforts have met with variable levels of success. To date, none of the potential control techniques have been attempted in Oregon's marine and estuarine waters. The Oregon Invasive Species Council has included *D. vexillum* (and several other tunicates) on the statewide list of 100 worst invasive species (OISC 2014).

Species Identity: *Didemnum vexillum*, colonial ascidian, tunicate, sea squirt

- Taxonomic Position:
    - Kingdom: Animalia
    - Phylum: Chordata
    - Subphylum: Tunicata
    - Class: Ascidiacea
    - Order: Enterogona
    - Suborder: Aplousobranchia
    - Family: Didemnidae
    - Genus and species: *Didemnum vexillum*

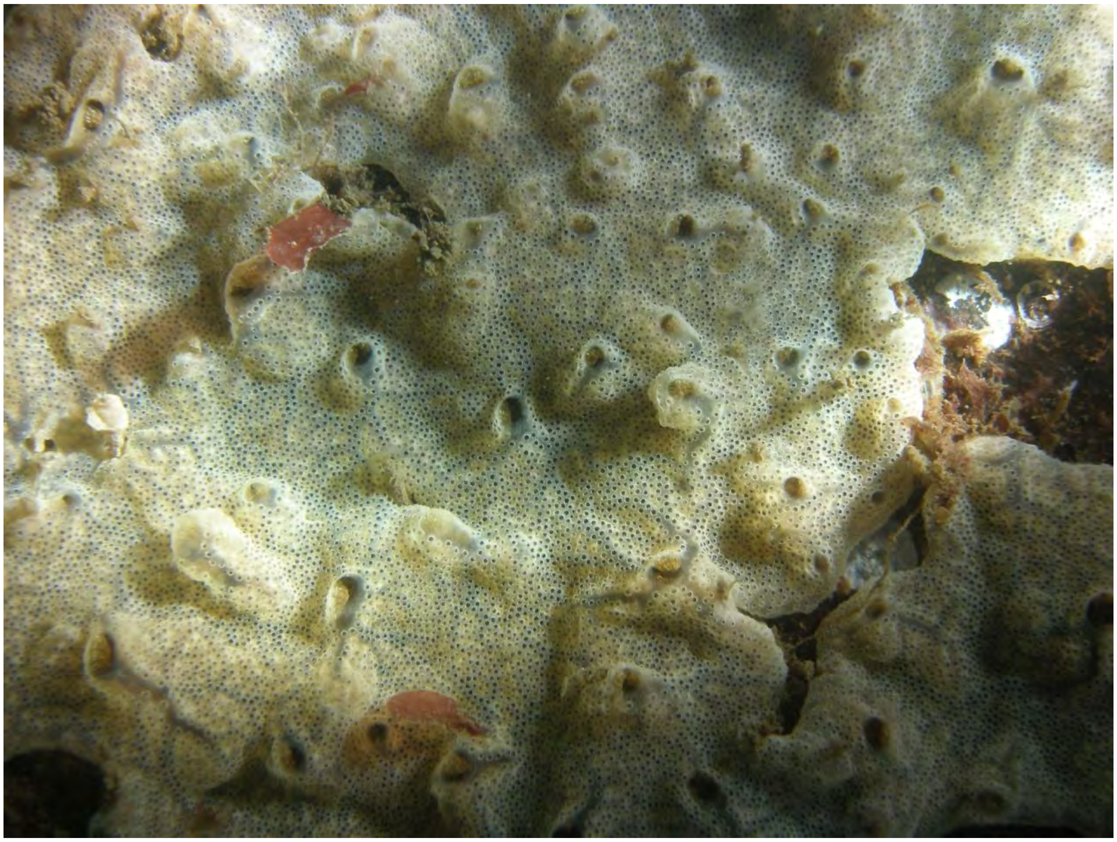

Figure 1. Colonies of *Didemnum vexillum* were first observed in Oregon on sub-tidal rock jetties at the Umpqua Triangle, Winchester Bay, OR (Feb 2010; photo: L. Curran)

Overall Risk Rating: High Risk (83%)
Numerical Risk Factor Score: 12.5 (on a scale from 1-15)

Table 1. Summary of risk types, statements of risk, ratings, and risk factors associated with invasion by *Didemnum vexillum* in Oregon waters.

RISK TYPE	STATEMENT OF RISK	RISK RATING	RISK FACTOR (1-3)
Establishment of New Colonies	Ambient environmental parameters are conducive to reproduction in Oregon waters	High	3
Dispersal and Spread	Colonies are spread locally by fragmentation and larval dispersal, and over longer distances by recreational and commercial boating/shipping activities and shellfish mariculture operations	High	3

Ecological Impact	Colonies can become spatially-dominant competitors that overgrow native organisms and occupy available marine and estuarine habitats	High	3
Economic Impact	Expansion of tunicate colonies may result in moderate economic impacts to shellfish mariculture operations, and increase costs to combat fouling in marinas and on the hulls of recreational boats and commercial vessels	Moderate/High	2.5
Human Health	Human health risks are not expected to increase due to colonization by *Didemnum vexillum*	Low	1

Analysis of the different types of risk associated with establishment of *Didemnum vexillum* indicates that the tunicates constitute an overall High Risk in Oregon waters. Uncertainties regarding the likelihood for successful invasions by *D. vexillum* in Oregon fall into four categories: (1) ability of the colonies to persist within new habitats; (2) ability to spread after establishment; (3) capacity for dispersal and statewide establishment within multiple bays, estuaries, and marine habitats; and (3) potential ecological and economic impacts. The strength and direction of local water currents determine how far larvae and/or colony fragments can disperse within a discrete body of water, while human-mediated transport is the dominant factor in long-distance dispersal and redistribution given the limited ability to disperse within the plankton. Water temperature and salinity both affect growth and reproduction of *D. vexillum*, however ascidians can tolerate a wide range of environmental conditions and become dormant when conditions are unfavorable. The ecological effects of *D. vexillum* infestations on biodiversity and productivity are widely variable across the regions where they have invaded, and their potential economic impacts in Oregon are highly uncertain.

PART 1. Introduction and Overview

A. Background Information

Colonial ascidians (*aka* tunicates, sea squirts) are sessile marine invertebrates that are common worldwide in temperate waters where they attach to rocks, shells, pilings, boat hulls, and many other types of substrata (Barnes 1980; Newberry and Grosberg 2007). *Didemnum vexillum* (Figure 2) is a compound colonial ascidian originally thought to be native to the central coast of Japan (Lambert 2009). Over the past decade this species has been recognized to be a global invader that has established viable populations in temperate waters along the northeast Pacific coast (Alaska, British Columbia, Washington, Oregon, California), the Atlantic coast of the United States (Maine, New Hampshire, Massachusetts, Rhode Island, Connecticut, New York, New Jersey), throughout northern Europe (Netherlands, France, Ireland, Wales, Scotland, England, Italy), and in New Zealand (Bullard *et al.* 2007; Herborg and O'Hara 2009; McCann *et al.* 2013; USGS 2014). The encrusting body-form of *D. vexillum* is frequently described as "sponge-like" allows the amorphous yellow-orange colonies of aggregated tiny zooids to adhere to each other within the gelatinous tunic. Colonies of *D. vexillum* occur on the bottom and attach themselves to a variety of substrata in the form of broad sheets, mats, elaborate lobes, or rope-like structures. The typical lifespan of *D. vexillum* colonies is about 1-3 years but it is difficult to determine the precise age of the colonial organism due to periodic growth, fragmentation, and senescence of parts of the colony (Barnes 1980; Kott 1989).

Figure 2. Colonies of *Didemnum vexillum* attached to the shells of native mussels (*Mytilus trossellus*), where continued growth of the tunicates can overgrow and smother the mussels.

Like other colonial ascidians, *Didemnum vexillum* possess two distinct modes of reproduction. First, the hermaphroditic *D. vexillum* can reproduce sexually by producing eggs and sperm that are retained and develop internally in brood chambers. A well-developed "tadpole" larva is held within the atrium and eventually released, and the larvae swim and disperse for 6-24 hrs before settlement out of the water column (Daley and Scavia 2008, Lengyel *et al*. 2009). This short-term larval form is adapted for site-selection prior to settlement rather than long distance dispersal (Kott 1989). Prior to settlement the photosensitive larvae are attracted to shaded habitats such as those found in crevices, underneath rocks, docks and boats (Bates 2005, Bingham 1997) and they often settle in areas of light wave action (Holloway and Connel 2002). At similar latitudes, recruitment typically occurs in the late summer and early fall (Whitlatch and Osman 2007, Bullard et al. 2007a). The settled zooid becomes sexually mature within a few weeks after settlement (Lambert 2002). Second, colonies of *D. vexillum* are also capable of asexual reproduction via the process of fragmentation and propagative budding (Kott 2002; Valentine *et al*. 2007b, Bullard *et al*. 2007b).

The first introductions of *Didemnum vexillum* into the U.S. were discovered simultaneously in 1993 at Damariscotta River, Maine and San Francisco Bay, California (Daley and Scavia 2008). Since then, *D. vexillum* has been found covering the benthos of Georges Bank (an important scallop fishing area off of the coast of Massachusetts), throughout the Salish Sea / Puget Sound, along the west coast of Vancouver Island, at numerous locations in California, and in Sitka (AK). During the winter/spring of 2010, colonies of *D. vexillum* were discovered for the first time in Oregon at two locations, the Umpqua Triangle (Winchester Bay, Umpqua River estuary / L. Curran) and the Charleston Marina (Coos Bay / R. Emlet). Surveys of the *D. vexillum* colonies (2010 to 2013) on floating docks in the Charleston Marina, and monitoring of the populations on oyster aquaculture mooring lines inside the Umpqua Triangle, has indicated a progressive, but seasonal, increase of colonies (B. Hansen, US Forest Service, Corvallis, pers. comm.). During 2012-13, new colonies of *D. vexillum* were discovered on stationary pier pilings in the Charleston Marina, and on subtidal surfaces of large jetty boulders along the north jetty of the Umpqua Triangle (Umpqua River mouth). These new discoveries are alarming because they indicate that the *D. vexillum* populations have gained sufficient propagule pressure to successfully recruit locally to adjacent habitats, and because they have now made the jump onto stationary objects (*i.e.,* pier pilings and jetty boulders) that cannot be readily removed as a potential control measure. However, the populations of *D. vexillum* have not yet spread beyond the Charleston Marina nor expanded their distribution from the Umpqua Triangle to the nearby Winchester Bay marina. Restricted occurrence and limited spread of *D. vexillum* within the Charleston Marina and the Umpqua Triangle relative to other sites (*e.g.*, New Zealand, New England, and many more locations) highlights the opportunity and pressing need to take actions designed to remove newly established colonies from stationary objects, reduce the propagule pressure, and to develop an integrated management strategy for prevention and control of this species in Oregon waters.

B. Risk Rating Details

B1. Potential for Establishment of New Colonies is High

Ambient environmental parameters are conducive to reproduction in Oregon waters

Rationale:

Colonies of *Didemnum vexillum* can establish readily when three conditions are met: (1) the colonies collectively generate sufficient propagule pressure to allow successful reproduction (sexual or asexual); (2) the preferred substrata (boulders, rocks, gravel, underwater structures, moorings, pilings, docks, shellfish, shellfish lines, kelp, crabs, fishing gear, boat hulls, seaweeds, etc.) are available for attachment; and (3) ambient environmental parameters are within the physiological tolerance of the species. *D. vexillum* seems to prefer hard substrata with at least some degree of fouling and can also attach to plants, algae and invertebrates. The colonies can live to a depth of 80m (continental shelf) and also survive in shallow tidal pools (Kleeman 2009). All of these substrata can be found in Oregon's coves, marinas, tidal zones, estuaries and bays.

Seawater temperature and salinity are both major factors that contribute to survival of *Didemnum vexillum* in marine habitats, bays, and estuaries. Colonies can survive from -2 to 25˚C (Valentine *et al.* 2009) and withstand daily fluctuations of up to 11˚C (Valentine *et al.* 2007a). Optimal growth occurs at 14-18˚C (57-64˚F) (Kleeman 2009) and optimal recruitment takes place at 14-20˚C (57-68˚F). Previous studies have shown that both growth and reproduction can occur from 10-25˚C (Daley and Scavia 2008). Water temperatures along the Oregon coast typically fluctuate seasonally between 5.5 and 20˚C (42-68˚F) with both maximum and minimum temperatures recorded off of Astoria (NOAA 2010a). Sea surface temperatures off the mouth of the Umpqua River (Buoy 139) have been measured to fluctuate up to 10˚C between the seasonal maximum and minimum values (C.D.I.S. 2010a). Average monthly sea surface temperatures (SST) at the Umpqua Buoy 139 fluctuated between 9.5˚C (February) and 13.5˚C (August) over the period from 7/1/06 to 4/29/10 (Figure 3). Although the mean sea surface temperatures recorded at Umpqua Buoy 139 do not encompass values corresponding to the optimal ability of *D. vexillum* to grow and spread (14-18˚C), it must be noted that maximum temperature values can exceed the mean values by up to 5˚C. Consequently, it is recognized that *D. vexillum* is capable of growth and reproduction at any time during the year with the most likely time period extending from June to October. During the warmer summer and autumn months, *D. vexillum* has ample opportunity to grow and reproduce.

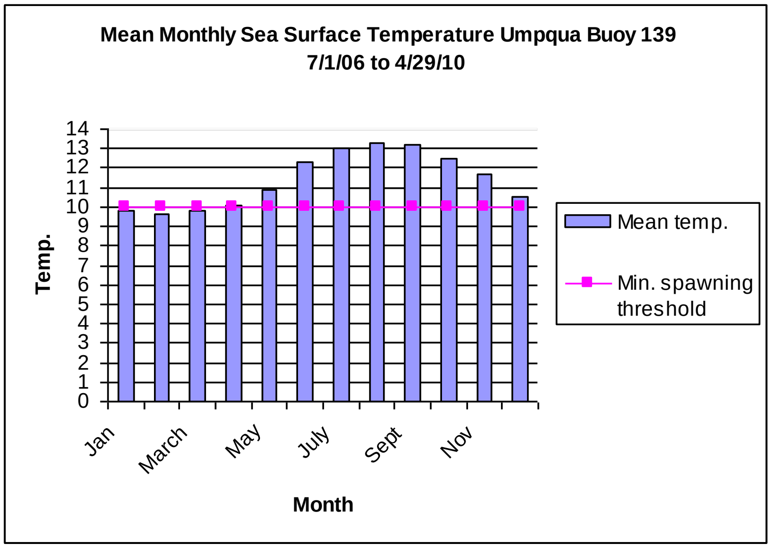

Figure 3. Average monthly sea surface temperatures (SST) at the Umpqua Buoy 139 fluctuated between 9.5˚C (February) and 13.5˚C (August) over the period from 7/1/06 to 4/29/10. Line with boxes indicates the minimum threshold temperature for spawning of *Didemnum vexillum* at 10˚C.

Sea surface temperatures recorded at Coos Bay Buoy 126 closely resemble the thermal pattern recorded at the Umpqua River Buoy 139 (CDIS 2010b). Average monthly sea surface temperatures offshore from Coos Bay fluctuated between 10.4˚C (February) and 14.8˚C (June) over the period from 3/1/05 to 2/28/06 (Figure 4). Fewer data are available from Coos Bay Buoy 126 because it was decommissioned after February 2006. Nevertheless, the limited data set indicates elevated sea surface temperatures during the period between May and October.

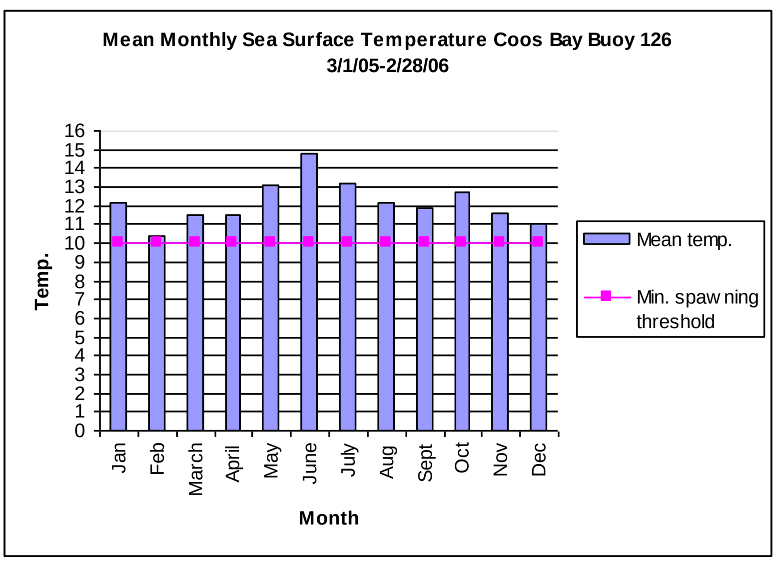

Figure 4. Average monthly sea surface temperatures (SST) at the Coos Bay Buoy 126 fluctuated between 10.4˚C (February) and 14.8˚C (June) over the period from 3/1/05 to 2/28/06. Line with boxes indicates the minimum threshold temperature for spawning of *Didemnum vexillum* at 10˚C.

The temperature cycle for surface waters within the Umpqua Triangle was similar to the pattern observed further offshore at Umpqua Buoy 139. SCUBA divers organized by the US Forest Service (B. Hansen) placed dataloggers inside the tidal lagoon of the Umpqua Triangle to record time-series measurements of surface water temperature and salinity values over 2012-2013 (Figure 5). Within the Umpqua Triangle, sea water temperatures were cold in winter (January to March 2012 / 8.0 to 9.5˚C), increased over the spring (April to May / 9.0 to 13.0˚C), and were elevated and variable over the summer (June to October / 10.0 to 16.0˚C). Sea water temperatures increased again in fall (November / 10.0 to 13.0˚C) and then declined again in winter (December / 9.0 to 13.0˚C). Salinities within the Umpqua Triangle also followed a seasonal cycle, although the pattern is more complex. Salinity values were generally high in winter (January to February 2012 / 25 to 30 ppt), and became highly variable over the spring as freshwater flowed down the Umpqua River and entered the protected waters of the Umpqua Triangle (March to April / 8 to 25 ppt), and then stabilized over the summer (May to August / 20 to 25 ppt). Salinity values were elevated in fall (September to November / 24 to 28 ppt) and then became highly variable with the onset of rainstorms in the early winter (December to January / 5 to 25 ppt). Taken together, the time-series data for these *in situ* temperature and salinity measurements also indicate that conditions are conducive to reproduction by *Didemnum vexillum* during May to November within the Umpqua Triangle.

Historical time-series temperature and salinity data is also available from Coos Bay via the Charleston Bridge monitoring station operated by the South Slough National Estuarine Reserve / System-wide Monitoring Program (SSNERR 2010). Mean monthly sea surface temperatures recorded at the Charleston monitoring station were averaged over a period from 4/1/05 to 4/30/10. The seasonal pattern of temperature and salinity fluctuations in Coos Bay is similar to the pattern observed for Winchester Bay due to its close geographic proximity and similar timing of flood events. It should be noted however, that Winchester Bay exhibits a higher rate of fresh water influx and has less than half the cross sectional area at the river mouth. Both of these factors could potentially affect salinity and temperature values between the two locations where colonies of *Didemnum vexillum* occur. In Coos Bay, seawater temperatures are elevated above 10˚C from April through November (Figure 6), and indicate that *D. vexillum* are probably able to grow and reproduce from May to September when temperatures approach an optimum level of 13 to 14˚C.

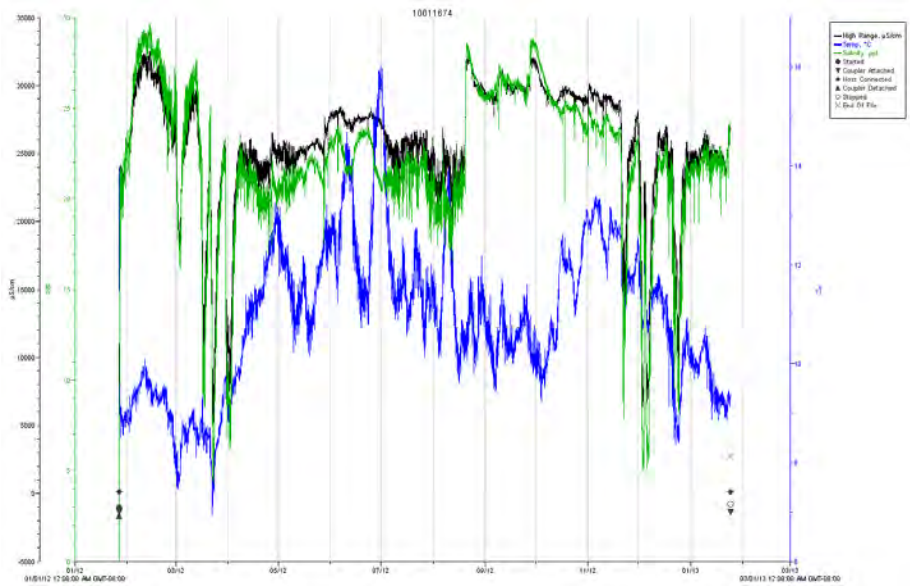

Figure 5. Time-series data records for ambient sea water surface temperature (blue) and salinity (green) measurements recorded by a datalogger attached to a mooring line within the Umpqua Triangle over 2012-2013.

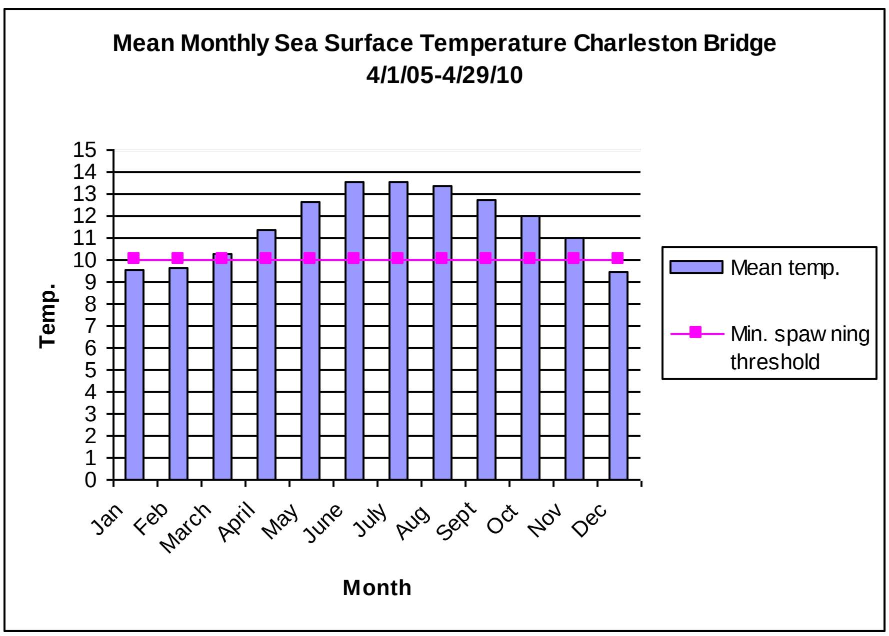

Figure 6. Mean monthly sea surface temperatures recorded at the Charleston SWMP monitoring station (Coos Bay, OR). Values indicate monthly averages from 4/1/05 to 4/30/10, and line with boxes indicates the minimum threshold temperature for spawning of *Didemnum vexillum* at 10˚C.

Monthly mean salinity values recorded at the Charleston monitoring station (Coos Bay) indicate that salinity values within the marine-dominated region of the estuary remain above the minimum threshold (25 ppt) for spawning by *Didemnum vexillum* for much of the year (Figure 7). The colonies of *Didemnum vexillum* prefer salinities of 25 parts per thousand or higher. The ascidians have been documented to die back in shallow water during high, fresh water runoff events (Daley and Scavia 2008). These records indicate that the salinity levels in Charleston are most likely sufficient for survival of *D. vexillum* during most of the year.

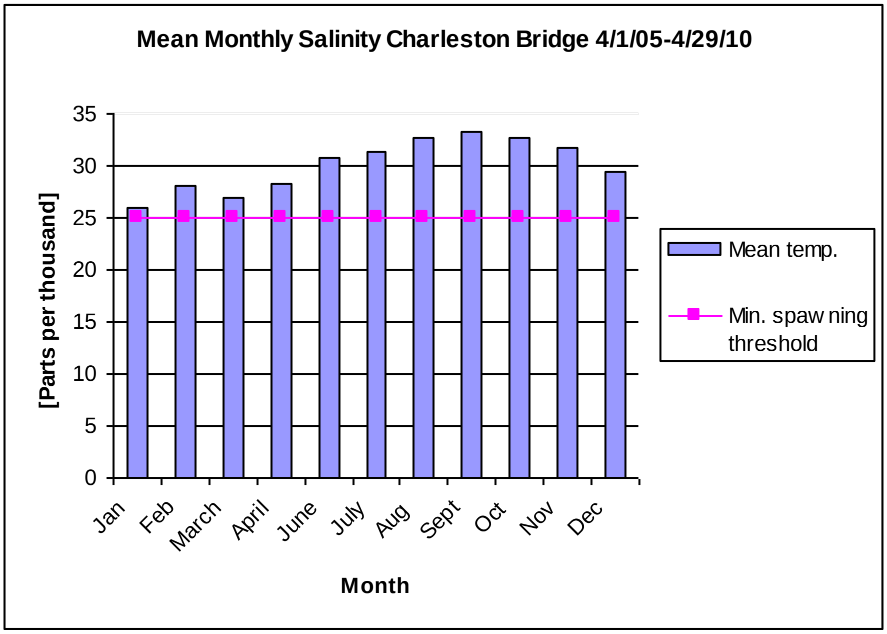

Figure 7. Mean monthly sea surface salinity values recorded at the Charleston SWMP monitoring station (Coos Bay, OR). Values indicate monthly averages from 4/1/05 to 4/30/10, and line with boxes indicates the minimum threshold salinity for spawning of *Didemnum vexillum* at 25 ppt.

B2. Potential for Tunicate Colonies to Spread is High

Colonies are spread locally by fragmentation and larval dispersal, and over longer distances by recreational and commercial boating/shipping activities and shellfish mariculture operations

<u>Rationale</u>:

The potential for colonies of *Didemnum vexillum* to spread after initial establishment is determined: (1) over short distances by fragmentation and localized larval dispersal; and (2) over longer distances primarily by recreational and commercial boating/shipping activities and shellfish mariculture operations. The short duration of the tadpole larval phase (6-24 hrs; Daley and Scavia 2008; Lengyel *et al*. 2009; Figure 8) restricts the spatial scale of both dispersal (distribution away from the parent) and dispersion (scattering of propagules away from each other) to local levels. In many species of tunicates the tadpole larvae typically disperse only a few meters (Davis and Butler 1989) although dispersal distances are presumably lengthened considerably in areas of strong tidal flow. Natural dispersal of *D. vexillum* may also occur through the spread of detached fragments of colonies either in water currents, rolling along the bottom or by attachment to the bodies of motile invertebrates (*i.e.*, crab; Bernier *et al.* 2009). Risk associated with dispersal by fragmentation becomes more serious when the colony develops to the large pendulous stage (Kleeman 2009; Figure 9). Rafting of colony fragments may also occur, and up to 15% of the fragments of *D. vexillum* have been documented to survive suspension in the water column for 30 days (Carman 2008) and fragments have been observed to attach themselves to substrata within six hours of contact (Bullard *et al*. 2007b; Morris and Carmen 2012). Localized spread of the tunicate colonies along docks and piers, and within boat basins and marinas is thought to occur largely through the processes of fragmentation and short-distance larval dispersal.

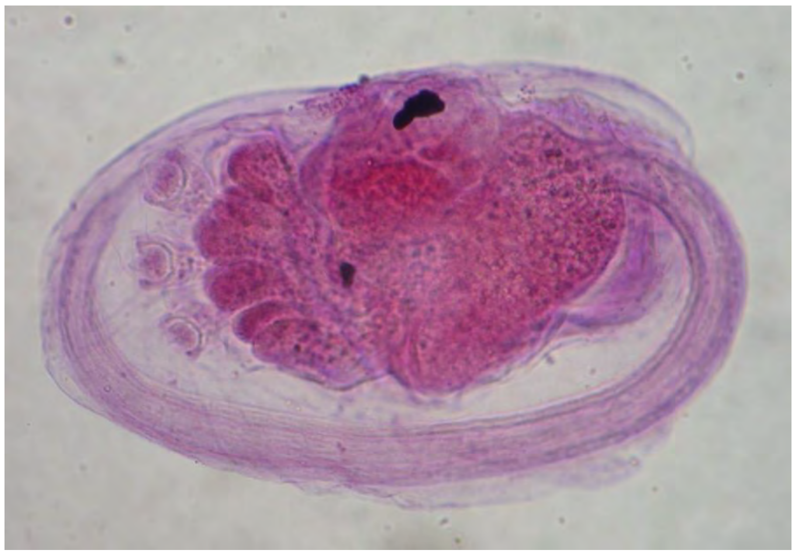

Figure 8. The tadpole larval form of *Didemnum vexillum* possesses a muscular tail for swimming and anterior adhesive papillae for attachment to benthic substrata (photo: www.WoodsHole.USGS.gov)

Figure 9. Colonies of *Didemnum vexillum* can develop into pendulous lobes that detach and are transported over short distances to new locations. The process of fragmentation contributes to localized spread of this invasive species.

The risk of spreading *Didemnum vexillum* over longer distances is associated with a variety of anthropogenic vectors. Recreational and commercial boating are widely considered as the primary vector for initial introduction of *D. vexillum* into new areas (Kleeman 2009; Gittenberger 2010), and inadvertent transport of viable colonies associated with shellfish mariculture operations is also an important pathway for new introductions (Dijkstra *et al.* 2007; Switzer *et al.* 2011). Hull fouling on commercial and recreational vessels allows the didemnid colonies to persist and grow for long periods in protected shallow water environments where the fragments and/or larvae have opportunities to become established on a variety of substrata such as other vessel hulls, floating docks, piers, pilings, mooring lines, rip-rap, and rocks (Figure 10). Long distance transport of *D. vexillum* colonies via ballast water is most likely of lesser importance because the short time period for the planktonic larvae makes it difficult for them to become entrained during the pumping of ballast fluids (Gittenberger 2010). Secondary fouling of commercial and recreational fishing gear such as nets and crab pots also poses a risk for the spread of *D. vexillum*. Commercial and recreational boating and fishing are popular activities in Coos Bay and Winchester Bay (Oregon) and hull fouling and vessel transport are likely vectors for spread of *D. vexillum* beyond the Charleston Marina and Umpqua Triangle. Periodic dredging activities that disrupt bottom communities within these harbors and marinas may also provide another pathway for anthropogenic fragmentation and dispersal.

Figure 10. Hull fouling by colonies of *Didemnum vexillum* (photo: AquaNIS / Information system on aquatic non-indigenous and cryptogenic species).

Commercial shellfish farming and harvest activities (movement of equipment and/or inadvertent transport of shellfish colonized by *Didemnum vexillum*) are another important vector for the spread of this species (Gittenberger 2010; Switzer *et al*. 2011; Rolheiser *et al*. 2012; McCann *et al*. 2013). Colonies of *D. vexillum* readily grow while attached to aquaculture nets, cages, mooring lines, and other equipment that are commonly used by shellfish growers during mariculture operations (Figure 11). These materials provide new substrata that are opportunistically colonized by *D. vexillum* while the mariculture activities are carried out in shallow marine and estuarine waters. Although mariculture products and equipment are typically cleaned prior to transport or sales, the processing of shellfish and cleaning of equipment at shoreside facilities may provide a pathway to reintroduce *D. vexillum* back into marine and estuarine waters. Transport of *D. vexillum* may also occur over longer distances (among and between commercial shellfish growing areas) by providing an avenue for inadvertent "hitch-hikers" that are attached to the shells of living oysters, mussels, and clams that are then transported by vessels, barges or trucks (Therriault and Herborg 2007). Shellfish dredging and other harvest activities provide yet another mechanism for the spread of *D. vexillum*. Colonial ascidians are more successful at occupying space on the bottom following disturbance by dredging (Kleeman 2009). Trawl nets or other harvest implements dragged along the bottom can also dislodge fragments of *D. vexillum*, suspend them in the water column and aid in their dispersal. Better understanding and management of these human-mediated transport vectors could be a key to limiting further spread of this invasive ascidian (Therriault and Herborg 2007).

Figure 11. Extensive growth of *Didemnum vexillum* colonies on mariculture nets that are used for the commercial cultivation of mussels in Okeover Inlet, British Columbia (photo: G. King).

B3. Potential for Ecological Impact is High
Colonies can become spatially-dominant competitors that overgrow native organisms and occupy available marine and estuarine habitats

Rationale:
Colonies of *Didemnum vexillum* are known to become spatially-dominant competitors in marine and estuarine environments where they can out-compete, overgrow, smother, and kill native organisms (Bullard *et al*. 2007; Mercer *et al*. 2009; Figures 12 and 13). Rapid growth and expansion of *D. vexillum* have been shown to cover the siphons of bivalves and other filter-feeders, and to interfere with the ability of demersal fishes to find prey (USGS 2010). The colonies of *D. vexillum* can also inhibit recruitment of other benthic invertebrates and reduce metrics of local biodiversity (Osman and Whitlatch, 1995). Significant loss of biodiversity may occur in areas where *D. vexillum* has become the dominant spatial competitor because the dense colonies directly inhibit larval settlement or indirectly decrease the survival of post-larval invertebrates (Morris *et al*. 2009). By smothering the benthos, other animals do not have the opportunity to compete for space or find prey. Spatial dominance by *D. vexillum* can cause predatory animals to migrate to other areas and increase predatory pressure in those associated habitats. A dense mat of *D. vexillum* on gravel substrates and/or seagrass beds may also inhibit Clupeids (herrings) from spawning and prevent juvenile Gadids (codlike fish) from finding cover and food (Smith and Morse 1993, Collie *et al*. 2000). Anecdotal accounts have been published of *D. vexillum* smothering scallops and mussels on Georges Bank and in New Zealand (Coutts 2002; USGS 2006; Valentine *et al*. 2007b). Didemnid tunicates possess an acidic gelatinous tunic and other chemical defenses (Pisut and Pawlik 2002), and few predators have been observed to feed directly on *D. vexillum* including a sea star, a sea urchin, a chiton, and a few species of marine snails (USGS 2009). In New Zealand, caging experiments suggested that spread of *D. vexillum* may be prohibited by benthic predation within subtidal cobble habitats (Forrest *et al*. 2013), however the specific predators were not identified. Deposition of suspended sediment loads may also be affected by the ability of *D. vexillum* to form dense mats of growth along the bottom, but this has yet to be explored. The precise mechanisms by which extensive infestation of *D. vexillum* colonies will affect the complexity, productivity and prey interactions of marine and estuarine communities are difficult to determine without directed research and manipulative field experiments.

Figure 12. *Didemnum vexillum* can become the dominant spatial competitor on a variety of substrata in marine and estuarine environments, and the tunicate colonies can overgrow other established organisms.

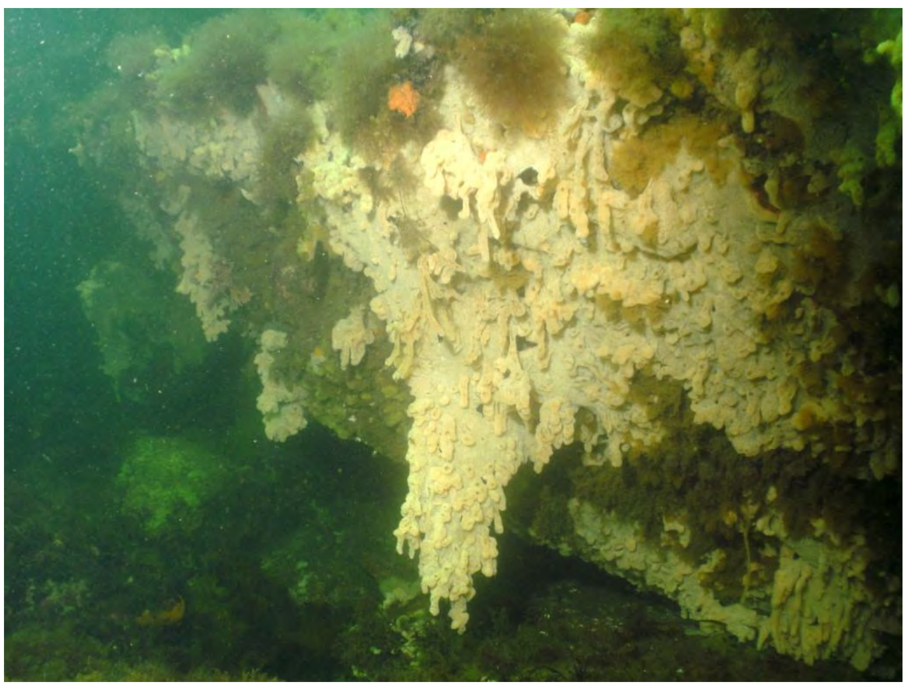

Figure 13. Colonies of *Didemnum vexillum* occupy extensive area on a sub-tidal boulder. In this photo the colonies have developed numerous prominent lobes that are capable of detachment and fragmentation to establish new colonies along the bottom.

B4. Potential for Economic Impacts is Moderate/High
Expansion of tunicate colonies may result in moderate/high economic impacts to shellfish mariculture operations, and increase costs to combat fouling in marinas and on the hulls of recreational boats and commercial vessels

Rationale:
Didemnum vexillum has the potential to cause economic harm to commercial shellfish farms by directly smothering the cultivated oysters and clams, and by increasing the time and costs associated with processing and handling (Cayer *et al*. 1999, Boothroyd *et al*. 2002). Because oysters must be cleaned prior to shipment and sale, heavy colonization by *D. vexillum* could result in substantial economic losses to commercial shellfish farms in Winchester Bay, Coos Bay, and other locations along the Oregon coast. Crab pots, fish traps, and shellfish mariculture mooring lines can also become covered with *D. vexillum* and must be cleaned upon retrieval. In some cases, dense monocultures of *D. vexillum* have been observed to develop along the mooring lines of mussel culture operations (Gittenberger 2010) and precipitated the need for replacement. In New Zealand, governmental agencies and aquaculture operators have spent over $1M over the past decade in their attempts to eradicate *D. vexillum* from economically important shellfish growing areas and mussel lines. In the event that predatory fish move away from areas infested by *D. vexillum* and juvenile fish rearing areas are lost (as predicted), loss to bottom fishing operations could occur due to a lower catch-per-unit-effort. Marinas, private and commercial boat owners would incur the costs associated with cleaning and removal of *D. vexillum*. Commercial and recreational boat owners may decide to avoid contaminating their vessels by choosing to berth their vessels elsewhere, resulting in financial loss to marina owners. Heavy hull fouling by *D. vexillum* can also affect the hydrodynamic drag on the hulls of boats and thereby result in higher fuel costs. Recreational fishing opportunities could decrease if fish emigrate away from areas infested with sea squirts. Recreational and commercial shellfish harvesters may experience decreased access to shellfish beds that are impaired by heavy encrustation by the tunicates. In addition, recreational SCUBA divers may choose to avoid areas where there has been a significant loss of biodiversity due to *D. vexillum* because the dense colonies inhibit recruitment (Morris *et al*. 2009) and fewer interesting fish and other marine life may persist. The public will pay higher taxes to cover the costs of eradication, inspection, monitoring and removal. It is also conceivable that, in the future, wave powered generators and associated equipment located in Oregon's nearshore waters could become fouled by a *D. vexillum* infestation. This could result in increased cleaning and maintenance costs.

B5. Potential for Adverse Human Health Impacts is Non-existent
Human health risks are not expected to increase due to colonization by Didemnum vexillum

Rationale:
At this time, there are no confirmed risks or threats to human health associated with expansion and colonization by *Didemnum vexillum*. It is possible that mariculture operators may be exposed to a very low-level of human health risk during repeated

handling, processing, and cleaning of shellfish and mooring lines that are heavily colonized by the tunicates because the gelatinous coverings are known to contain chemical defenses and toxic proteins (Pisut and Pawlik 2002; Forrest 2013). For example, oyster shuckers in British Columbia have reported onset of an asthmatic condition associated with the opening of shellfish that are fouled with the solitary tunicate *Styela clava* in poorly-ventilated areas (Clarke and Therriault 2007). Lambert (2009) reported that efforts have been completed to successfully sequence the genome structure of *D. vexillum*, and this species offers the potential for using tunicates to investigate the process of vertebrate evolution. In addition, Rinkevich and Fidler (2014) have recently developed laboratory methods for the culture of *D. vexillum* that will allow for propagation of genetically well-defined stocks and strains in this species. Tunicates and their bacterial/cyanobacterial symbionts are also of interest for the discovery of possible pharmaceutical drugs. It is possible that these intensive studies of genetic structure and culture requirements may reveal potential hazards or benefits for human health.

C. **Overall Assessment of Risk**:

Analysis of the different types of risk associated with establishment of *Didemnum vexillum* indicates that the colonial tunicates constitute an overall High Risk in Oregon waters (Table 1, above). Concern over the potential for *D. vexillum* to become invasive in Oregon's marine habitats, bays, and estuaries stems primarily from the physiological, ecological and life-history traits that are common to other bioinvasive species, including: (1) tolerance to a wide range of environmental conditions; (2) high reproductive and population growth rates; (3) ability to spread by colony fragmentation and short-distance larval dispersal; (4) the ability to become a spatially-dominant competitor and overgrow benthic organisms; (5) apparent scarcity of predators; and (6) the ability to survive in human dominated habitats. The Oregon Invasive Species Council has included *D. vexillum* (and several other tunicates) on the statewide list of 100 worst invasive species (OISC 2014).

In other locations, the overall invasion risk for *Didemnum vexillum* has been assigned risk factor scores of High (Dutch Wadden Sea; Gittenberger 2010), High Risk (British Columbia; Therriault and Herborg 2007; Herborg *et al*. 2008); Serious Risk (Wales; Kleeman 2009); a Priority Concern (Washington; Pleus *et al*. 2008), a High Profile Pest Species (New Zealand; Morrisey and Miller, 2008), and a Marine Pest (Western Australia; Munoz and McDonald 2014), and the species is characterized to have a high potential for Negative Impact in the United States; (Daley and Scavia 2008). Taken collectively these assessments of risk factors indicate that *D. vexillum* is widely regarded as an organism of high concern which presents an unacceptable level of risk in Oregon waters (Leung and Dudgeon 2008). Deliberate introduction of species that pose unacceptable risks should be prohibited, and new introductions should not be left unchecked. In cases where new introductions are at an early stage of invasion, proactive management measures should be taken to control and eradicate the established colonies, and concurrent steps should be focused on prevention of further introductions (Leung and Dudgeon 2008).

PART 2. Methods for Eradication and Removal

A. Previous Control Efforts

Several methods have been proposed and tested for the removal and eradication of *Didemnum vexillum* in locations outside of Oregon including New Zealand (Coutts and Forrest 2007; Pannell and Coutts 2007), Scotland (Nimmo *et al.* 2011), Wales (Kleeman 2001, Holt and Cordingley 2011), England (Cook 2010), Ireland (Kelly and Maguire 2008), Canada (Switzer *et al.* 2011), Washington (Pleus *et al*. 2008; Puget Sound Partnership 2011), and Alaska (McCann *et al*. 2013). These efforts have included a combination of mechanical and chemical methods that are designed to physically remove the fouled substrata and colonies from the marine environment, remove the colonies from fouled substrata, kill the tunicates *in situ* via suffocation, immersion in biocides, and burial. More recent efforts have been made to remove colonies of *Didemnum vexillum* from living shellfish, mariculture equipment, mooring lines, and the undersides of docks and pilings (Switzer *et al*. 20111; Rolheiser *et al*. 2021; McCann *et al*. 2013). These efforts primarily include the application of wraps and immersion in noxious fluids (*e.g.*, acetic acid, bleach, freshwater, brine, hypoxic seawater), and other techniques. The control efforts have met with variable levels of success, and to date, the potential control techniques have only been attempted on a small scale in Oregon's marine and estuarine waters.

B. Mechanical and Chemical Control Efforts

Two general approaches are currently used in the field to control the spread of non-native tunicates: (1) Mechanical Control; and (2) Chemical Control (Table 2). Mechanical approaches include active removal or destruction of the tunicate colonies by hand or by the use of equipment such as high-pressure water jets, scraping or suction devices, desiccation, and asphyxiation/starvation. In contrast, chemical approaches use toxic substances or induced changes to the physical properties of the water caused by altering temperature, pH, or salinity (Coutts 2002; Coutts 2005; Coutts 2006; Forrest 2007; McCann *et al*. 2013). Biological control, including the introduction of living organisms such as parasites, disease agents, and predators constitute another approach that has been used to control or eliminate other non-native species, but is not currently used for control of non-native tunicates.

Table 2. Comparison of various control methods to remove or kill colonies of *Didemnum vexillum*.

APPROACH	METHOD	APPLICATION	EFFICACY	BENEFITS	DRAWBACKS
Mechanical	Plastic wrap and leave in place	Pilings, pontoons, buoys, lines and hulls	100% if done properly and no tears in plastic	Cheap, no chemicals, reusable plastic	Takes up to 48 days, stinks as animals rot, plastic tears, recolonization on outer plastic, SCUBA divers

Mechanical	Filter fabric tarps, weighted down	Rocky substrata, boulders, rip-rap and jetties	Variable, depends on seal against substrate, ≤100%	Reusable fabric, non-toxic	Expensive, time consuming, hard to seal completely, wave action can dislodge, SCUBA divers
Mechanical	Underwater scrape and/or removal	Pilings, docks, boat hulls, plants, boulders, mooring lines, structures, rip-rap, jetties	80%, depends on total discovery and collection	Cheap, quick, reduces immediate biomass, good for follow up removals	Spreads by fragmentation, hard to discover and collect all specimens, SCUBA divers
Mechanical	Remove colonies from water, pressure wash and dry	Pontoons, buoys, lines, and hulls	100% total kill	Very effective	Expensive and time consuming to remove boats and pontoons
Mechanical	Removal of shellfish	Adult shellfish	≤100%	Can seriously reduce spread	Loss of immature product, hard to get all infected adults
Mechanical	Smother with dredged materials and sand	Gravel and flat, softer substrates	100%	Other marine life grows back in time	Need dredge, tailings wash out of larger boulder crevices
Mechanical	Rotating brush w/ vacuum	Hulls	80%	Cleans large surfaces, collects *D. vexillum* as it cleans	Misses crevices and non-flat surfaces, most colonies re-grow afterwards, need special equipment, SCUBA divers
Chemical	Plastic wrap, infuse w/ fresh water	Pilings, pontoons, buoys, lines and hulls	Variable, depends on length of treatment 0-100%	Cheap, no chemicals, reusable plastic	No better than wrap and leave, *D. vexillum* has high tolerance to fresh water, SCUBA divers
Chemical	Plastic wrap, infuse w/ acetic acid 5-10% or bleach 0.5-1%	Pilings, pontoons, buoys, lines and hulls	100% if done properly and no tears in plastic	Takes < 30 min., cheap chemicals, reusable plastic	Subject to tears in plastic, recolonization on outer plastic, SCUBA divers
Chemical	Plastic wrap, infuse w/ NaOH 6%	Pilings, pontoons, buoys, lines and hulls	100% if done properly and no tears in plastic	Takes only 48 hrs, reusable plastic	Subject to tears in plastic, recolonization on outer plastic, need divers, more toxic
Chemical	Dipping in acetic acid or bleach	Shellfish spat	≤100% depends on concentration	Can be quite effective at limiting	Some shellfish killed

	solutions		and amt. of time dipped	shellfish infection	
Chemical	Anti-fouling paint	Hulls	100% as long as paint lasts and is not rubbed off	Great vector control, prevents spread	Depends on voluntary cooperation of boat owners, expensive and time consuming, must haul boat
Chemical	Underwater use of pressurized acetic acid 40%	Pilings, pontoons, buoys, lines, boulders and hulls	50%	Direct application to hard to reach colonies	Acid rapidly dilutes in water, not 100% effective, need special equipment, SCUBA divers
Mechanical & Chemical	Lime, concrete, torch burning, other experimental methods	Various experimental substrates	Variable up to 50%	Ingenious, inventive, good for helping understanding of eradication techniques	Expensive and not sufficiently effective

C. Control Efforts for *Didemnum vexillum* along the West Coast

In 2011, a multi-agency group undertook an effort to remove colonies of *Didemnum vexillum* that had become established on the underside of a finger-dock in the Charleston Boat basin (Coos Bay, OR). The International Port of Coos Bay, the Oregon Institute of Marine Biology, and Oregon Sea Grant worked together to wrap the finger-dock in polyethylene tarp and tow the dock into a freshwater region of the estuary. The dock was re-inspected after the wrap-and-tow procedure, and the epifouling organisms (including *D. vexillum*) perished after a period of two weeks. Although this technique proved effective, it will not be possible to wrap-and-tow the stationary pilings and boulders that have recently been inhabited by new colonies of *D. vexillum*. In addition, the financial cost to the Port of Coos Bay, other ports and marinas, and boat owners will be very high in the event that they are required to wrap all docks and hulls and tow them to freshwater to kill fouling organisms.

Experimental work was recently conducted in Sitka (AK) to determine the efficacy of different chemical control and eradication techniques (McCann *et al*. 2013). These efforts all achieved 100% mortality of *Didemnum vexillum* colonies after the following chemical treatments: (1) <u>Acetic acid</u> (10%) for 2 min; (2) <u>Bleach</u> (1%) for 10 min; (3) <u>Freshwater</u> for 4 hrs; and (4) <u>Brine solution</u> (62 ppt) for >4 hrs. It is important to note that these experimental efforts, although 100% effective, were conducted with isolated colonies that were fully exposed to the treatment solutions in zip-lock bags. It is unclear how these results will extrapolate to the colonies of *D. vexillum* that inhabit living shellfish, mariculture equipment, mooring lines, the undersides of docks, vertical surfaces of pilings and bulkheads, gravel, rocks, and other substrata on the seafloor.

Three mechanical methods for the removal of invasive tunicates from docks and watercraft hulls were tested recently by the Washington Department of Fish and Wildlife: (1) removal-by-hand; (2) pressure washing; and (3) asphyxiation/starvation (Pleus *et al*. 2008). Removal of solitary tunicates by hand was a viable technique that was very effective on floating structures with firm surfaces such as docks, buoys, and watercraft hulls. However, it was a labor-intensive and time-consuming process that was not 100% effective at removing all tunicates. Removal of the tunicates by hand requires transporting the animals from the water to an off-site terrestrial disposal area in order to eliminate the potential for redistribution through gamete dispersal, reattachment of whole animals, or asexual budding from tissue fragments. This removal method is highly selective and among the least destructive to neighboring plants and animals. To date (2014), large-scale removal-by-hand efforts have focused only on solitary tunicates (i.e., *Styela clava*). Colonial tunicates such as *Didemnum vexillum* may respond favorably to this method of control, but the method has not yet been attempted on a large spatial scale (Pleus *et al*. 2008). Use of pressurized water is also highly effective at removing nearly all living organisms from a surface. However, high pressure jets can only be used on non-deteriorated surfaces made from concrete, metal, or other materials without compromise to structural or aesthetic integrity, and when the treatment will not result in the release of pollutants (e.g. creosote) into the water. Containment of the resulting biological debris is also difficult with this technique and may lead to further spread through gamete dispersal, reattachment of whole animals, or asexual budding from tissue fragments. Complete wrapping of infested structures with polyethylene tarps, plastic wrap, or other materials effectively starves and asphyxiates the colonial tunicates and other epifouling organisms. The State of Hawaii has had limited success using the method to eradicate invasive corals from several marinas, and workers in New Zealand have experienced some success with localized control of *Didemnum vexillum* using this technique. The Washington Department of Fish and Wildlife tested the asphyxiation method and found that the technique was not 100% effective because it is difficult to completely seal structures from the outside environment, and wrapping odd shaped structures can be cumbersome (Pleus *et al*. 2008).

Underwater suction dredges or venturi pumps are widely used to remove objects, organisms or sediment from the seabed or underwater structures. This technique is frequently widely employed in underwater research, archeology, salvage, and construction. Similar to a vacuum cleaner, the intake hose may be fitted with a wide nozzle to remove wide swaths of organisms from underwater surfaces, or fitted with a narrow nozzle to allow for removal of objects from confined or odd shaped structures. Suction dredges also require the use of mesh netting to contain the fragments of organisms, and they are currently being used to control non-native algae from Hawaiian reefs. This method has not been attempted for the removal of non-native tunicates along the west coast, but warrants serious consideration and testing.

D. Biological Control Efforts

Tunicates have few known predators (Castilla *et al*. 2004) and it is thought that most predation occurs during the larval stage or very shortly after settlement and metamorphosis (Osman and Whitlach 2004; Simkanin *et al.* 2012; Forrest *et al.* 2013). Predators have the potential to limit or restrict the establishment and/or spread of tunicate populations in natural seabed habitats (Osman and Whitlach 2007), but artificial structures and suspended populations may serve as an important refuge from predation. In Marlborough Sound (New Zealand), populations of *D. vexillum* proliferate only on suspended structures, and the colonies failed when they were experimentally transplanted to seabed cobble and macroalgae habitats (Forrest *et al*. 2013). When benthic predators were excluded from the bottom by cages, the colonies survived at a rate that was comparable to that observed on suspended structures. These results suggest that in some locations benthic predation (by crab, seastars, chitons, snails etc.) has the potential to restrict establishment of new colonies of *D. vexillum*. In Oregon, however, it is not clear which natural predators (if any) will readily prey upon established colonies of *D. vexillum* or the newly settled recruits.

Deliberate biological control of non-native tunicates has not yet been attempted. The use of biological tools to control or eradicate non-desired species has a long and contentious history (Messing and Wright 2006). Success stories are few and there are many well-known case studies that illustrate the potential for disastrous consequences to the environment, industry, and human health following the deliberate or inadvertent introduction of foreign predators or pathogens. One method of biological control that has been effective for some species while being relatively environmentally benign is the use of induced sterility through genetic manipulation. This usually involves some form of selective breeding of captive animals and reintroduction into the wild and is not likely to be feasible for colonial tunicates. Biological control for non-native tunicates will only be considered as a last resort when other more practicable means of control have been exhausted and the consequences of continued proliferation of the target species are dire.

<u>Note</u>: The safety of personnel involved with any deployed method will be of primary concern. Additionally, any harm to organisms or the environment as a result of using any of the eradication techniques mentioned here will be given the utmost consideration before implementation. The effects of these eradication methods on threatened and endangered species will be thoroughly considered and the proper permits regarding them must be obtained prior to use of any control measure.

E. Recommendations

To date (June 2014) populations of *Didemnum vexillum* have been detected at two locations in Oregon (Charleston Boat Basin and Umpqua Triangle). Monitoring of the oyster aquaculture mooring lines and sub-tidal jetty boulders inside the Umpqua Triangle has indicated a progressive increase in spatial coverage and redistribution by the colonies over the past several years (B. Hansen, pers. comm.). During 2012-13, new colonies of *D. vexillum* were discovered on stationary pier pilings in the Charleston Marina. These new discoveries are alarming because they indicate that the *D. vexillum* populations have gained sufficient propagule pressure to successfully recruit locally to adjacent habitats. They also represent a significant increase in the level of risk because the colonies have become established on stationary objects (*i.e.,* pier pilings and jetty boulders) that cannot be readily removed as a potential control measure.

Despite the disturbing changes in the distribution of *Didemnum vexillum* observed at the Charleston Marina and Umpqua Triangle sites, it is encouraging that the colonies have not yet spread beyond Charleston nor expanded to the nearby Winchester Bay Marina. Restricted distribution of this invasive species at the two sites during this early stage of the invasion process provides an opportunity for resource managers to take pro-active steps to control the spread before the colonies of *D. vexillum* become widespread in Oregon's waters.

Given the current status of the invasion by *Didemnum vexillum* in Oregon, the following recommendations are in order:

- Management Strategy for Invasive Tunicates: The Oregon Invasive Species Council (OISC) should work with the relevant natural resource agencies (*i.e.*, ODA, ODFW, OMB, ODEQ, port and harbor districts), commercial mariculture operators, and other stakeholders to develop a statewide management strategy for detection and control of invasive tunicates. The management strategy should take a bioregional approach to assess and evaluate the risk of invasion by multiple species of non-indigenous tunicates, and give full consideration to the status of populations, vectors for continued and new introductions, and control measures along the west coast of North America. The OISC Invasive Tunicate Committee should be charged with development of the Oregon Management Strategy for Invasive Tunicates, and the strategy should include statements of policies, coordinated planning, agency responsibilities, risk assessment, and descriptions of the procedures for monitoring, control measures, follow-up evaluations, vector identification, preventative actions, and stakeholder outreach/education. The management strategy should also include estimates of the statewide personnel requirements needed to fully address the issues associated with invasive tunicates in state waters, and identify the time-schedule and budget for management actions.

- Monitoring Program for Invasive Tunicates in Oregon Bays, Estuaries, and Marine Habitats: The state of Oregon should design and implement a monitoring program designed to routinely conduct surveys to quantify the distribution and abundance of invasive tunicates within infected and uninfected sub-tidal habitats. The statewide surveys should focus on priority bays and habitats where the likelihood of establishment of new colonies is particularly high, including harbors and marinas with substantial vessel traffic as well as bays and estuaries that are used for commercial mariculture of shellfish. The monitoring program should identify a series of carefully-chosen index sites that serve as representatives of conditions all along the Oregon coast, as well as sentinel areas that can provide ecological data and other information that are needed to understand the ecological and economic impacts. The monitoring program should also include periodic surveys that are designed to follow-up on the efficacy of deliberate control measures taken at specific sites to reduce or eradicate colonies of *Didemnum vexillum* from estuarine or marine habitats.

- Risk Assessment for Invasive Tunicates: The OISC / Invasive Tunicate Committee should develop a risk assessment document that provides a statewide summary of the current status of invasions, a review of the relevant technical information and literature, declarations of ecological and socio-economic impacts, identifies thresholds for action, and a response plan including recommended options for control measures. The risk assessment for invasive tunicates should be revised and updated periodically on an as needed basis (*i.e.*, every five years).

- Action Plan for Control of Invasive Tunicates at Infected Sites: The OISC / Invasive Tunicate Committee should periodically develop and implement an Action Plan designed to address pressing information needs and urgent management actions that are identified for high risk sites and characteristics of the tunicate populations. The Action Plan should identify and make recommendations for the immediate steps and proactive management measures that can be taken to help document the status and condition of invasive tunicate populations, control and eradicate tunicates in marine and estuarine habitats, identify specific vectors for transport, increase stakeholder awareness, and help decrease the likelihood of future invasions.

- New Research: The OISC / Invasive Tunicate Committee should periodically identify the need for new research efforts and activities that focus on the biology, ecology, and socio-economics of invasive tunicates in Oregon waters. These research needs should be conducted to generate information about the growth, reproductive biology, propagule pressure, dispersal, ecology (susceptibility of competitors and predators), environmental tolerances, genetic stocks, efficacy of control measures, vectors for transport, estimation of ecological and economic damages, and viability of new populations that are particularly relevant to the ongoing invasion of Oregon's bays, estuaries, and marine habitats. In response to a specific emergency, the Invasive Tunicate Committee may provide recommendations to the OISC in support of research activities that may be part of an appropriate response plan (OISC 609-010-0130).

- Education / Outreach: The OISC / Invasive Tunicate Committee should periodically develop and implement a focused series of stakeholder outreach and education activities designed to increase appreciation and awareness of the invasion of Oregon waters by non-indigenous tunicates. These education/outreach activities may include meetings with stakeholders, workshops, production of printed materials, development of fact-sheets and websites, and demonstrations, as well as other outreach activities. In response to a specific emergency, the Invasive Tunicate Committee may provide recommendations to the OISC in support of stakeholder outreach and education activities that may be part of an appropriate response plan (OISC 609-010-0130).

Taking action to eradicate *Didemnum vexillum* from infested bays and estuaries in Oregon at this time is highly complementary to the goals of the Oregon Invasive Species Council (OISC 2014), to the Oregon Department of Fish and Wildlife / Oregon Conservation Strategy (ODFW 2005), and the Oregon Nearshore Strategy (ODFW 2006). The mission of the Oregon Invasive Species Council is to "*protect Oregon's economy and natural resources by conducting a coordinated and thorough effort to keep invasive species out of Oregon and to eliminate, reduce, or mitigate the impacts of invasive species already established in Oregon.*" The goal of the Oregon Conservation Strategy is to "*reduce the scale and spread of priority invasive species infestations*," and the strategy identifies suitable actions and "*early response mechanisms to facilitate swift containment of new introductions using site appropriate tools*." The Oregon Conservation Strategy (2005) goes further and places a "*focus on eradication of invasive species in strategy habitats (estuaries) and other high priority areas where there is a clear threat to ecosystems and a high probability of success*." Similarly, the Oregon Nearshore Strategy seeks to "*promote actions that will conserve ecological functions and nearshore marine resources to provide long-term ecological, economic, and social benefits for current and future generations of Oregonians*." The ODFW Nearshore Strategy also identifies marine invasive species as a priority threat, and calls for "*detailed inventories of native and non-indigenous species by trained taxonomists.*" The Nearshore Strategy specifically highlights the need for new research that focuses on suspected introduced coastal species, on non-native species already introduced to other coastal environments of the world that could be introduced to the Oregon coast, and the need for process-oriented research to improve the understanding of population lags among invading and native species. Development and implementation of an integrated management approach and control strategy for *D. vexillum* and other invasive tunicates will help protect Oregon's coastal ecosystems and prevent economic damages to coastal communities. In addition, careful documentation and follow-up of any deliberate management measures will also generate valuable data and information that will contribute to the management and control of tunicate invasions in other locations along the west coast and in other regions of the world.

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Format: This pest risk assessment is based on the format used by the Exotic Forest Pest Information System for North America. For a description of the evaluation process used, see Step 3 – Pest Risk Assessment under Guidelines at:
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