

Biological Synopsis of the Invasive Tunicate *Didemnum* sp.

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DIDEMNUM sp.**

by

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ABSTRACT

Daniel, K.S., and Therriault, T.W. 2007. Biological synopsis of the invasive tunicate *Didemnum* sp. Can. Manuscr. Rep. Fish. Aquat. Sci. 2788: vi + 53 p.

The invasive tunicate *Didemnum* sp. has been reported in Canadian waters and has the potential to negatively impact native flora and fauna. This biological synopsis has been prepared to aid in the risk assessment which should be completed in 2006. There is considerable uncertainty in *Didemnum* taxonomy making it impossible to confirm the identity of the species invading Canadian waters. Genetic analyses should be undertaken to resolve the current taxonomic debate around *Didemnum*. *Didemnum* sp. is believed to be native to Japan and has been found in Canada, France, New Zealand, the Netherlands, and the United States. In British Columbia, limited surveys have found *Didemnum* sp. in the Strait of Georgia and at sites along the west coast of Vancouver Island. This tunicate possesses several traits that likely enhance its invasion success including its ability to grow and sexually reproduce quickly, smother competing or co-occurring organisms, and its tolerance of a wide variety of environmental conditions. *Didemnum* sp. has the potential to negatively impact water quality, macrophytes, invertebrates, fishes, and aquaculture facilities. This report summarizes available information on *Didemnum* sp. including a taxonomic description, biological characteristics, its distribution (native and non-native ranges), and potential impacts of its invasion.

RÉSUMÉ

Daniel, K.S., et Therriault, T.W. 2007. Biological synopsis of the invasive tunicate *Didemnum* sp. Can. Manuscr. Rep. Fish. Aquat. Sci. 2788: vi+ 53 p.

Des tuniciers envahissants du genre *Didemnum* ont été signalés dans les eaux canadiennes. Ces animaux peuvent affecter la flore et la faune indigènes. Ce synopsis biologique vise à faciliter l'évaluation des risques, qui devrait être réalisée en 2006. Une grande incertitude entoure la taxinomie des *Didemnum*, ce qui empêche de confirmer l'identité des espèces qui envahissent les eaux canadiennes. Des analyses génétiques doivent être menées pour clore le débat taxinomique entourant les *Didemnum*. Les espèces du genre *Didemnum* sont réputées être indigènes au Japon. On en trouve au Canada, en France, en Nouvelle-Zélande, aux Pays-Bas et aux États-Unis. En Colombie-Britannique, peu de relevés ont trouvé des espèces de *Didemnum* dans le détroit de Georgia et dans des sites le long de la côte ouest de l'île de Vancouver. Les tuniciers de ce genre possèdent plusieurs caractères qui améliorent probablement leur capacité d'invasion, notamment la capacité de croître et de se reproduire sexuellement rapidement, la capacité d'étouffer les organismes compétiteurs ou sympatriques et la tolérance à une grande variété de conditions environnementales. Les *Didemnum* peuvent nuire à la qualité de l'eau, aux macrophytes, aux invertébrés, aux poissons et aux stations aquacoles. Ce rapport résume l'information existante sur les *Didemnum* (description taxinomique, caractéristiques biologiques, répartition des espèces indigènes et exotiques, impact potentiel de l'invasion).

1.0. INTRODUCTION

Aquatic invasive species (AIS) pose an enormous risk to native biodiversity and can compromise ecosystem function (e.g., Sala *et al.* 2000). In addition, once AIS become established there are often significant economic impacts, as is the case for biofouling organisms like mussels or tunicates. For example, in the Great Lakes zebra mussels (*Dreissena polymorpha*) have fouled municipal and industrial water intake pipes that now require substantial cleaning. In the marine environment, invasive tunicates are responsible for fouling aquaculture gear and product.

Currently six invasive tunicate species are known in Canadian waters. Some are solitary (e.g., *Styela clava*, *Molgula* sp., *Ciona intestinalis* on the east coast of Canada) while others are colonial (e.g., *Botryllus schlosseri*, *Botrylloides violaceus*, *Didemnum* sp.). Further, most of the invasive tunicate species have been identified in both Atlantic and Pacific Canadian waters. Invasive tunicates are not restricted to Canadian waters, however, as tunicate invasions have become a global concern and in April 2005 a conference was held to discuss invasive tunicates worldwide (Bailey and Carman 2005). *Didemnum* sp. was identified as a species of concern and has been reported in British Columbia, Canada. The smothering capabilities of *Didemnum* sp. and other invasive tunicate species have potential impacts on aquatic macrophytes, invertebrates, water quality and fishes. These impacts will be discussed in the Impacts Associated with Introductions section (see below).

The Family Didemnidae remains taxonomically complex and controversial. There is no general consensus on what species of *Didemnum* is invading coastal waters worldwide (e.g., Valentine 2003, Cohen 2005). Kott (2002) described *Didemnum* collected from waters of New Zealand as new species *D. vexillum* and reports this species as being indigenous to New Zealand despite going unnoticed until collections made late in 2001. In contrast, Coutts (2002) identified *D. vexillum* as an "unwanted exotic organism" introduced to New Zealand waters. Coutts (2002) presented both Kott's view that the tunicate is native and Gretchen Lambert's view that it is a non-native and may originate from Japan. Moreover, the New Zealand Mussel Industry has deemed *D. vexillum* a serious biosecurity risk despite the disagreement about its origin (Coutts and Sinner 2004). Currently, there are no reports of *D. vexillum* outside of New Zealand. Similar controversy surrounds the *Didemnum* species reported from Portsmouth Harbour, New Hampshire, also described as a new species *D. vestum* by Kott (2004), who claims it is native to the North Atlantic. Kott (2004) reported *D. vestum* was conspecific to *D. candidum* whose range extends from the New England coast to the Gulf of Mexico. However, Gretchen Lambert (pers. comm.) believes that this *Didemnum* sp. is identical to *D. vexillum* and may be native to Japan, with one possible type-location at Ise Bay, near Nagoya. Molecular tools might allow confirmation of species identity but until such genetic analyses have been completed we hypothesize that the invasive *Didemnum* species reported from Canadian waters originated in Japan. For clarity we will refer to this invasive species as *Didemnum* sp. which is different from native *Didemnum* species previously reported from Canadian waters (Table 1).

1.1. NAME AND CLASSIFICATION

After Valentine (2003), Cohen (2005) and Bullard *et al.* 2007:

Kingdom: Animalia
Phylum: Chordata
Subphylum: Tunicata
Class: Ascidiacea
Order: Aplousobranchia
Family: Didemnidae
Genus and species: *Didemnum* sp.

Common English Name: colonial tunicate, sea squirt, ascidian, the blob

Common French Name:

Similar Species: *Didemnum carnulentum*, *Didemnum albidum*, *Trididemnum opacum*, *Didemnum candidum*

1.2. DESCRIPTION

Didemnum sp. (Figure 1) shares many characteristics common among colonial tunicate species. Characteristics such as colony shape and colour, where the colony grows, zooid structure, and spicule shape have been used previously to separate and identify different *Didemnum* species with varying levels of success. However, Monniot *et al.* (1991) suggest colony form, colour and consistency only help with the identification of the most distinct didemnids and more advanced tools are required to separate very similar species.

Didemnum sp., like all *Didemnum* species, is characterized by: many small zooids, 1-2 mm in length, embedded in a sheet-like, gelatinous matrix called a tunic or test (Berrill 1935, Van Name 1945, Berrill 1950, Lambert 1974, Goodbody 1974, Kott 1989, Monniot *et al.* 1991, O'Clair and O'Clair 1998, Kott 2001, Tyree 2001, Pederson 2004, Cohen 2005, Lambert 2005, Lambert and Lambert 2005). Colonies form thin encrusting sheets or irregularly lobed encrusting mounds (Berrill 1935, Van Name 1945, Berrill 1950, Lambert 1974, Lambert *et al.* 1987, Kott 2001 and 2002, Mather 2002, Valentine 2003, Cohen 2005, Geerlofs and Gordon 2005). White, calcareous, stellate spicules are embedded within the tunic's surface among zooids that contain individual oral siphons while atrial siphons discharge into a common cloacal aperture maintained in deep crevices within the colony (Berrill 1935, Van Name 1945, Berrill 1950, O'Clair and O'Clair 1998, Kott 1989, Monniot *et al.* 1991, Tyree 2001, Kott 2002, Mather 2002, Kott 2004, Cohen 2005, Lambert and Lambert 2005). Didemnid spicules are 40 µm in diameter on average, but can reach 100 µm (Monniot *et al.* 1991). This rather unique combination of spicules and zooids give the tunic's surface an overall appearance

described as “small, white dots and pinhole-sized pores” (Geerlofs and Gordon 2005a and 2005b).

Due to the small size of individual zooids within a colony, most *Didemnum* species characteristics must be viewed microscopically. These include: zooids divided into a thorax containing the zooid apertures and branchial sac, and an abdomen containing the digestive and reproductive organs and the heart (Berrill 1935, Van Name 1945, Berrill 1950, Monniot *et al.* 1991, O’Clair and O’Clair 1998, Lambert and Lambert 2005); thorax with 4 rows of stigmata (Van Name 1945, Berrill 1950 and 1951, Gosner 1971, Lambert 1974, Lambert *et al.* 1987, Monniot *et al.* 1991, Kott 2001, Lambert and Lambert 2005); sperm duct coiled around testis (Kott 2001 and 2004, Monniot *et al.* 1991); six lobes of branchial aperture (Monniot *et al.* 1991); one or two vesicles in the testis (Monniot *et al.* 1991); cloacal aperture just a broad opening in dorsal mantle (Monniot *et al.* 1991); and no vascular ampullae in the tunic (Lambert and Lambert 2005).

Some have suggested *Didemnum* sp. can be separated from other similar, often co-occurring colonial tunicates by examination of colony shape. Descriptions of *Didemnum* sp. colony shape include: long, ropey, or beard-like colonies; low, undulating mats with short appendages that encrust or drape; sponge-like colonies that move like macro-algal fronds in the currents; extensive, thin sheets overgrowing themselves and other epibionts; or flexible, irregular, long, flat, frond-like branched outgrowths projecting from the surface of the colony (Coutts 2002, Kott 2002, Valentine 2003, Cohen 2005, Geerlofs and Gordon 2005a and 2005b). However, it should be noted that *Didemnum* sp. colony shape changes with age. Young *Didemnum* sp. colonies usually present as thin mats (Geerlofs and Gordon 2005a and 2005b) but as the colony matures, irregular lobes are formed, thereby greatly increasing surface complexity (Valentine 2003, Kott 2004, Geerlofs and Gordon 2005a and 2005b).

Didemnum sp. colonies vary in colour from tan, cream, yellow, orange, or pinkish (Van Name 1945, Gosner 1971, Coutts 2002, Kott 2002 and 2004, Cohen 2005, Geerlofs and Gordon 2005a and 2005b, Lambert and Lambert 2005, Valentine *et al.* 2005a). During April and May, when *Didemnum* sp. are dormant, colonies appear as small patches on the undersides of rocks such as observed in Sandwich, Maine (Valentine *et al.* 2005a). As *Didemnum* sp. colonies mature they became pale pink, pale yellow or pale orange in colour (Valentine *et al.* 2005a). Colony colour does not indicate ascidian genus because it can vary among colonies or colony location (Monniot *et al.* 1991), possibly due to local environmental conditions or available trace elements.

Little is documented in regards to the size of *Didemnum* sp. colonies. In New Zealand, *Didemnum* sp. averaged 22 cm in circumference and 50-100 cm in length, with some colonies as long as 220 cm (Coutts 2002). On Georges Bank, in the United States, *Didemnum* sp. colonies covered 103.6 km² (40 square miles) in 2003 and 2004 (Valentine *et al.* 2005b). By 2005 *Didemnum* sp. covered 259 km² (100 square miles) of Georges Bank (Cohen 2005). The 2006 summer cruise found that *Didemnum* sp.

colonies had doubled at 75% of the survey sites (Dawn Sephton, pers. comm.), but had not spread into Canadian waters on Georges Bank.

Larval ascidians are brooded in the tunic and released as swimming non-feeding tadpoles (Berrill 1950, Millar 1971, Abbott 1975, Svane and Young 1989, Morgan 1995, Jeffery 1997, O'Clair and O'Clair 1998). Key characteristics of *Didemnum* larvae are the presence of a tubular notochord composed of 40 discoidal cells, dorsal central nervous system, locomotory tail, a head, three rows of incompletely developed stigmata, and three adhesive papillae (Berrill 1950, Berrill 1955, Cloney 1982, Monniot *et al.* 1991, Jeffery 1997, O'Clair and O'Clair 1998, Kott 2001 and 2004, Lambert 2005a). Colonial ascidian larvae are usually large. *Botrylloides violaceus* tadpoles are more than 1 mm in length; *Didemnum* sp. unhatched larvae are about 600-700µm in length (Lambert and Lambert 2005).

Similar species found on the Pacific Coast of North America include *D. carnulentum*, *D. albidum* and *Trididemnum opacum* (Van Name 1945, Cohen 2005, Lamb and Hanby 2005). On the Atlantic coast of North America *D. candidum* is similar to the invasive *Didemnum* sp. (Van Name 1945, Smith 1964, Kott 2004). Table 1 details the differences between *Didemnum* sp. and similar native species found on both coasts of North America.

2.0. BIOLOGY AND NATURAL HISTORY

2.1. AGE AND GROWTH

Colonial ascidians typically live from 1 to 3 years, generally developing during the first year followed by breeding and death in subsequent years (Berrill 1950, O'Clair and O'Clair 1998). Age determination is difficult due to the periodic regeneration and reduction the colony undergoes (Millar 1971, Berrill 1951, Tyree 2001). Regeneration is typically accomplished by younger zooids while older zooids typically undergo tissue disintegration, resorption and autolysis (Berrill 1951, Birkeland *et al.* 1981).

Didemnum sp., like most invasive tunicates, is capable of rapid growth (Bak *et al.* 1996, Kott 2001, Tyree 2001, Lambert 2002, Valentine 2003, Cohen 2005, Lambert 2005b, Pederson *et al.* 2005). Under experimental conditions, *Didemnum* sp. colony fragments 5-9 cm² grew rapidly by budding; increasing in size 6- to 11- fold in the first 15 days (Valentine *et al.* 2005a). However, many colonial tunicates have reduced growth rates as the colony grows (Tyree 2001). Factors such as season, temperature, and habitat type each affect the extent of *Didemnum* sp. colony growth. *Didemnum* sp. rapidly grew from July to September in a tidepool in Sandwich, Maine (Valentine *et al.* 2005a). During winter months growth is very slow or nonexistent, with the colony reducing mass to a dormant bud that awaits favourable conditions before growth is re-initiated (Millar 1971, Nakauchi and Kawamura 1990, Monniot *et al.* 1991). In laboratory experiments, however, *Didemnum* sp. grew faster at cooler conditions (ambient water temperature or below) than temperatures above ambient (McCarthy 2005). Habitat type

also affects *Didemnum* sp. colony growth with faster growth reported from open coastal habitats due to an apparent competitive advantage over other co-occurring species that can become overgrown by the colony (Osman and Whitlatch 2005). As with other invasive species, *Didemnum* sp. is highly opportunistic and will colonize free space or grow over animals, substrate and plants, and the colonies can either fuse or reject each other when they meet (Monniot *et al.* 1991, Carman 2005, Lambert 2005a, Osman and Whitlatch 2005, Valentine *et al.* 2005a). When a *Didemnum* sp. colony regresses the potential exists that other species can invade the now vacated space (Birkeland *et al.* 1981). This regression may be seasonal occurring during the winter months when the tunicate colony dies back.

Colony growth is achieved via asexual reproduction or propagative budding (Berrill 1955, Millar 1971, Abbott 1975, Berrill 1975, Nakauchi 1982, Stoner 1989, Nakauchi and Kawamura 1990, Kott 2001, Tarjuelo and Turon 2004, Bates 2005, Cohen 2005, Valentine *et al.* 2005b). The first individual of a colony is an oozoid which begins to bud soon after it settles (Monniot *et al.* 1991). The new bud forms one or more genetically identical individuals called blastozooids (Monniot *et al.* 1991). During the budding process didemnids form two kinds of buds, thoracic and abdominal, from a single zooid (Figure 2)(Nakauchi 1982, Monniot *et al.* 1991, Kott 2001). The abdominal half of the original zooid grows a new thorax and the thorax of the original zooid forms a new abdomen (Berrill 1951, Nakauchi 1982, Monniot *et al.* 1991, Kott 2001). As the two buds grow they separate (Monniot *et al.* 1991). Unlike other ascidians, didemnids can bud while the gonads are maturing (Monniot *et al.* 1991). Didemnidae also undergo precocious budding where blastozooids are produced in the larvae within the tunic (Kott 2001).

2.2. PHYSIOLOGICAL TOLERANCES

Didemnum sp. tolerates a wide range of environmental conditions including temperature, salinity, and water quality (Millar 1971, Lambert and Lambert 1998, Lambert 2002, Lambert 2005b). *Didemnum* sp. colonies are found in water temperatures ranging from -2 °C - 24 °C (Cohen 2005, Lambert 2005b, Valentine *et al.* 2005a). In the United States (US) *Didemnum* sp. has tolerated daily water temperature fluctuations of 9 °C (Valentine *et al.* 2005a). Ascidians tend to hibernate, die off, or go dormant when temperatures are not favourable and resume growth and reproduction when favourable conditions return (Millar 1971, Nakauchi and Kawamura 1990, Monniot *et al.* 1991). For example, when exposed to 6 °C water, typically during October to March, *Didemnum* sp. colonies tend to die off or become dormant (Gittenberger 2005, Valentine *et al.* 2005a). In the Netherlands temperature is thought to be the main cause for the population increase in 1998. In 1997 the minimum water temperature was -2 °C and after 1997 the minimum water temperature was 4 °C (Gittenberger 2005). Thus, it appears that colder winter temperatures were limiting colony size. In contrast to nearshore *Didemnum* sp. colonies, the Georges Bank colony is found in 4-15 °C water and does not die off during the winter months (Valentine *et al.* 2005a), possibly because temperatures don't become cold enough to initiate the dormancy period. This

hypothesis is supported by a colony in Long Island Sound where *Didemnum* sp. is most abundant in deeper water sites with slightly cooler summer water temperatures (McCarthy 2005). Based on colonies in the Netherlands and the US, it appears that 4 °C may be a critical temperature where cooler temperatures limit spread or growth, and warmer temperatures enhance spread or growth of *Didemnum* sp. colonies.

Didemnum sp., like most ascidians, is rarely found in salinities less than 25‰; however, they can tolerate wide fluctuations in salinity (Millar 1971, Vázquez and Young 2000, Lambert 2005b). In San Francisco Bay *Didemnum* sp. has not been found in salinities less than 26‰ (Cohen 2005). At salinities lower than 20‰ ascidians close their siphons which eventually can lead to zooid death (Stoner 1992, Tyree 2001). In areas where the water is affected by heavy rains and has a large freshwater lens the intertidal tunicates disappear (Monniot *et al.* 1991). Ascidian larvae also are affected by salinity. Ascidian larvae will not enter a halocline but instead sink away from the freshwater layer (Vázquez and Young 1996). In low salinity waters colonial tunicate larvae do not metamorphose or metamorphose slowly, resulting in reduced survival (Vázquez and Young 2000).

Non-indigenous ascidians tend to tolerate all types of water quality, yet do not tolerate extended periods of air exposure (desiccation). Many ascidians are tolerant of high water pollution, high particulate and dissolved organic matter, and may even consume organic pollutants like heavy metals and hydrocarbons (Monniot *et al.* 1991, Tyree 2001, Lambert 2005b). *Didemnum* sp., however, was only present in fair water quality and moderate nitrogen levels in Massachusetts waters during 2003 and 2004 (Carman *et al.* 2005). Moreover, areas with very heavy sedimentation cause ascidians to close their oral siphons to prevent the siphon and branchial filtering wall from clogging and ceasing respiration to avoid suffocation (Monniot *et al.* 1991, Tyree 2001). Moreover, when exposed to air for more than 3 hours per day for 28 consecutive days *Didemnum* sp. died (Valentine *et al.* 2005a).

2.3. REPRODUCTION

Didemnum sp., like all Didemnidae, are hermaphroditic and ovoviviparous (Van Name 1945, Berrill 1950, Millar 1971, Abbott 1975, Berrill 1975, Svane and Young 1989, Monniot *et al.* 1991, Osman and Whitlatch 1995a, Lambert and Lambert 1998, Kott 2001, Tyree 2001, Lambert 2002, Tarjuelo and Turon 2004, Cohen 2005, Lambert 2005a, Lambert and Lambert 2005). Sperm are released into the sea out the atrial siphon, travel into the oral siphon of another zooid and fertilize the egg internally (Monniot *et al.* 1991, O'Clair and O'Clair 1998, Kott 2001, Lambert and Lambert 2005). The egg is released from the abdomen into the tunic for fertilization and the larvae brood in the tunic below the zooid (Figure 2) (Millar 1971, Abbott 1975, Berrill 1975, Svane and Young 1989, Monniot *et al.* 1991, Osman and Whitlatch 1995a, Lambert and Lambert 1998, Kott 2001, Tyree 2001, Lambert 2002, Tarjuelo and Turon 2004, Cohen 2005). Once the tadpole larvae are fully developed they are released from the colony

into the common cloacal cavities and then into the water column (Millar 1971, Svane and Young 1989, Tyree 2001).

The eggs in the ovary grow to full size, developing one at a time, with a typical zoid producing 1-20 eggs (Berrill 1950, Lambert and Lambert 2005). Eggs can mature within the colony in several weeks (Lambert and Lambert 2005). *Didemnum* sp. has 1 testis lobes, covered by a coiled sperm duct with 9-10 coils (G. Lambert, pers. comm.). There are conflicting reports on the location of the Didemnidae gonads. Monniot *et al.* (1991) and Berrill (1950) believe the gonads are contained in the gut loop; whereas, O'Clair and O'Clair (1998) state developing gonads are beside the gut loop.

Ascidian gonad development and spawning are controlled by several factors but light and temperature, which change seasonally, appear most important (Millar 1971, Berrill 1975, Svane and Young 1989, Forward *et al.* 2000, Bates 2005, Lambert 2005a). The end of the breeding season is not linked to a critical temperature (Millar 1971) and spawning typically coincides with the period of maximum food production (Lambert 2005b). The preferred conditions of light, temperature, and food concentration needed for *Didemnum* sp. to maximize spawning are not currently known.

The time of year ascidians spawn depends on location. In Alaska, ascidian spawning is thought to be limited to the spring or summer (O'Clair and O'Clair 1998). For temperate ascidian species spawning typically occurs during the summer and in the fall for tropical species (Cohen 2005, Lambert 2005b). Berrill (1975) believed the typical spawning season was August to September which may be extended from May to October, depending on the species and its location.

Svane and Young (1989) identified water turbidity, depth, cloud cover, season, and other factors controlling underwater irradiance as factors that control larval release. *Didemnum* larvae tend to be released in the morning, after a period of darkness, ensuring the larvae are settled before it is dark (Olson 1983, Svane and Young 1989, Hurlbut 1992, Morgan 1995). Also, it has been suggested that the environmental conditions larvae use as settling cues vary during the day (Hurlbut 1992).

Once the larvae are released they only swim in the water column for minutes to hours before settling (Berrill 1955, Svane and Young 1989, Monniot *et al.* 1991, Hurlbut 1992, Morgan 1995, Osman and Whitlatch 1995a, O'Clair and O'Clair 1998, Tarjuelo and Turon 2004, Cohen 2005, Lambert and Lambert 2005, Valentine *et al.* 2005b). The short swimming period may limit predation on the larvae or currents sweeping the larvae away thereby increasing the probability larvae will find similar habitat as the parent colony to settle on nearby (Monniot *et al.* 1991).

Once released from the adult colony the larvae swim towards the light in a positively phototactic and negatively geotactic response (Berrill 1955, Millar 1971, Berrill 1975, Olson 1983, Hurlbut 1991, Monniot *et al.* 1991, Hurlbut 1992 and 1993, Morgan 1995, Oren and Benayahu 1998). This is followed by a period when the larvae move away from the light, in a negatively phototactic and positively geotactic response that

culminates in settling on the undersides of substrates (in the shade) (Berrill 1955, Millar 1971, Berrill 1975, Olson 1983, Hurlbut 1991, Monniot *et al.* 1991, Hurlbut 1992 and 1993, Morgan 1995, Oren and Benayahu 1998). During July to October 2003 in Long Island Sound, USA, the highest density of *Didemnum* sp. larval settlement on *in situ* sampling devices occurred during the day and into early evening (0900-1500 and 1500-2100) while the lowest settlement occurred during the morning and overnight (0300-0900 and 2100-0300) (Bullard *et al.* 2005). Furthermore, *Didemnum* sp. larval settlement was not affected by lunar or tidal cycles (Bullard *et al.* 2005). Possible benefits of larvae settling in shaded areas include protection from direct sunlight and less sedimentation in downward facing locations (Hurlbut 1993).

Once the larvae have settled they undergo metamorphosis and begin exogenous feeding. Metamorphosis involves a series of steps to transform the motile, non-feeding larvae into a non-motile, feeding individual (Cloney 1982, Burke 1983). Ascidian metamorphosis has been described in detail by Cloney (1982) and includes the following steps: “(1) Secretion of adhesives by the papillae or the epidermis of the trunk, (2) Eversion and retraction of papillae, (3) Resorption of the tail, (4) Loss of the outer cuticular layer of the larval tunic, (5) Emigration of blood cells or pigmented cells (many species), (6) Rotation of organs through an arc of about 90°; expansion of branchial basket; elongation of oozoid or juvenile, (7) Expansion, elongation or reciprocation of ampullae; reorientation of test vesicles; expansion of the tunic, (8) Retraction of the sensory vesicle, (9) Phagocytosis of visceral ganglion, sensory organs and cells of axial complex, and (10) Release of organ rudiments from an arrested state of development.”

2.4. FEEDING AND DIET

Prior to settlement, ascidian tadpole larvae, including *Didemnum*, do not actively feed (Berrill 1955, Jeffery 1997, Monniot *et al.* 1991, Tyree 2001, Lambert and Lambert 2005). Following settlement in areas of high suspended organic particles, *Didemnum* sp. become sessile marine filter feeders (Berrill 1975, Monniot *et al.* 1991, Bak *et al.* 1998, Lambert and Lambert 1998, Tyree 2001, Lambert 2001, Lambert 2002, Cohen 2005). After larval metamorphosis the diet consists primarily of phytoplankton, suspended particulate matter, diatoms, and suspended bacteria (Millar 1971, Monniot *et al.* 1991, Bak *et al.* 1998, O’Clair and O’Clair 1998, Tyree 2001). Particles as small as 1 micron are captured in the filtering feeding process (Tyree 2001) with factors such as the amount of food in the water, time spent filtering, food particle size, and filtering capacity determining how much food is obtained (Millar 1971, Kott 1989). Sediment falling into the oral siphons slows filtering efficiency (Kott 1989, Monniot *et al.* 1991) while for colonial species, increasing the number of zooids in a colony increases filtering efficiency (Tyree 2001).

Ascidians are capable of filtering large amounts of water to obtain food. Filtration rates range from 2-3 litres of water per hour to 18.5 litres of water per hour for a typical, solitary ascidian depending on size (Monniot *et al.* 1991, O’Clair and O’Clair

1998). Filtering capacity of colonial ascidians like *Didemnum* sp. is difficult to determine and remains unknown.

Ascidians filter feed by drawing water in the oral siphon with beating ciliated cells located on the rows of stigmata on the lateral walls of the branchial sac (Millar 1971, Goodbody 1974, Monniot *et al.* 1991, O'Clair and O'Clair 1998, Kott 1989 and 2001, Tyree 2001, Bone *et al.* 2003). Ascidians filter all suspended particles in the water column while feeding (Tyree 2001). Any large particles are removed by tentacles at the entrance to the branchial sac or by squirting (Goodbody 1974, Monniot *et al.* 1991, O'Clair and O'Clair 1998). The endostyle, found along the ventral margin of the branchial sac, produces mucus which covers the stigmata and traps food particles as the water passes through (Monniot *et al.* 1991, O'Clair and O'Clair 1998). The mucus is then wound into a strand and moved down into the esophagus and gut for absorption (Goodbody 1974, Monniot *et al.* 1991, O'Clair and O'Clair 1998). Finally, the water passes out through the atrial opening and the food waste exits the anus near the atrial opening (O'Clair and O'Clair 1998).

2.5. HABITAT

Didemnum sp. is a successful fouling organism due to its ability to grow on many natural and artificial structures. This tunicate grows on hard substrates or surfaces, and does not grow on mud or sand bottoms without gravel or cobble that prevent moving sediment (Berrill 1955, Coutts 2002, Cohen 2005, Valentine *et al.* 2005b). *Didemnum* sp. grow on natural substrates such as rock outcrops, gravel seabeds, pebbles, cobble, boulders, tunicates, sponges, macroalgae, hydroids, anemones, bryozoans, polychaetes, scallops, mussels, oysters, limpets, barnacles, other ascidians, shell, and hard clay with stones (Berrill 1950 and 1955, Monniot *et al.* 1991, Lambert 2002, Valentine 2003, Cohen 2005, Gittenberger 2005, Valentine *et al.* 2005a and 2005b). *Didemnum* sp. can colonize artificial structures including docks, floats, wood and metal pilings, moorings, rope, steel chain, automobile tires, plastic, ship hulls, buoys, jetties, concrete, iron, and wood (Millar 1971, Monniot *et al.* 1991, Tyree 2001, Lambert 2002, Valentine 2003, Cohen 2005, Geerlofs and Gordon 2005a and 2005b).

Didemnum sp. is found at depths from the intertidal zone to 65 m (Valentine 2003, Cohen 2005). On Georges Bank *Didemnum* sp. is found on pebble and cobble pavement at depths of 45-60 m and colonies are bound by moving sand ridges (Valentine *et al.* 2005b). Geerlofs and Gordon (2005b) report *Didemnum* sp. may be found at 6-12 m depths on hard substrates such as rock walls. During aquaculture site surveys in British Columbia in October and November 2005, *Didemnum* sp. was found at depths of 0.5-6 m on culture gear (Debbie Palzat, pers. comm.).

There is conflicting information about whether colonial ascidians tolerate low or high wave action. Osman and Whitlatch (2005) noted that *Didemnum* sp. along the eastern United States preferred the open coast and offshore deeper waters over protected embayments, harbours and marinas though it also thrives in protected areas

with reduced wave action and enhanced nutrient levels (Lambert 2002, Bullard *et al.* 2007).

2.6. INTERSPECIFIC INTERACTIONS

The sessile nature of *Didemnum* sp. does not allow it to physically move to escape predation so they have developed chemical and physical defences. The primary chemical defence is the acidic tunic (Stoecker 1980a and 1980b, Carman 2005). The tunic has a pH of 1-2 and acid is released from bladder cells when the ascidian is injured (Stoecker 1980a and 1980b, Carman 2005). Chemicals like didemnins reduce the palatability of ascidian adults (Lindquist *et al.* 1992, Tarjuelo *et al.* 2002, Joullié *et al.* 2003). Physical defence in adult ascidians is provided by the spicules in the tunic and the tunic itself (Kott 1989). In addition to protection from predation and wave action the tunic anchors the colony to the substrate and prevents the collapse of internal organs (Monniot *et al.* 1991). Lindquist *et al.* (2002) suggest that the stellate spicules may be more for defence against the elements than against predation. However, others believe spicules in the tunic reduce palatability to predators (Tarjuelo *et al.* 2002). Ascidian larvae avoid predation by quickly settling on preferred habitat, reduced pigmentation, brooding inside the adult colony's tunic, and chemical defence reducing its palatability (Kott 1989, Lindquist *et al.* 1992, Tarjuelo *et al.* 2002, Joullié *et al.* 2003).

Didemnum sp. has few known predators possibly due to the low nutritive value of the tunic (Tarjuelo *et al.* 2002, Lambert 2005b), but probably primarily due to noxious secondary metabolites. The common periwinkle, *Littorina littorea*, preyed upon a dying *Didemnum* sp. colony between October and March on the east coast of the United States (Valentine 2003, Carman 2005, Valentine *et al.* 2005a). In New Zealand, chiton, sea urchins and sea stars have preyed upon *D. vexillum* (Valentine 2003). Other probable predators of *Didemnum* sp. include general ascidian predators such as fish, flatworms, polychaete worms, echinoderms (mainly sea stars), gastropods, seals, and other cetaceans (Millar 1971, Monniot *et al.* 1991).

2.7. BEHAVIOUR AND MOVEMENTS

Larval dispersal is affected by wave action, wind exposure, direction of sun to the reef, and water temperature (Hurlbut 1992, Stoner 1992, Forward *et al.* 2000). Currents can change frequently causing the larvae to be swept away from the parent colony (Forward *et al.* 2000). The short time ascidian larvae spend in the water column before settling may limit the impact such factors exert on larval dispersal thereby limiting spread (Hurlbut 1992).

As an adult colony *Didemnum* sp. movement is limited, although the colony can expand through fragmentation or moving along with its habitat. Modes of transport, and possible introduction, include hull fouling of ships and boats, fouling of fishing gear,

fishing trawls, dredges, colony fragments in ballast water, movement of oyster and other shellfish stock or gear, and natural process like currents (Millar 1971, Monniot *et al.* 1991, Lambert 2002, Cohen 2005, Lambert 2005b, Valentine *et al.* 2005b).

2.8. DISEASES AND PARASITES

Little is known about the diseases and parasites specific to *Didemnum* sp. Carman (2005) found 18 species of benthic foraminifera on New Hampshire *Didemnum* sp. samples. All of the benthic foraminifera were dead, possibly due to the tunicate's acidic tunic (Carman 2005).

In general, ascidians have communal, parasitic and symbiotic organisms living in their tunics, branchial chambers, gut tracts, or atrial chambers (Millar 1971, Monniot 1990, Monniot *et al.* 1991, O'Clair and O'Clair 1998, Kott 2001, Tyree 2001). Copepods, amphipods, shrimps, polychaetes, molluscs, decapods, hydroids, algae, nematodes, ciliates, protozoans (gregarines), suctorian ciliates, and pea crab have been found living on or in ascidians (Millar 1971, Monniot 1990, Monniot *et al.* 1991, O'Clair and O'Clair 1998, Kott 2001, Tyree 2001, Coutts 2002). These organisms are thought to do relatively little harm to ascidians, although some parasitic copepods, nemertean worms and decapod crabs could cause minor damage (Monniot 1990, Monniot *et al.* 1991, O'Clair and O'Clair 1998).

3.0. DISTRIBUTION

3.1. NATIVE DISTRIBUTION AND ABUNDANCE

Given the ongoing taxonomic debate concerning species identity, we hypothesize that *Didemnum* sp. may be native to Japan (Cohen 2005, Gretchen Lambert, pers. comm.). One location where possible *Didemnum* sp. has been collected is Ise Bay, near Nagoya (Gretchen Lambert, pers. comm.), but we suspect other native locations exist (Figure 3). Genetic analyses and comparisons with other populations are underway.

In Ise Bay the temperature varies greatly over the year while salinity remains relatively constant. The mean annual water temperature is 19.9 °C at 0 m, with a range between 6.0 °C -31.1 °C (Japan Oceanographic Data Center 2006). The mean annual salinity is 33.41‰ at 0 m with a range between 29.28‰- 34.99‰ (Japan Oceanographic Data Center 2006). Moreover, at the mouth of Ise Bay a thermohaline front forms during the winter (Yanagi *et al.* 1997). During February 1995 the temperature ranged from 14.0 °C at the mouth to approximately 8.5 °C at the head of the bay (Yanagi *et al.* 1997). Salinity ranged between 34.6‰ at the mouth to 32.0‰ at the head during the same period (Yanagi *et al.* 1997).

3.2. NON-NATIVE DISTRIBUTION (EXCLUDING CANADA)

Didemnum sp. has been introduced (excluding Canada) worldwide: Europe, New Zealand, and the United States (Table 2 and Figures 4-10).

In Europe *Didemnum* sp. has been reported in France, Ireland, and the Netherlands. In northern France, *Didemnum* sp. populations were first discovered in 2002; the species has now been reported from numerous sites, although other sites supporting the species could exist (Valentine 2003, Cohen 2005, G. Lambert, pers. comm.). In the Netherlands, the first population was discovered in 1991 and the species has rapidly spread since 1998 to a total of 15 sites, although again, others could exist (Valentine 2003, Cohen 2005, Gittenberger 2005).

In New Zealand *Didemnum* sp. was first discovered in April 2001 in Picton, Marlborough Sound (Coutts 2002, Valentine 2003, Cohen 2005). This ascidian was discovered in Whangamata Harbour on wharf pilings in October 2001 and in December 2001 in Shakespeare Bay on the barge 'Steel Mariner' (Coutts 2002, Valentine 2003, Cohen 2005, Coutts and Taylor 2005). *Didemnum* sp. populations have since been identified in Tauranga Harbour, Waimahara Wharf, and, in January 2006, a second site in Shakespeare Bay (Valentine 2003). Interestingly, despite these rather recent sightings, Kott (2002) described this didemnid as a new species *D. vexillum*, claiming it to be native to New Zealand.

Didemnum sp. has been introduced on both coasts of the United States (Valentine 2003, Cohen 2005, Carman 2005, Bullard *et al.* 2007). The most prominent population of *Didemnum* sp. in the United States is on the Georges Bank fishing grounds first discovered in 2002 (Valentine *et al.* 2005b). With increased sampling the documented coverage has grown from 103.6 km² (40 square miles) in 2003 to 259 km² (100 square miles) in 2005 (Cohen 2005, Valentine *et al.* 2005b). The spread of this species on Georges Bank may have been accelerated due to human-mediated activities such as those associated with fishing trawls and dredges, in addition to natural dispersal of colony fragments (Valentine *et al.* 2005b).

Along the east coast of the United States the first report of *Didemnum* sp. was in the Damariscotta River, Maine, in 1993 (Valentine 2003, Cohen 2005). However, there have been reports of *Didemnum* sp. since the 1970's (Cohen 2005). *Didemnum* sp. distribution has spread to 37 sites in New York, Connecticut, Rhode Island, Massachusetts, New Hampshire, and Maine (Valentine 2003, Carlton 2004, Auker 2005, Carman 2005, Cohen 2005, Pederson *et al.* 2005, Valentine *et al.* 2005a).

Along the west coast of the United States *Didemnum* sp. has been introduced to California and Washington. In California, *Didemnum* sp. was first reported in San Francisco Bay in 1993 (Valentine 2003, Cohen 2005, deRivera *et al.* 2005). *Didemnum* sp. has been found since in California at Half Moon Bay in 1997, Elkhorn Slough – Moss Landing in 1998, Morro Bay and Bodega Harbor in 2000, Humboldt Bay and Tomales Bay (5 sites) in 2001, Port San Luis and Sausalito in 2003, and Horseshoe

Bay and at Fisherman's Wharf in San Francisco Bay in 2004 (Wasson *et al.* 2001, Fairey *et al.* 2002, Valentine 2003, Cohen 2005, deRivera *et al.* 2005, Bullard *et al.* 2007). In 2003, *Didemnum* sp. was discovered at Des Moines marina, Edmonds underwater park, and since 2000 on Totten Inlet mussel rafts within Puget Sound, Washington (Valentine 2003, Cohen 2005, G. Lambert, pers. comm.). *Didemnum* sp. has been found since in Dabob Bay in Hood Canal, Washington (Lambert 2005c). At this time there are no records of *Didemnum* sp. sightings in Oregon and it is not possible to determine if this is due to reduced search efforts or inhospitable conditions for *Didemnum* survival.

3.3. DISTRIBUTION IN CANADA

Didemnum sp. has been found in British Columbia, Canada (Table 3 and Figure 11). The first documented occurrence of the tunicate was Okeover Inlet on mussel cages in 2003 (Valentine 2003, Cohen 2005). *Didemnum* sp. has since been reported from Agamemnon Channel, Pendrell Sound, Jedediah Island, Trevenen Bay, Jarvis Inlet, Deep Bay, Lemmens Inlet, and Lions Rock (Valentine 2003, Cohen 2005). During October and November 2005 informal aquaculture site surveys were conducted to identify potential aquatic invasive species in the Strait of Georgia and the west coast of Vancouver Island. *Didemnum* sp. was present in Okeover Inlet, Deep Bay and Lemmens Inlet as previously reported. In addition, six new locations with *Didemnum* sp. were discovered including Tyee Cove, False Bay, Gorge Harbour; Village Bay, Teakerne Arm, Deep Bay and Nanoose Bay (Debbie Palzat, pers. comm.). Shellfish growers believe *Didemnum* sp. has been at their aquaculture sites long before the first documented sighting in 2003 (Anon. Shellfish Grower). Interestingly, *Didemnum* sp. found on mussel cages in Washington State and British Columbia were owned by the same company (Cohen 2005), but it is not possible to identify which population was the source at this time or whether these are independent invasions.

4.0. USE BY HUMANS

There are no known human uses documented for *Didemnum* sp. It has been suggested that *Didemnum* sp. could be used by the pharmaceutical industry (Gretchen Lambert, pers. comm.). Other ascidians have been used for human consumption, bait, and medicinal purposes. For example, *Pyura chilensis*, *P. praeputialis*, *Microcosmus*, *Halocynthia roretzi*, *H. aurantium*, and *Styela clava* are used for human consumption (Monniot *et al.* 1991, Lambert 2005b). *Pyura praeputialis* also is used as bait in Australia (Monniot *et al.* 1991). *Trididemnum* species and most other didemnids including *Lissoclinum patella* and *L. bistratum* (Family Didemnidae) contain didemnins and other cyclic peptides with a high level of antiviral and anti-cancer activity which are extremely cytotoxic (Monniot *et al.* 1991, Saito 1996).

5.0. IMPACTS ASSOCIATED WITH INTRODUCTIONS

There are a variety of potential impacts associated with introductions, especially biofouling organisms like *Didemnum* sp. Ascidians generally possess traits that allow them to be successful fouling organisms. They are efficient filter feeders, grow fast, reproduce quickly, are opportunistic and can out compete many native organisms, readily colonize both artificial and natural substrates, are tolerant to a wide variety of environmental conditions and have few predators (Berrill 1950, Berrill 1955, Millar 1971, Berrill 1975, Monniot *et al.* 1991, Lambert and Lambert 1998, Tyree 2001, Coutts 2002, Lambert 2002, Valentine 2003, Dunstan and Johnson 2004, Carman 2005, Cohen 2005, Geerlofs and Gordon 2005b, Gittenberger 2005, Lambert 2005b, Osman and Whitlatch 2005, Pederson *et al.* 2005, Valentine *et al.* 2005a and 2005b). Thus, *Didemnum* sp. has many potential impacts on aquatic macrophytes, water quality, plankton, invertebrates, and fishes.

5.1. IMPACTS ON AQUATIC MACROPHYTES

The only known impact of *Didemnum* sp. on aquatic macrophytes is its ability overgrow and smother seaweeds (Tyree 2001, Cohen 2005, Pederson *et al.* 2005).

5.2. IMPACTS ON WATER QUALITY

Colonial tunicates often occur in areas with high levels of suspended organic particles and pollution (Millar 1971, Bak *et al.* 1998, Tyree 2001, Lambert 2005b). It is possible that *Didemnum* sp. could lower suspended organic particles in the water column while filter feeding (Lambert and Lambert 1998).

5.3. IMPACTS ON FAUNA

5.3.1. Plankton

Didemnum sp. consumes plankton as part of its filter feeding diet (Millar 1971, Tyree 2001). Due to the efficiency of filter feeding it is possible that *Didemnum* sp. may change the plankton composition and/or decrease the amount of plankton available to other organisms, including invertebrates and fish (see below) (Lambert and Lambert 1998, Pederson *et al.* 2005).

5.3.2. Invertebrates

The ability to smother other invertebrates is the greatest potential impact of *Didemnum* sp., especially as it pertains to shellfish aquaculture, but also its ability to

rapidly cover large areas of the natural benthos. This colonial ascidian overgrows sponges, hydroids, anemones, limpets, oysters, mussels, scallops, barnacles, bryozoans, corals, coelentrates, other ascidians, and other fouling community species (Birkeland *et al.* 1981, Monniot *et al.* 1991, Tyree 2001, Coutts 2002, Kott 2002, Cohen 2005, Lambert 2005b, Pederson *et al.* 2005, Valentine *et al.* 2005a). Mussel and oyster farms have been negatively affected by *Didemnum* sp. growth with some bivalves losing condition and suffering increased mortality (Lambert and Lambert 1998, Tyree 2001, Coutts 2002, Coutts and Sinner 2004, Auker 2005, Cohen 2005).

In addition to potential competition for food (Lambert and Lambert 1998, Pederson *et al.* 2005), other impacts on invertebrates by *Didemnum* sp. include the prevention of benthic larval settlement (Osman and Whitlatch 1995a, Cohen 2005, Pederson *et al.* 2005), out competing native species for space (Osman and Whitlatch 1995b, Oren and Benayahu 1998, Pederson *et al.* 2005), and changing species composition within fouling communities (Valentine *et al.* 2005b). Valentine *et al.* (2005b) found an increase in the polychaete, *Nereis zonata*, and a slight decrease of the hermit crab, *Pagurus acadianus*, at *Didemnum* sp. sites on Georges Bank in 2003.

5.3.3. Fishes

The spread of *Didemnum* sp. colonies worldwide may have a variety of potential impacts on fish populations. As *Didemnum* sp. grows over native food organisms fish may resort to eating the ascidian, which offers very little nutritional value (Valentine *et al.* 2005b), but more likely will leave the area. The acidic tunic could kill any fish eggs or larval fish settling on *Didemnum* sp. (Osman and Whitlatch 1995a, Cohen 2005, Pederson *et al.* 2005). There also is concern that *Didemnum* sp. could reduce refuges for juvenile rockfish on Georges Bank (Pederson *et al.* 2005). Moreover, there is potential that *Didemnum* sp. could affect salmon aquaculture cages. *Didemnum vexillum* has been found on salmon cages in East Bay, New Zealand, but no negative impacts were noted at the time (Sinner and Coutts 2003, Coutts and Sinner 2004)

5.4. IMPACT SUMMARY

Due to the aggressive colonizing abilities of *Didemnum* sp. it could alter plant, invertebrate and fish communities. Not only does *Didemnum* sp. have negative impacts on invertebrates in the wild, but also those being farmed. As *Didemnum* sp. spreads over large areas, like Georges Bank, native populations are likely to be displaced or lost. More research needs to be completed to fully understand the effects of introduced *Didemnum* sp. on native flora and fauna.

6.0. AREAS OF POTENTIAL RESEARCH

There are many areas which need to be further examined to better understand *Didemnum* sp. worldwide. First and foremost the taxonomy of *Didemnum* sp. needs to be resolved. Given the often contradictory or ambiguous descriptions based on traditional morphological characteristics it seems the taxonomy will only be resolved through the application of molecular tools. More photographic work also should be completed as detailed pictures of *Didemnum* sp. larvae, zooid and spicule structure would aid in identification and taxonomic clarification. Once the taxonomy is resolved such outstanding issues as native range, population structure, and environmental tolerances will be resolved. Moreover, with a known taxonomy a comprehensive guide can be made for the identification of *Didemnum* sp. at future invasion sites.

Further research on *Didemnum* sp. needs to be conducted to improve our knowledge about this invasive tunicate's biology. Possible research questions to examine include: what is the lethal temperature and salinity amounts for larvae and adult colonies, what is the ideal temperature range and amount of light needed for spawning, when does *Didemnum* sp. spawn, how do temperature and salinity changes affect the ascidian's growth, how long does *Didemnum* sp. take to reach sexual maturity, what can be used to control or eliminate *Didemnum* sp., and do the control mechanisms used for *Styela clava* on the east coast of Canada work on *Didemnum* sp.

7.0. SUMMARY

A biological review of the invasive tunicate *Didemnum* sp. has been compiled to aid in the risk assessment for *Didemnum* sp. scheduled for completion in 2006. We have examined the description, distribution, life history, and impacts due to invasion by *Didemnum* sp. Little is known specifically about *Didemnum* sp. biology due to limited research on this tunicate species. The invasion of *Didemnum* sp. has a variety of potential negative impacts on the ocean habitat, native species diversity, and the aquaculture industry.

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Table 1 cont'd

Table 1. Characteristic differences between *Didemnum* sp. and native Didemnidae species found on both coasts of North America. Numbers in brackets refer to authors as listed at the bottom of the table. For the full reference refer to the Literature Cited section.

| Characteristic | <i>Didemnum</i> sp. (2, 8, 14,16) | <i>Didemnum vestum</i> (6, 16) | <i>Didemnum vexillum</i> (5, 16) | <i>Didemnum carnulentum</i> (1, 4, 9, 10, 13, 15, 16) | <i>Didemnum albidum</i> (3, 4, 7, 9, 13, 15, 16) | <i>Trididemnum opacum</i> (1, 9, 15, 16) | <i>Didemnum candidum</i> (1, 3, 9, 11, 12, 13, 15, 16) |
|-----------------------|--|---|---|--|--|---|---|
| Colony Form | - Thin, flat encrusting, large colonies may form long slender lobes - Small, white dots and pinhole-sized pores on tunicate's surface - Meandering dark lines visible on colony surface between zooid groups | - Encrusting or lobed; meandering dark lines visible on colony surface between zooid groups | - Thin encrusting sheets, sometimes overgrow themselves and fuse together Have long flat leaf-, frond-, or flag-like outgrowths or cylindrical processes - Meandering dark lines visible on colony surface between zooid groups | - Thin encrusting colony, 1mm thick - Thin flat colonies less than 6mm high to 12.5mm across - Literature reports meandering dark lines visible on colony surface between zooid groups | - Thin encrusting colony 1-2mm thick - Not over 3mm thick - Occasionally measuring more than 100 m across - No meandering dark lines - Dense spicules in tunic | - Thin encrusting colonies; whitish due to dense spicules in tunic. | - Flat encrusting colony 2-4mm thick, up to 10cm in diameter, irregularly lobed - Borders of colony – thin to thick and rounded - Colony surface sometimes smooth and sometimes wrinkled - 1.5mm thick |
| Colony Colour | - Tan, cream, yellow, orange or pinkish - April and May – colonies small olive green patches under rocks in parts of New England but not elsewhere. | - Pale yellow | - Yellowish cream | - Pink varying to opaque pink, chalky white and opaque, grey tunic | - Yellowish salmon or rosy, opaque whitish or greyish - white usually | - Pale flesh colour tinged with greyish and dotted with whitish specks, greyish or white often tinged with lavender | - White, yellowish, less frequently reddish or red, in turbid water more or less coloured or discoloured as mud. - Uneven white or off-white |
| Distribution | - Native: Japan (?), Introduced: Netherlands, New Zealand, British Columbia, France, Ireland, California, Washington, Eastern US | -Portsmouth Harbour, Newcastle, New Hampshire | - New Zealand – Whangamata Harbour, Queen Charlotte Sound, | - BC to Panama | - Arctic region lying to the north of Europe and Atlantic Ocean - Bering Sea to California - Arctic South to Cape Cod, but in | British Columbia to California | - Atlantic side of North America (New Hampshire – to Brazil), Red Sea to Indian Ocean, Philippine Islands, Palau Islands, Hawaiian Islands, Gilbert Islands, |

Table 1 cont'd

| Characteristic | <i>Didemnum</i> sp. (2, 8, 14,16) | <i>Didemnum vestum</i> (6, 16) | <i>Didemnum vexillum</i> (5, 16) | <i>Didemnum carnulentum</i> (1, 4, 9, 10, 13, 15, 16) | <i>Didemnum albidum</i> (3, 4, 7, 9, 13, 15, 16) | <i>Trididemnum opacum</i> (1, 9, 15, 16) | <i>Didemnum candidum</i> (1, 3, 9, 11, 12, 13, 15, 16) |
|--|---|---|---|---|--|--|---|
| | | | | | shallow water only near northern range - Temperate – north Atlantic and Asia. | | Galapagos Islands - Bay of Fundy south to Brazil - Hawaii |
| Spicules form | stellate | stellate | stellate | Starburst, pointed | Blunt-rayed globular spicules | stellate | stellate |
| Spicule size | To 0.035 mm usually with occasional larger ones | To 0.035mm, usually 0.02mm | Up to 0.058 mm but to 0.035 mm usually | 0.019-0.065 mm | 0.08mm, some not over 0.05mm, 0.05 mm or larger | 0.03 to 0.035 mm | 0.025mm to 0.04 or 0.05mm, 35-48µ - less than 0.02mm 15-20 microns |
| # of Spicule Rays | Very variable, but usually 8-11 conical rays in optical cross section | 5-7, occasionally 9 sharply pointed conical rays in optical cross section | 9-11 conical rays in optical cross section | Short thick rays | Stubby rays, usually bluntly rounded | | 7-9 club shaped or cylindrical rod-like rays |
| Distribution of bladder cells in test | Surface | Surface | Surface | Surface | Surface | Surface | Present in test in varying quantity |
| Branchial siphon Length | | Short | Short | | | | |
| Atrial Languet (Present/Absent) | Absent | Absent | Absent | Absent | Absent | Present | Absent |
| Lateral Organ Form | | Pair of small ventrally directed cups | | | | | |
| Zooid Size | ~ 1-2 mm | 1-2 mm | 1-2 mm | 1-2mm | 1.5 – 1.7mm long | < 2mm | 1 mm, 1.6 mm tot. length |
| # of stigmata | Thorax with 4 rows of stigmata | 6-7 stigmata in anterior row of the branchial sac; 4 rows of stigmata | 4 rows of stigmata, 8 or 9 in the anterior row of the branchial sac | 4 rows of stigmata in branchial sac, with about 6 in each row | Pharynx with 4 rows of stigmata | Pharynx with 3 rows of stigmata, with about 12 in first 2 and apparently 1 or 2 less in the last row | Pharynx with 4 rows with 7 stigmata per row 4 rows with 6 per half row |

Table 1 cont'd

| Characteristic | <i>Didemnum</i> sp. (2, 8, 14,16) | <i>Didemnum vestum</i> (6, 16) | <i>Didemnum vexillum</i> (5, 16) | <i>Didemnum carnulentum</i> (1, 4, 9, 10, 13, 15, 16) | <i>Didemnum albidum</i> (3, 4, 7, 9, 13, 15, 16) | <i>Trididemnum opacum</i> (1, 9, 15, 16) | <i>Didemnum candidum</i> (1, 3, 9, 11, 12, 13, 15, 16) |
|---|--------------------------------------|-----------------------------------|--------------------------------------|--|---|---|---|
| # of vas deferens coils | 8-10 | 8 | 9 | | | | 7, proximal part of vas deferens coils 4-5 to 8-9 times. |
| Gut Length | | | | | | | |
| Flexure of posterior pyloric of loop | | | Flexed ventrally forming double loop | | | | |
| # of larvae ectodermal ampullae | 6 pairs | 6 pairs | 6 pairs | | | | 4 pairs |

| | |
|--|--|
| <ol style="list-style-type: none"> 1. Abbott D.P. 1975. 2. Cohen, A.N. 2005. 3. Gosner, K.L. 1978. 4. Harbo, R.M. 1999. 5. Kott, P. 2002. 6. Kott, P. 2004. 7. Kozloff, E.N. 1974. 8. Lambert G. and Lambert, C. 2005. | <ol style="list-style-type: none"> 9. Lambert, C.C., Lambert, G., and Kozloff, E.N. 1987. 10. O'Clair, R.M., and O'Clair, C.E. 1998. 11. Smith, R. I. (ed). 1964. 12. Tokioka, T. 1967. 13. Tyree, S. 2001. 14. Valentine, P.C. 2003. 15. Van Name, W.G. 1945. 16. G. Lambert, pers. comm. |
|--|--|

Table 2. *Didemnum* sp. records for France, the Netherlands, New Zealand, and the United States. (Valentine 2003, Cohen 2005, DeRivera *et al.* 2005, Lambert 2005c). The majority of the information, including table format, is from the USGS website last accessed March 6, 2006 (Valentine 2003). Refer to Figures 4 to 10 for corresponding maps.

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|----------------------------|-------------|---------|-----------------|-------------------|---|---|----------------|
| FR-1 | Brest | Brest | FR | <1 | | marina floating dock | Marina floating dock, Brest. Aug. 28, 2005. Identified by G. Lambert, 2005. | Valentine 2003 |
| FR-2 | Perros-Guirec | Brittany | FR | | | ropes | Located at the marina in Perros-Guirec, a small town in Brittany facing the English Channel. Aug. 7, 2002. Identified by Francoise Monniot. | Valentine 2003 |
| FR-3 | Port of Le Havre | Le Havre | FR | 3 | | brick quay, barnacles, mussels, tunicates | Pertuis Vauban-Barre, Port of Le Havre. Dec. 1, 2001. Identified by Francoise Monniot. | Valentine 2003 |
| FR-4 | Port of Le Havre | Le Havre | FR | 2 | | brick quay, barnacles, mussels, tunicates | Bassin Vauban, Port of Le Havre. Apr. 21, 2002. Identified by Francoise Monniot. | Valentine 2003 |
| FR-5 | Port of Le Havre | Le Havre | FR | <1 | | floating dock in the port | Floating dock, Port of Le Havre. Aug. 26, 2005. Identified by G. Lambert and G. Breton. | Valentine 2003 |
| NE-1 | Bommenede | Grevelingen | NE | 4 | | algae, tunicates | Bommenede, Grevelingen, Zeeland. Dec. 31, 1999. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-2 | Brouwersdam | North Sea | NE | 5 | | tunicates, rocks | Brouwersdam, North Sea, Zeeland. Dec. 23, 2000. Identified by Arjan Gittenberger. | Valentine 2003 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|----------------------------|---------------|---------|-----------------|-------------------|---------------------|--|----------------|
| NE-3 | Den Osse harbor | Grevelingen | NE | 10 | | boulder | Den Osse harbor, Grevelingen, Zeeland. Oct. 31, 1999. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-4 | Den Osse harbor | Grevelingen | NE | 10 | | tunicate | Den Osse harbor, Grevelingen, Zeeland. X and Y are Amersfoort coordinates. Summer 2001. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-5 | Goes | Oosterschelde | NE | 6 | | oysters | Goes, Sas van, Oosterschelde, Zeeland. July 8, 2000. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-6 | Goes | Oosterschelde | NE | 7 | | anemones | Goes, Sas van, Oosterschelde, Zeeland. June 2003. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-7 | Ouddorp Inlage | Grevelingen | NE | 8 | | tunicates | Ouddorp Inlage, Grevelingen, Zeeland. Apr. 7, 2000. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-8 | Scharendijke | Grevelingen | NE | 6 | | rock | Scharendijke harbor, Grevelingen, Zeeland. X and Y are Amersfoort coordinates. Spring 1999. Identified by Arjan Gittenberger | Valentine 2003 |
| NE-9 | Scharendijke | Grevelingen | NE | 12 | | red algae, hydroids | Scharendijke harbor, Grevelingen, Zeeland. X and Y are Amersfoort coordinates. Summer 2003. Identified by Arjan Gittenberger | Valentine 2003 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|----------------------------|--|---------|-----------------|-------------------|----------------------------------|---|----------------|
| NE-10 | Zierikzee | Oosterschelde | NE | 4; 6 | | algae, sponges, anemones, oyster | Zierikzee, de Val, Oosterschelde, Zeeland. June 25, 2000. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-11 | Zierikzee | Oosterschelde | NE | 7; 8 | | tunicates, hydroids | Zeelandbrug, Zierikzee, Oosterschelde, Zeeland. July 1, 2000. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-12 | Zierikzee | Oosterschelde | NE | 8 | | | Zeelandbrug, Zierikzee, Oosterschelde, Zeeland. X and Y are Amersfoort coordinates. Spring 2003. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-13 | Zijpe | Oosterschelde | NE | 3 | | tunicates, mussels | Zijpe mussel farm, Oosterschelde, Zeeland. Oct. 17, 1999. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-14 | Zijpe | Oosterschelde | NE | 4 | | tunicates | Zijpe mussel farm, Oosterschelde, Zeeland. Oct. 23, 1999. Identified by Arjan Gittenberger. | Valentine 2003 |
| NE-15 | Zoeters-bout | Oosterschelde | NE | 9 | | anemones | Zoetersbout, Oosterschelde, Zeeland. Dec. 26, 2000. Identified by Arjan Gittenberger. | Valentine 2003 |
| NZ-1 | Picton | Picton, Marlborough Sounds, South Island | NZ | 1 | 8-20 | vessel hull | Collected and identified by Ashley Coutts as <i>D. vexillum</i> . May 2003. Colony found on a barge (Waimarie I) that was infected during anchorage next to the barge Steel Mariner between | Valentine 2003 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|----------------------------|--|---------|-----------------|-------------------|---|---|----------------|
| | | | | | | | January and March 2001. | |
| NZ-2 | Shakespeare Bay | Picton, Marlborough Sounds, South Island | NZ | 0-16 | 8-20 | wharf piles, mooring lines, vessel hulls, and rocky/cobble seabed | Collected by Mike Page; identified by Patricia Mather (Kott) as <i>D. vexillum</i> . Prefers artificial structures in shaded areas (e.g. under wharfs, vessels). 2002-2003. For specific dates, see image captions. | Valentine 2003 |
| NZ-3 | Shakespeare Bay 2 | Picton, Marlborough Sounds, South Island | NZ | | | mussel lines and mussels on seabed | Overgrowing mussel lines and encrusting mussels on seabed; Queen Charlotte College Aquaculture Academy mussel lines. Identified by A. Coutts, January 30, 2006. | Valentine 2003 |
| NZ-4 | Tauranga Harbour | Tauranga, North Island | NZ | 0-10 | 9-21 | wharf piles, mooring lines, vessel hulls | Collected by Brian Coffey; identified by Brian Coffey and Ashley Coutts as <i>D. vexillum</i> . No images from this location shown on website. | Valentine 2003 |
| NZ-5 | Waimahara Wharf | Shakespeare Bay, Picton, South Island | NZ | 1-5 | | metal and concrete wharf pilings | On metal wharf pilings under Waimahara Wharf, Shakespeare Bay, Picton, New Zealand. Photographed Mar 24, 2005. Identified by A. Coutts. | Valentine 2003 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|----------------------------|---------------------------|---------|-----------------|-------------------|--|---|----------------|
| NZ-6 | Whangamata Harbour | Whangamata, North Island | NZ | 0-4 | 9-21 | wharf piles, mooring lines, vessel hulls | Collected by Brian Coffey. Oct. 2001. Identified by Patricia Mather (Kott) as <i>D. vexillum</i> . | Valentine 2003 |
| US-EC-1 | 311020 | Georges Bank, NW Atlantic | USA | 47 | | pebble gravel | Northern Edge of Georges Bank, in an area open to fishing; U.S. waters. Nov. 1, 2003. | Valentine 2003 |
| US-EC-2 | 311021 | Georges Bank, NW Atlantic | USA | 41 | | pebble gravel | Northern Edge of Georges Bank, in an area open to fishing; U.S. waters. Nov. 1, 2003. | Valentine 2003 |
| US-EC-3 | 311022 | Georges Bank, NW Atlantic | USA | 43 | | pebble gravel | Northern Edge of Georges Bank, in an area open to fishing; U.S. waters. Nov. 1, 2003. | Valentine 2003 |
| US-EC-4 | 311023 | Georges Bank, NW Atlantic | USA | 41 | | pebble gravel | Northern Edge of Georges Bank, in an area open to fishing; U.S. waters. Nov. 1, 2003. | Valentine 2003 |
| US-EC-5 | 311024 | Georges Bank, NW Atlantic | USA | 45 | | pebble gravel | Northern Edge of Georges Bank, in an area open to fishing; U.S. waters. Beam trawl sample. Nov. 1, 2003. | Valentine 2003 |
| US-EC-6 | 311032 | Georges Bank, NW Atlantic | USA | 47 | | pebble gravel | Northern Edge of Georges Bank, in an area open to fishing; U.S. waters. Naturalists dredge sample. Nov. 1, 2003. | Valentine 2003 |
| US-EC-7 | Beavertail Point | Conanicut Island, RI | USA | intertidal | | rock surfaces | Intertidal rock surfaces, eastern shore of Beavertail Point, Conanicut Island, RI. Photographed and identified by L. Auker, Oct. 4, 2005. | Valentine 2003 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|--------------------------------------|-----------------------------------|---------|-----------------|-------------------|------------------------------|---|----------------|
| US-EC-8 | Chatham Harbor | Chatham, MA | USA | intertidal | | wood pilings, blue mussels | Dock in Chatham Harbor, MA. Photographed Aug. 7, 2003. | Valentine 2003 |
| US-EC-9 | Cobscook Bay | Eastport, ME | USA | 5-6 | | rocky seabed | Rocky seabed in Cobscook Bay near location of old toll bridge from Eastport (Old Toll Bridge Road) to Perry ME. Photographed Aug. 6, 2005. Identified by L. Harris, 2005. | Valentine 2003 |
| US-EC-10 | Eel Pond | Woods Hole, MA | USA | intertidal | 2.2 | floating docks, ropes | Photographed specimens collected at water depths of 0.5 to 2 m. Jan. 7, 2004 | Valentine 2003 |
| US-EC-11 | Eel Pond | Woods Hole, MA | USA | intertidal | -2 | floating docks, ropes | Photographed specimens collected at water depths of 0.5 to 2 m. Jan. 15, 2004 | Valentine 2003 |
| US-EC-12 | Fort Island Narrows | Fort Island Narrows, Maine | USA | 12 | | shell hash, gravel | Fort Island Narrows; in the Damariscotta River between Fort Island and the western river bank. July 19, 1993. Identified by G. Lambert, Jan. 2004. | Valentine 2003 |
| US-EC-13 | Fort Point | Portsmouth Harbor, New Castle, NH | USA | 5 | | ropes hanging from pier | Ropes attached to Coast Guard pier at Fort Point, Portsmouth Harbor, New Castle, NH. Photographed Aug 2002. Identified by L. Harris, 2002. | Valentine 2003 |
| US-EC-14 | Graduate School of Oceanography, URI | Narragansett, RI | USA | intertidal | | wood pilings, mussels, rocks | Beneath and adjacent to the dock at GSO. Photographed Dec. 1, 2002. | Valentine 2003 |
| US-EC-15 | Graduate School of Oceanography, URI | Narragansett, RI | USA | intertidal | | wood pilings, mussels, rocks | Beneath the dock at GSO. removed by ice action. Photographed Mar. 5, 2003 | Valentine 2003 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|----------------------------|------------------------|---------|-----------------|-------------------|-------------------------------|--|----------------|
| US-EC-16 | Iselin Dock | Woods Hole, MA | USA | 3-10 | | steel dock pilings | Pilings of the Iselin Dock at WHOI, Woods Hole, MA. Photographed 2002, 2004. Identified by P. Valentine, 2005. | Valentine 2003 |
| US-EC-17 | Iselin Dock | Woods Hole, MA | USA | 18-20 | | gravel | Seabed near the Iselin Dock at WHOI, Woods Hole, MA. Photographed Spring 1998. Provisional identification from photo. | Valentine 2003 |
| US-EC-18 | Oak Bluffs | Oak Bluffs, MA | USA | 1-2 | 0 | rope, steel bulkhead | Harbor entrance and at the head of the harbor. Photographed Jan. 22, 2004 | Valentine 2003 |
| US-EC-19 | Old Ponquogue Bridge | Shinnecock Bay, LI, NY | USA | 0.1 | | floating dock | Floating dock attached to south end of Old Ponquogue Br, east of new Ponquogue Bridge, Shinnecock Bay, LI, NY. Photographed Dec. 3, 2004. | Valentine 2003 |
| US-EC-20 | Osterville Grand Island | West Bay, MA | USA | 0-25 | | plywood, styrofoam, tunicates | Over growing solitary tunicate <u>Styela clava</u> and encrusting plywood and styrofoam of channel markers in West Bay, east of Osterville Grand Island, MA. Photographed Nov. 15, 2005. Identified by P. Valentine and M. Carman, 2005. | Valentine 2003 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|----------------------------|------------------|---------|-----------------|-------------------|-------------------------------|--|----------------|
| US-EC-21 | Osterville Grand Island | North Bay, MA | USA | 0-.25 | | plywood, styrofoam, tunicates | Over growing solitary tunicate <u>Styela clava</u> and encrusting plywood and styrofoam of channel markers in North Bay, north of Osterville Grand Island, MA. Photographed Nov. 15, 2005. Identified by P. Valentine and M. Carman, 2005. | Valentine 2003 |
| US-EC-22 | Osterville Grand Island | Cotuit Bay, MA | USA | 0-.25 | | plywood, styrofoam, tunicates | Over growing solitary tunicate <u>Styela clava</u> and encrusting plywood and styrofoam of channel markers in Cotuit Bay, west of Osterville Grand Island, MA. Photographed Nov. 15, 2005. Identified by P. Valentine and M. Carman, 2005. | Valentine 2003 |
| US-EC-23 | Piscataqua River | Newington, NH | USA | 12-15 | | rock surface | Piscataqua River bed, in NH part of river, off Newington, NH. Oct. 29, 2002. Provisional identification from photo (M. Carman, P. Valentine). | Valentine 2003 |
| US-EC-24 | Provincetown Harbor | Provincetown, MA | USA | intertidal | | metal pilings, tires, ropes | Macmillan Wharf (Provincetown town dock). Photographed Sep. 14, 2003. | Valentine 2003 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|----------------------------|---------------------------|---------|---------------------------------|-------------------|---|---|----------------|
| US-EC-25 | Sandwich tide pool | Sandwich, MA | USA | intertidal, 11 ft (3.4 m) range | | boulder | Located just south of the east entrance of the Cape Cod Canal at a Sandwich town beach. Photographed June, 2003. | Valentine 2003 |
| US-EC-26 | Sandwich tide pool | Sandwich, MA | USA | intertidal, 11 ft (3.4 m) range | | pebbles, cobbles, boulders, coarse sand | Located just south of the east entrance of the Cape Cod Canal at a Sandwich town beach. Photographed Dec. 4, 2003. | Valentine 2003 |
| US-EC-27 | Sandwich tide pool | Sandwich, MA | USA | intertidal, 11 ft (3.4 m) range | 1 | boulder | Located just south of the east entrance of the Cape Cod Canal at a Sandwich town beach. Photographed Feb. 3, 2004 | Valentine 2003 |
| US-EC-28 | Strong Island | Pleasant Bay, Chatham, MA | USA | 2-3 | | rock surface | Boulders off the NW part of Strong Island, Pleasant Bay, Chatham, MA. Photographed Sep. 4, 2005. Identified by M. Carman, 2005. | Valentine 2003 |
| US-EC-29 | Thrumcap Island | Damariscotta River, ME | USA | 10-15 | | rock surface | Off Thrumcap Island, at the mouth of the Damariscotta River, ME. Photographed October 7, 2002. Identified by R. Miller. | Valentine 2003 |
| US-EC-30 | Thrumcap Island | Damariscotta River, ME | USA | 10-15 | | rock surface | Off Thrumcap Island, at the mouth of the Damariscotta River, ME. Photographed Aug. 22, 2003. Identified by R. Miller. | Valentine 2003 |
| US-EC-31 | Town Cove | Orleans, MA | USA | 0.1 | | floating dock with styrofoam floats | Floating dock at Nauset Marine, Town Cove, Orleans, MA. Photographed Oct. 26, 2004. | Valentine 2003 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|----------------------------|--------------------|---------|-----------------|-------------------|---------------------------------|--|----------------|
| US-EC-32 | Town Cove | Orleans, MA | USA | 0.1 | | floating dock | Floating dock at Goose Hummock Shop, Town Cove, Orleans MA. Photographed Oct. 26, 2004. | Valentine 2003 |
| US-EC-33 | Town Cove | Orleans, MA | USA | 0.1 | | floating dock | Floating dock north of Nauset Marine on western shore of Town Cove, Orleans, MA. Photographed Oct. 26, 28, 2004. | Valentine 2003 |
| US-EC-34 | Town Cove | Orleans, MA | USA | 0.1 | | floating dock | Floating dock west of Hopkins Island on western shore of Town Cove, Orleans, MA. Photographed Oct. 26, 2004. | Valentine 2003 |
| US-EC-35 | Vineyard Haven | Vineyard Haven, MA | USA | 0.5 | 0 | rubber floats of floating dock | Floating dock adjacent to the ferry pier. Photographed Jan. 22, 2004 | Valentine 2003 |
| US-EC-36 | Wellfleet Harbor | Wellfleet, MA | USA | subtidal | | plastic mesh bags | Growing on plastic mesh bags and on oysters on subtidal sand flat in Wellfleet Harbor, MA. Photographed Nov. 14, 2005. Identified by P. Valentine and M. Carman, 2005. | Valentine 2003 |
| US-EC-37 | West Falmouth Harbor | Falmouth, MA | USA | 0.3 | | chain attached to floating dock | Floating dock at town pier off Nonamesset Rd., Falmouth, MA. Photographed Dec. 9, 2004. | Valentine 2003 |
| US-WC-1 | Des Moines Marina | Des Moines, WA | USA | 5 | | crab cage (plastic-coated wire) | At marina in Des Moines, WA. Nov. 2004. Identified by G. Lambert. | Valentine 2003 |
| US-WC-2 | Edmonds Underwater Park | Edmonds, WA | USA | 9-12 | | sunken wooden boat | In an underwater marine park on the coast of Puget Sound in Edmonds, WA. Sept. | Valentine 2003 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|------------------------------|---------------------|---------|-----------------|-------------------|------------------|---|----------------|
| | | | | | | | 2004. Identified by G. Lambert | |
| US-WC-3 | Gallagher Cove | Totten Inlet, WA | USA | 0.5-1.0 | | mussels, rope | Taylor Shellfish Farms, Totten Inlet, WA. Nov. 2004. Identified by G. Lambert | Valentine 2003 |
| US-WC-4 | Dabob Bay | Hood Canal, WA | USA | | | | Taylor Shellfish Farms, on mussel lines. | Lambert 2005c |
| US-WC-5 | Bodega Harbor | Bodega Bay, CA | USA | | | floating dock | Floating dock, Bodega Harbor, in the town of Bodega Bay, CA. Photographed May 29, 2003. Identified by G Lambert, 2003. | Valentine 2003 |
| US-WC-6 | Elkhorn Slough, Monterey Bay | Moss Landing, CA | USA | | | | First documented occurrence in California. Collected by Laura Bedinger, April 30, 1998. California Academy of Sciences specimen no. 144184. No image. | Valentine 2003 |
| US-WC-7 | Half Moon Bay | San Mateo, CA | USA | | | | discovered in 1997 | Cohen 2005 |
| US-WC-8 | Horseshoe Bay | Sausalito, CA | USA | | | mussels, bryozoa | Horseshoe Bay; dock at Presidio Yacht Club, Sausalito, CA. May 25, 2003. Identified by G. Lambert, 2004. | Valentine 2003 |
| US-WC-9 | Morro Bay | CA | USA | | | | discovered in 2000 | Cohen 2005 |
| US-WC-10 | Port San Luis | San Luis Obispo, CA | USA | | | | discovered in 2003 | Cohen 2005 |

Table 2 cont'd

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|--------------------------------|-------------------|---------|-----------------|-------------------|---------------------------------------|---|-----------------------------|
| US-WC-11 | San Francisco Bay | Fisherman's Wharf | USA | | | rope on floating dock | Fisherman's Wharf, San Francisco, CA. May 23, 2004. Identified by G. Lambert, 2004. | Valentine 2003 |
| US-WC-12 | San Francisco Bay | Sausalito, CA | USA | | | rope | Rope attached to dock at a marina in Sausalito, CA. May 28, 2003. | Valentine 2003 |
| US-WC-13 | Tomales Bay Clarke | CA | USA | | | | May 2003 | deRivera <i>et al.</i> 2005 |
| US-WC-14 | Tomales Bay Marshall | CA | USA | | | | May 2003 | deRivera <i>et al.</i> 2005 |
| US-WC-15 | Tomales Bay Miller Park | CA | USA | | | | May 2003 | deRivera <i>et al.</i> 2005 |
| US-WC-16 | Tomales Bay Sacramento Landing | CA | USA | | | | May 2003 | deRivera <i>et al.</i> 2005 |
| US-WC-17 | Tomales Bay Thomas Station | CA | USA | | | | May 2003 | deRivera <i>et al.</i> 2005 |
| US-WC-18 | Woodley Island | Humboldt Bay, CA | USA | 1 | | PVC fouling panels, 4x6 in (10x15 cm) | Dock at Woodley Island marina, Humboldt Bay, Eureka, CA. Photographed Sep. 2003; Oct. 2004; Jan. and Mar. 2005. | Valentine 2003 |

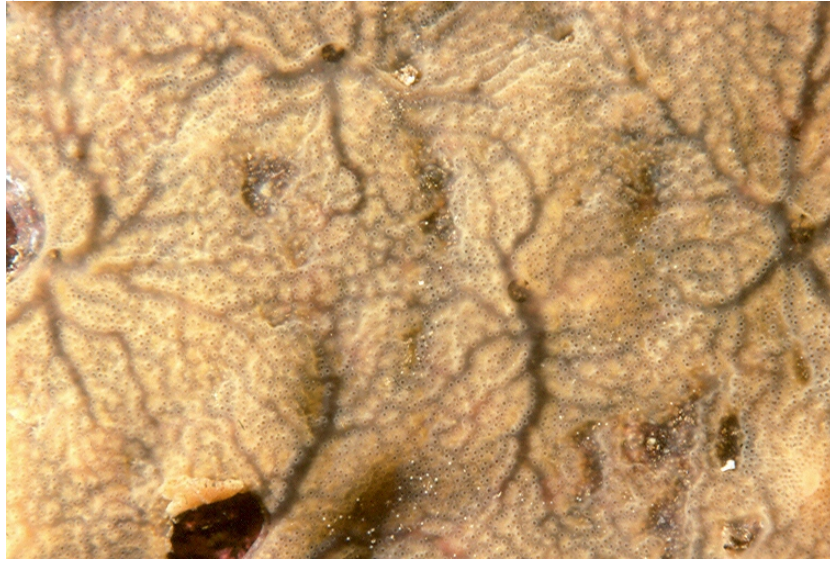
Table 3. *Didemnum* sp. records for British Columbia, Canada (Valentine 2003, Debbie Palzat pers. comm.). The majority of the information, including table format, is from the USGS website last accessed on March 6, 2006 (Valentine 2003). Refer to Figure 10 for the corresponding maps.

| Map Location | Location name or Station # | Region | Country | Water depth (m) | Water temp (degC) | Substrate type | Comments | Reference |
|--------------|----------------------------|-----------------------------------|---------|-----------------|-------------------|-------------------|---|---------------------------|
| CA-1 | Agamemnon Channel | Nelson Island, British Columbia | Canada | 6-12 | | rock wall | Rock wall in Agamemnon Channel, a fjord that separates the east and west parts of Nelson Island, BC. Photographed Dec. 2004. Identified by G. Lambert, Jan. 2005. | Valentine 2003 |
| CA-2 | Deep Bay | Baynes Sound, British Columbia | Canada | | | vexar oyster tray | Oyster farm in Deep Bay, B.C. No photo available. Specimen identified by G. Lambert, Nov. 2005. | Valentine 2003 |
| CA-3 | Deep Bay | Baynes Sound | Canada | 0.5-6 | 8-10 | | aquaculture site surveys, growing on the culture gear including the oysters, mussels, hydroids. Discovered Nov. 9, 2005 | Debbie Palzat pers. comm. |
| CA-4 | False Bay | Lasqueti Island | Canada | 0.5-6 | 8-10 | | aquaculture site surveys, growing on the culture gear including the oysters, mussels, hydroids. Discovered Oct. 19, 2005 | Debbie Palzat pers. comm. |
| CA-5 | Fanny Bay Oysters | Nanoose Bay | Canada | 0.5-6 | 8-10 | | aquaculture site surveys, growing on the culture gear including the oysters, mussels, hydroids. Discovered Nov. 25, 2005 | Debbie Palzat pers. comm. |
| CA-6 | Gorge Harbour | Cortes Island | Canada | 0.5-6 | 8-10 | | aquaculture site surveys, growing on the culture gear including the oysters, mussels, hydroids. Discovered Nov. 2, 2005 | Debbie Palzat pers. comm. |
| CA-7 | Jedediah Island | Sabine Channel, Strait of Georgia | Canada | 0-1 | | rock surfaces | On vertical rock surfaces along the west side of Jedediah Island, especially in Little Bull Passage, Strait of Georgia, BC. Photographed Sep. 1, 2005. Identified by S. Geerlofs, 2005. | Valentine 2003 |
| CA-8 | Jervis Inlet | Jervis Inlet, British Columbia | Canada | 6 approx. | | rocky seabed | On rocky seabed near an unnamed islet east of Captain Island in Jervis Inlet, BC. Photographed Nov. 25, 2005. Identified by G. Lambert, 2005. | Valentine 2003 |
| CA-9 | Lemmens Inlet | Meares Island, British Columbia | Canada | | | oyster string | Oyster farm in Lemmens Inlet, B.C. No photo available. Specimen identified by G. Lambert, Nov. 2005. | Valentine 2003 |
| CA-10 | Lemmens Inlet | Meares Island | Canada | 0.5-6 | 8-10 | | aquaculture site surveys, growing on the culture gear including the oysters, mussels, hydroids. Discovered Nov. 14, 2005 | Debbie Palzat pers. comm. |

Table 3 cont'd

| | | | | | | | | |
|-------|----------------|---------------------------------------|--------|------------|------|---------------|--|---------------------------|
| CA-11 | Lions Rock | Okeover Inlet, British Columbia | Canada | 10 approx. | 12.8 | rock surfaces | On sloping and vertical rock surfaces at Lions Rock, southwest of Edith Island, Okeover Inlet, B.C. Photographed Sep. 4, 2005. Identified by G. Lambert, 2005. | Valentine 2003 |
| CA-12 | Okeover Inlet | Malaspina Peninsula | Canada | subtidal | | mussel cages | On subtidal mussel cages in Okeover Inlet, Malaspina Peninsula, BC. Photographed 2003. Identified by G. Lambert, 2004. | Valentine 2003 |
| CA-13 | Okeover Inlet | Taylor Shellfish | Canada | 0.5-6 | 8-10 | | aquaculture site surveys, growing on the culture gear including the oysters, mussels, hydroids. Discovered Nov. 27, 2005 | Debbie Palzat pers. comm. |
| CA-14 | Okeover Inlet | | Canada | 0.5-6 | 8-10 | | aquaculture site surveys, growing on the culture gear including the oysters, mussels, hydroids. Discovered Nov. 27, 2005 | Debbie Palzat pers. comm. |
| CA-15 | Pendrell Sound | East Redonda Island, British Columbia | Canada | 6-15 | | rock wall | Rock wall on south side of Christy Island in Pendrell Sound, a fjord that incises East Redonda Island, BC. Photographed Aug. 10, 2004. Identified by G. Lambert, May 2005. | Valentine 2003 |
| CA-16 | Teakerne Arm | W. Redonda | Canada | 0.5-6 | 8-10 | | aquaculture site surveys, growing on the culture gear including the oysters, mussels, hydroids. Discovered Nov. 5, 2005 | Debbie Palzat pers. comm. |
| CA-17 | Trevenen Bay | Malaspina Peninsula, British Columbia | Canada | 12-15 | 12.2 | rock surfaces | On rock surfaces near C-King Seafood aquaculture site, west side of Trevenen Bay, BC. Photographed Sep. 4, 2005. Identified by G. Lambert, 2005. | Valentine 2003 |
| CA-18 | Tyee Cove | Nanoose Bay | Canada | 0.5-6 | 8-10 | | aquaculture site surveys, growing on the culture gear including the oysters, mussels, hydroids. Discovered Oct. 15, 2005 | Debbie Palzat pers. comm. |
| CA-19 | Village Bay | Quadra Island | Canada | 0.5-6 | 8-10 | | aquaculture site surveys, growing on the culture gear including the oysters, mussels, hydroids. Discovered Nov. 2, 2005 | Debbie Palzat pers. comm. |

A)



B)



Figure 1 *Didemnum* sp. from Agamemnom Channel, British Columbia. A) Closeup of *Didemnum* sp. colony surface, and B) *Didemnum* sp. colony. Both photos by Bernard Hanby. (<http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/htm/brit4a.htm>)

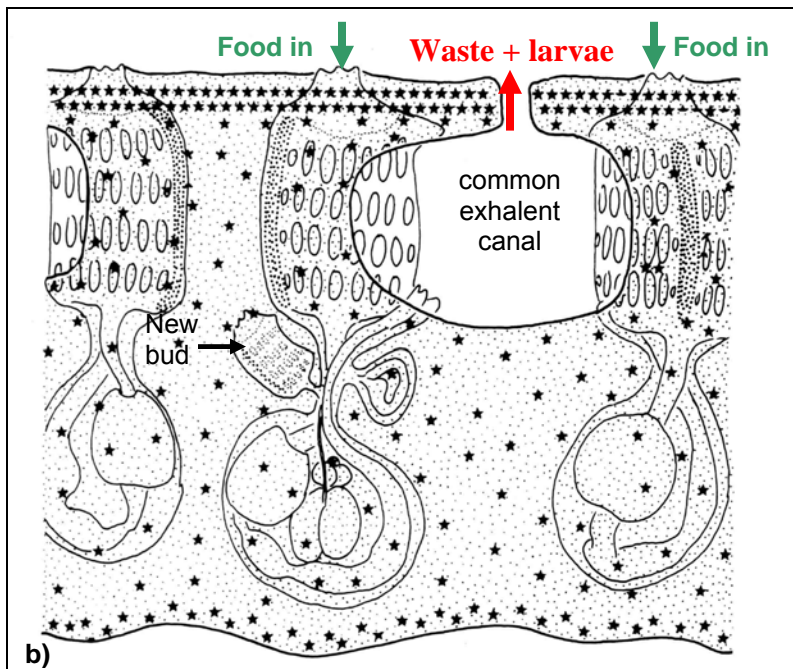
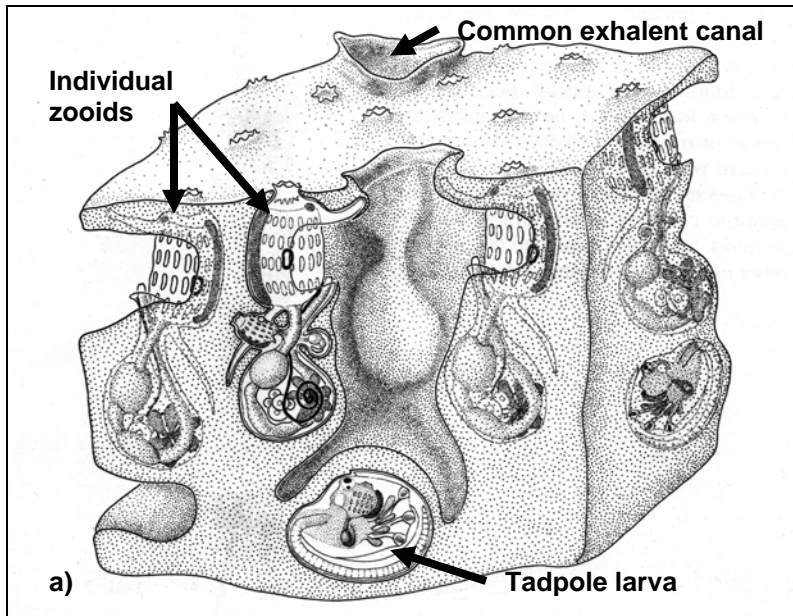


Figure 2. General 3-D (a) and 2-D (b) schematic views of a didemnid (from Monniot *et al.* 1991).

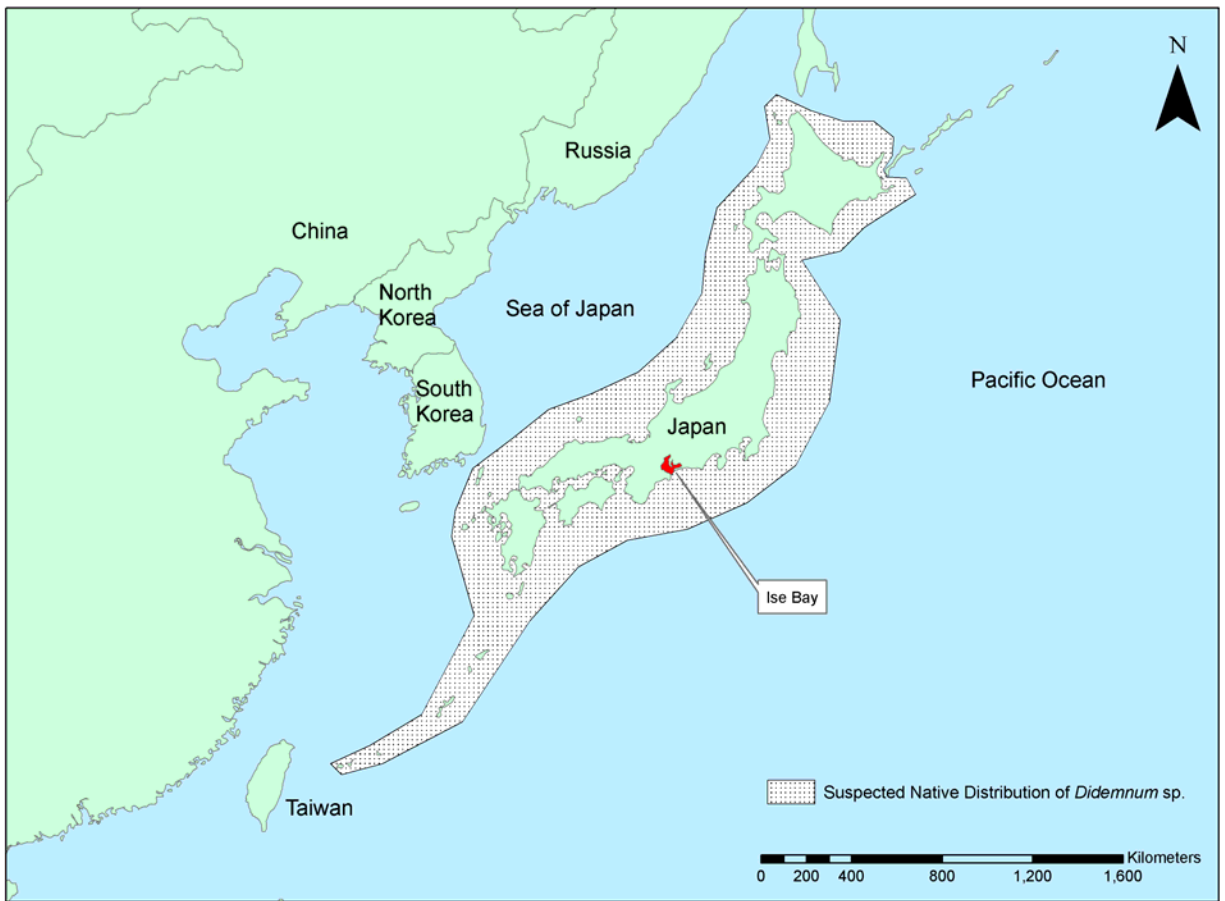


Figure 3. Suspected native distribution of *Didemnum* sp. (Gretchen Lambert, pers. comm.)

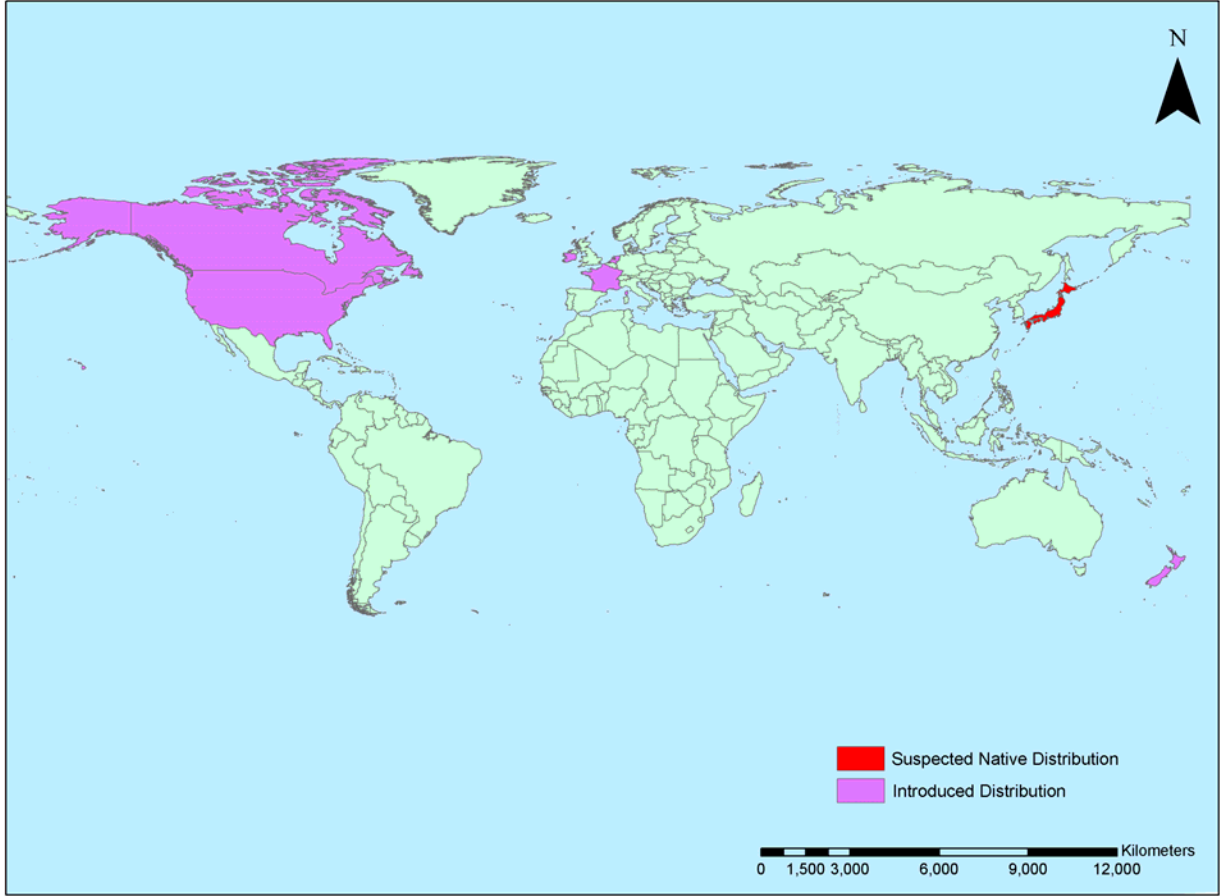


Figure 4. *Didemnum* sp. global distribution by country.

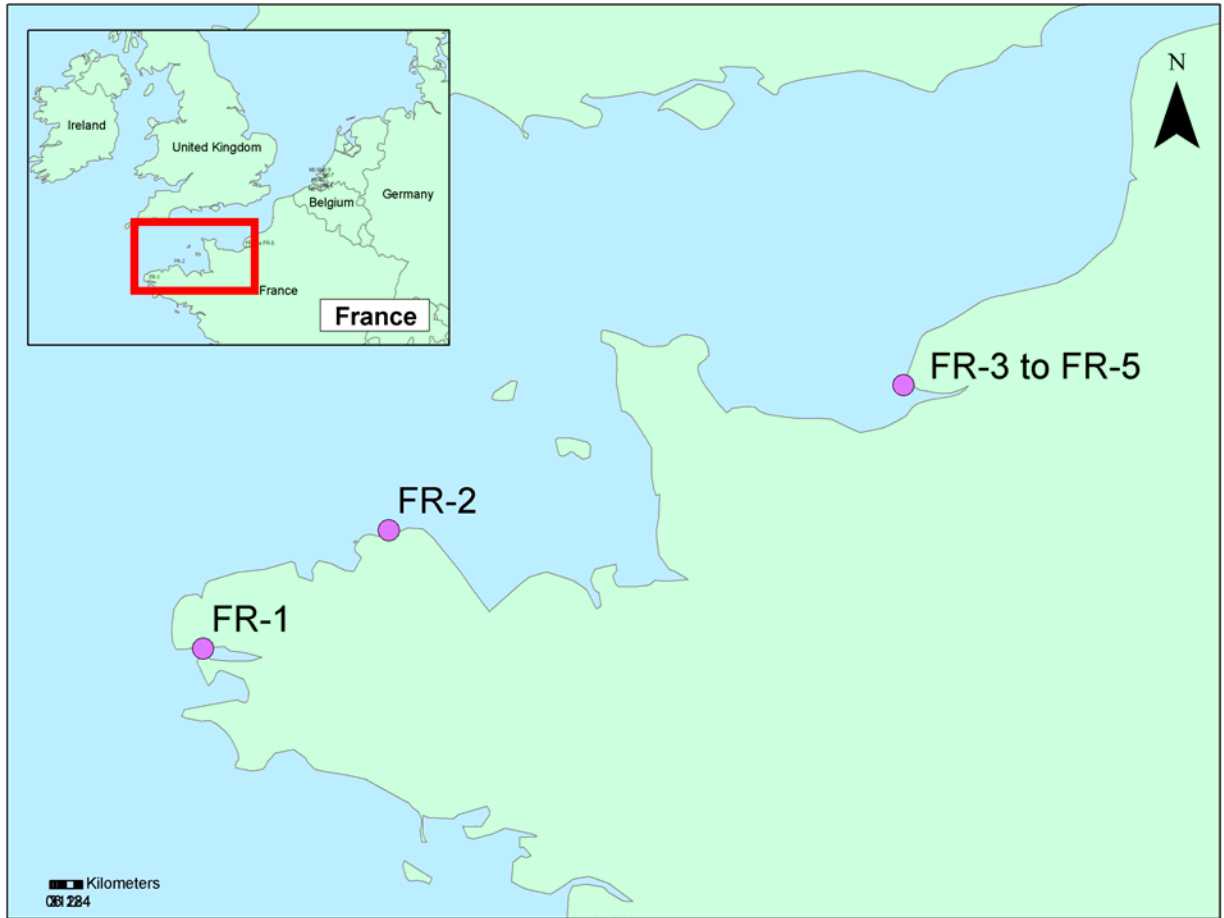


Figure 5. *Didemnum* sp. records in France (Valentine 2003). For more detailed information about each record see Table 2.

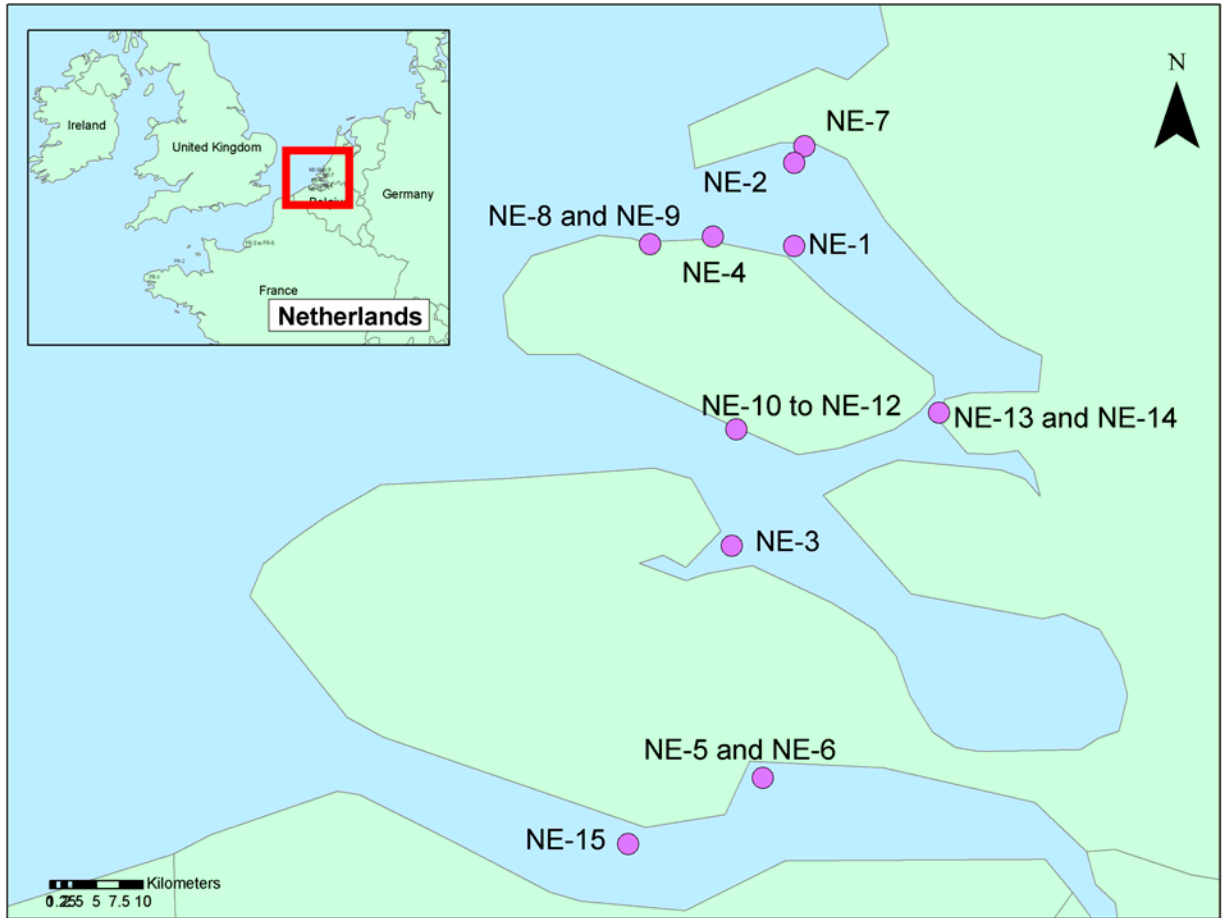


Figure 6. *Didemnum* sp. records in the Netherlands (Valentine 2003). For more detailed information about each record see Table 2.

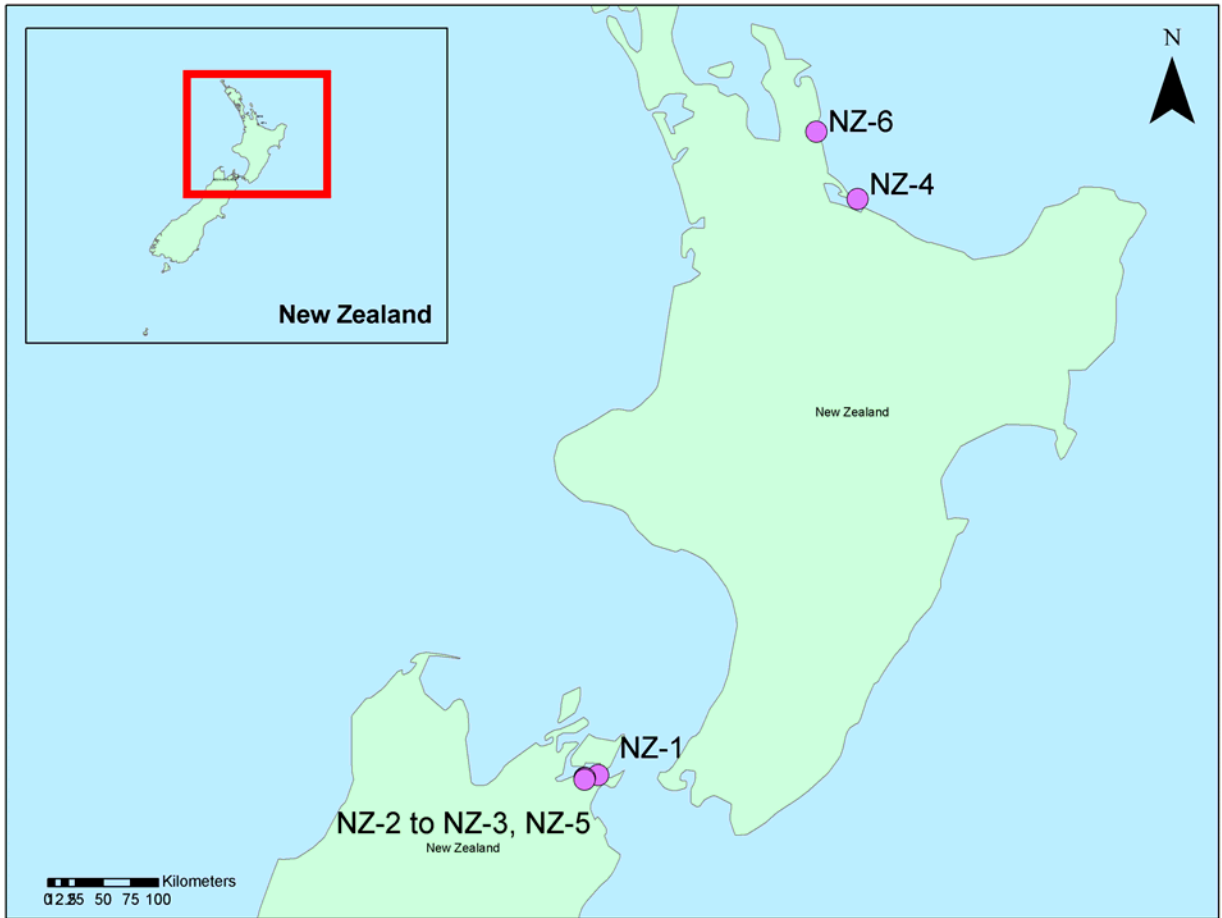


Figure 7. *Didemnum* sp. records in New Zealand (Valentine 2003). For more detailed information about each record see Table 2.

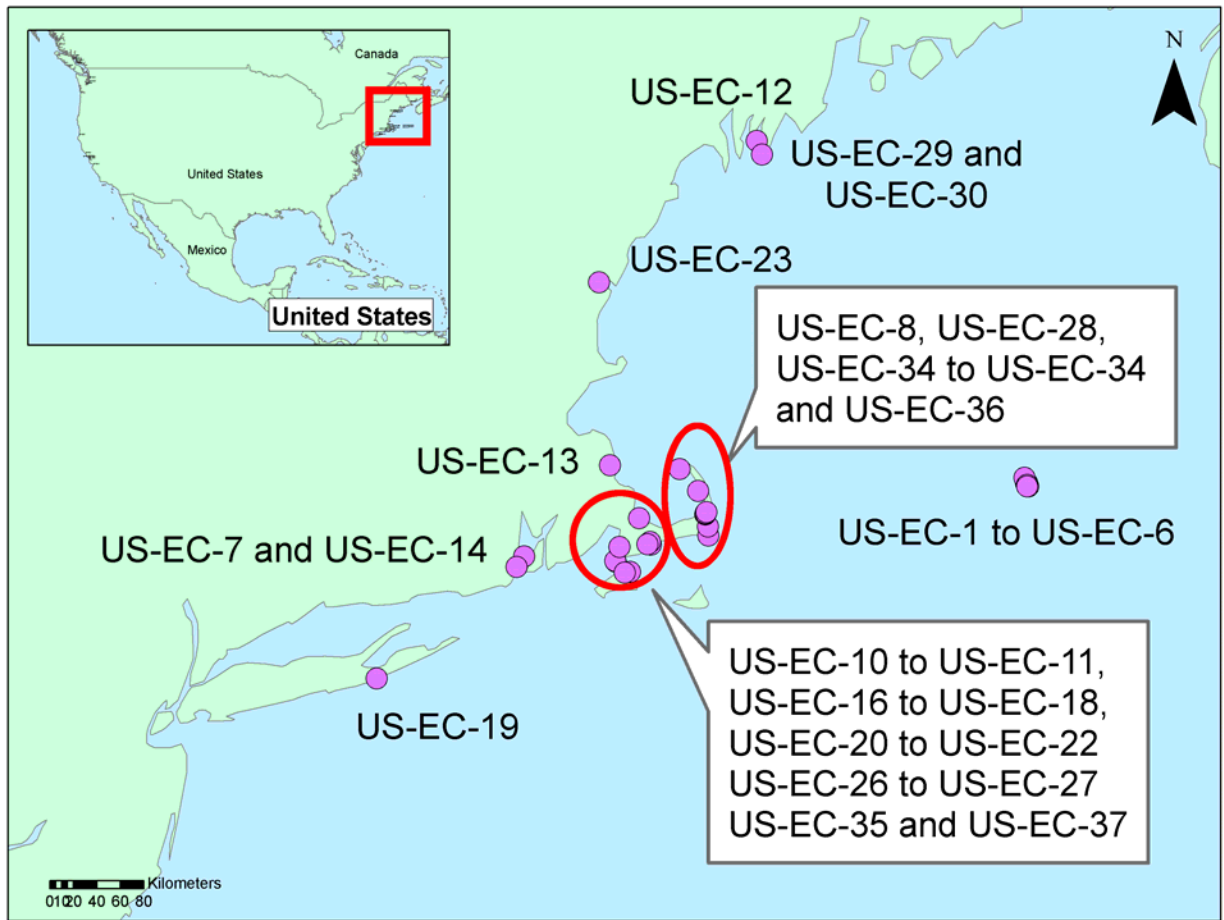


Figure 8. *Didemnum* sp. records along the eastern United States (Valentine 2003). For more detailed information about each record see Table 2.

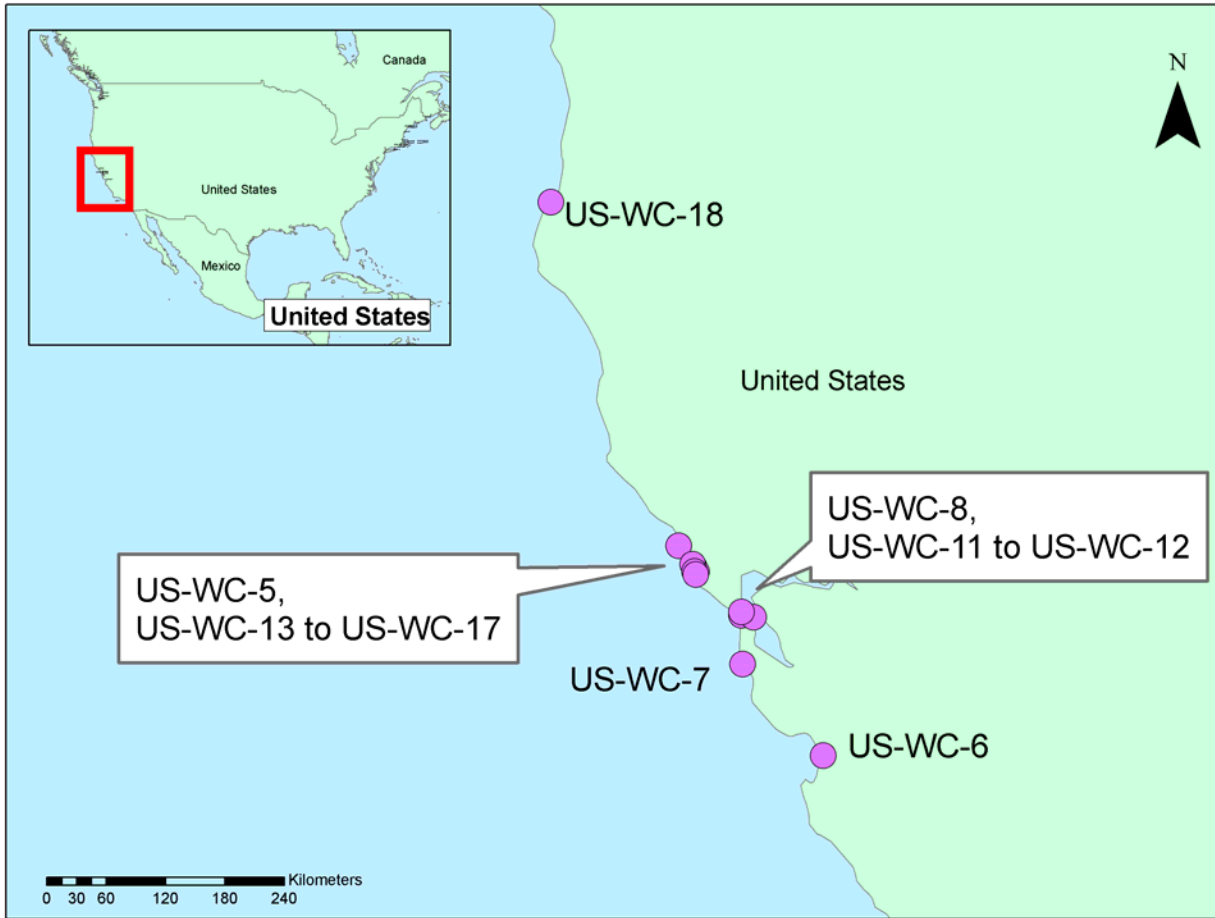


Figure 9. *Didemnum* sp. records in California, United States (Valentine 2003, Cohen 2005, DeRivera *et al.* 2005). For more detailed information about each record see Table 2.

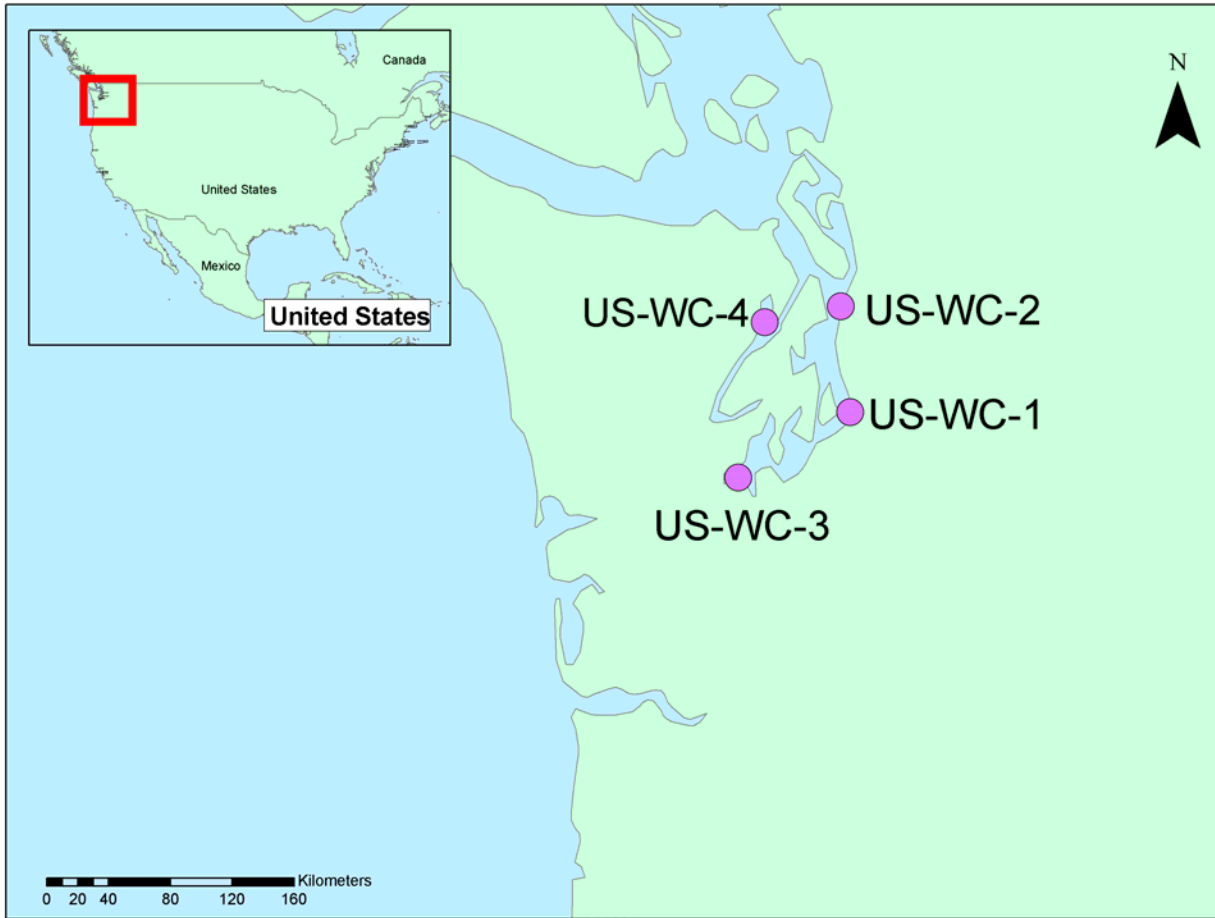


Figure 10. *Didemnum* sp. records in Washington state, United States (Valentine 2003). For more detailed information about each record see Table 2.

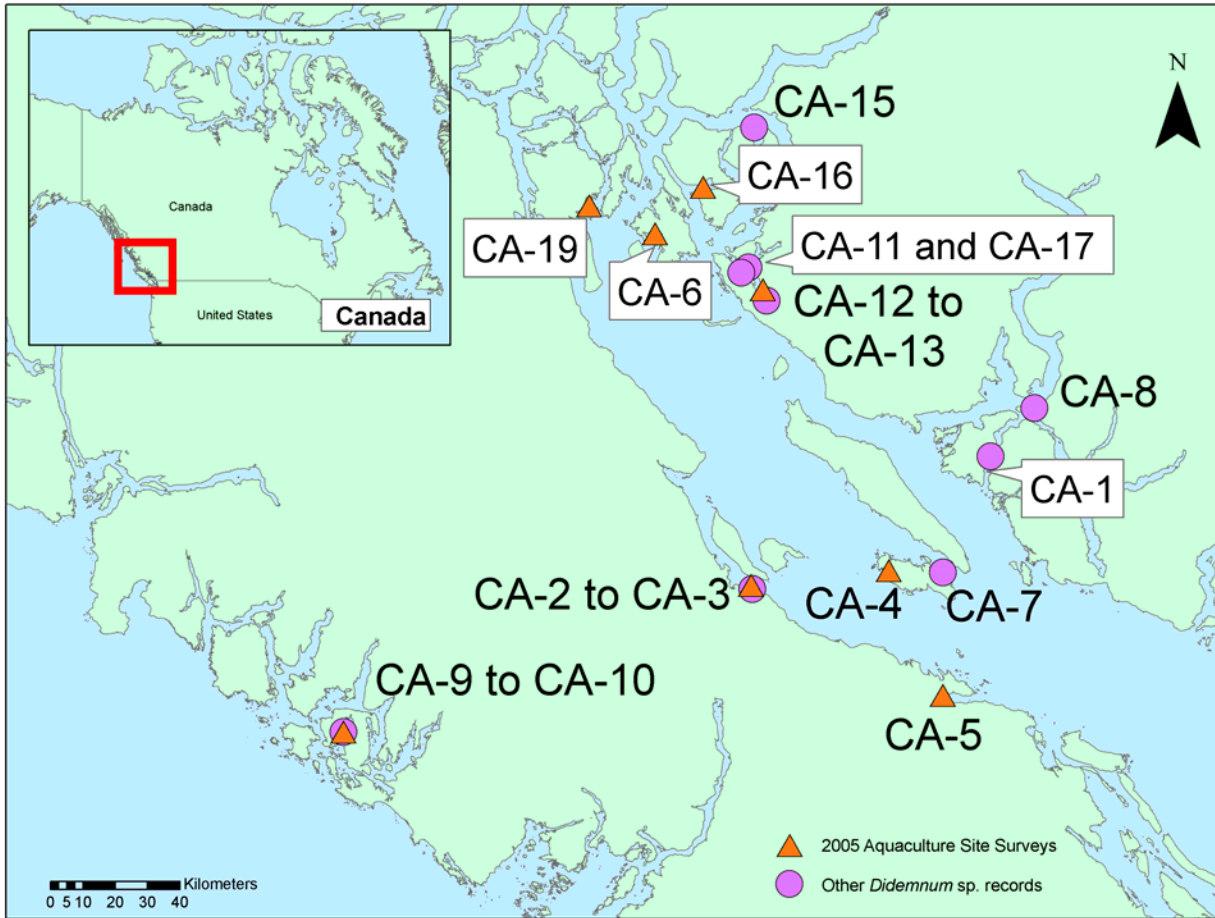


Figure 11. *Didemnum* sp. records in British Columbia, Canada. The orange triangles represent the aquaculture survey records from October and November 2005 (Debbie Palzat pers. comm.). The purple circles are *Didemnum* sp. records from the USGS website (Valentine 2003). For more detailed information about each record see Table 3.