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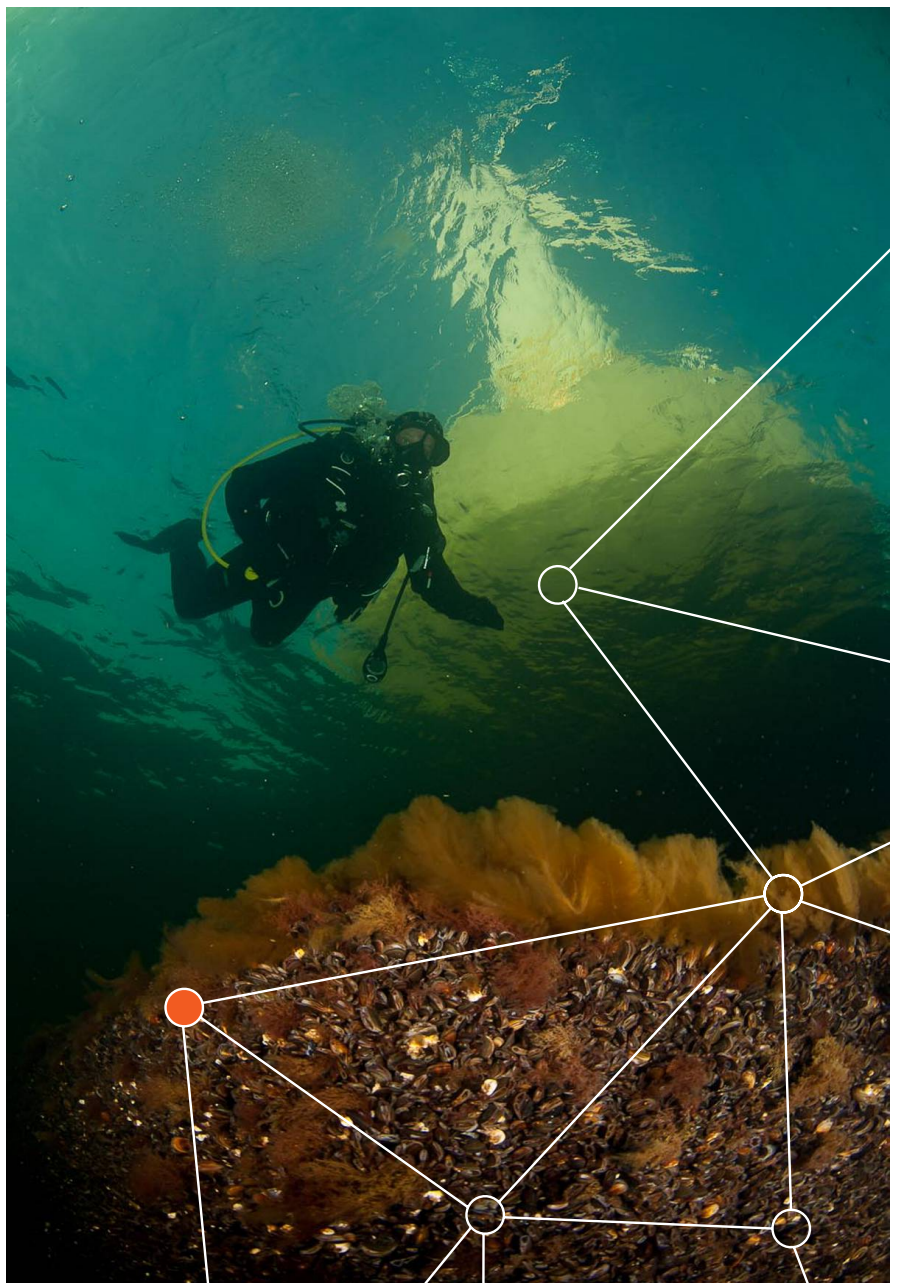# Alien Species Alert: Didemnum vexillum Kott, 2002: Invasion, impact, and control

ICES COOPERATIVE RESEARCH REPORT

RAPPORT
DES RECHERCHES
COLLECTIVES

Alien Species Alert: *Didemnum vexillum* Kott, 2002: Invasion, impact, and control

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Acknowledgements

1   Introduction

Didemnum vexillum Kott (2002) is a high-impact, globally-invasive, colonial tunicate species that is native to Japan (Lambert, 2009; Stefaniak *et al*., 2012). It is generally a temperate cold-water organism, and its introduced range currently includes New Zealand, the Netherlands, France, Ireland, United Kingdom, Spain, Italy, and both the west and east coasts of the United States and Canada (Lambert, 2009; Stefaniak *et al*., 2012; Tagliapietra *et al*., 2012; Ordóñez *et al*., 2015, Vercaemer *et al.,* 2015). Like other invasive tunicates, *D. vexillum* has the capacity to reproduce rapidly, outcompete native species, deteriorate environmental integrity, and cause significant economic harm (Lambert, 2005; Blum *et al*., 2007; Daniel and Therriault, 2007; Langyel *et al*., 2009; Cordell *et al*., 2013). For these reasons, this document aims to increase awareness of *D. vexillum*, with a focus on identification, natural history, current global distribution, potential impacts, and prospects for management and control where introductions occur.

2   Identification and taxonomy

Identification of *Didemnum vexillum* Kott (2002) (Table 2.1) is challenging due to the vast number of species within the genus *Didemnum*. The inability to confidently confirm identification of *D. vexillum* led researchers to use the "umbrella" name *Didemnum* sp. A; therefore, both *Didemnum vexillum* and *Didemnum* sp. A were used interchangeably prior to 2009. Genetic analyses have been used to demonstrate that samples of *Didemnum* sp. A and *D. vexillum* from Europe, the east and west coasts of North America, Japan, and New Zealand were the same species (Stefaniak *et al.*, 2009). Ongoing work to develop genetic primers may also assist future detection of invasive ascidians, such as *D. vexillum* (Stewart-Clark *et al.*, 2009, Vercaemer *et al.*, 2015). A comprehensive record of historical geographical spread and identification of this species was completed by Lambert (2009).

Table 2.1. The taxonomic statue of *Didemnum vexillum*.

Phylum	Chordata
Class	Ascidiacea
Order	Aplousobranchia
Family	Didemnidae
Genus	*Didemnum*
Species	*Didemnum vexillum* Kott, 2002

D. vexillum can form encrusting mats or lobate masses, depending on the environment. Each colony is composed of morphologically and genetically identical individuals, termed zooids, which are enclosed in a common tunic (Figure 2.1A). Colonies can vary in colour, but most are tan, cream, or yellow and can cover both non-living and living substrates (Kott, 2002; Lambert, 2009). The species can settle on and over-grow other sessile animals, such as other ascidians, bivalves, marine grasses, and seaweeds (Figure 2.1B; Carmen and Grunden, 2010). *D. vexillum* has also been observed overgrowing itself, fusing with other parts of the colony surface and forming thick sponge-like masses (Kott, 2002). Colonies tend to form mats on hard substrates and any other surface where currents are strong (Figure 2.1C), but hang in lobate clumps off structures such as wharves or kelp blades where currents are weaker (Figure 2.1D). These clumps tend to form long tendrils that easily break off living tissue which floats away, settles, and grows into new colonies, thus leading to further dispersal (Lambert, 2009; Valentine *et al.*, 2009). Outgrowths from the surface of the colony may occur from colony overlap or result from overgrowing 3-dimensional structures of other substrates (Kott, 2002). The gelatinous tunic itself is colourless. However, the colony gets its colour from the yellow gut loop, eggs, and embryos and from the white calcareous spicules, which are unevenly distributed throughout the tunic surface (Kott, 2002; Lambert, 2009). Additionally, the spicules can be used to distinguish *D. vexillum* from other *Didemnum* spp. and are stellate in shape, with 9–11 pointed rays in optical transverse sections, ranging in size between and within a colony, but generally measuring 20–30 μm (Kott, 2002; Lambert, 2009).
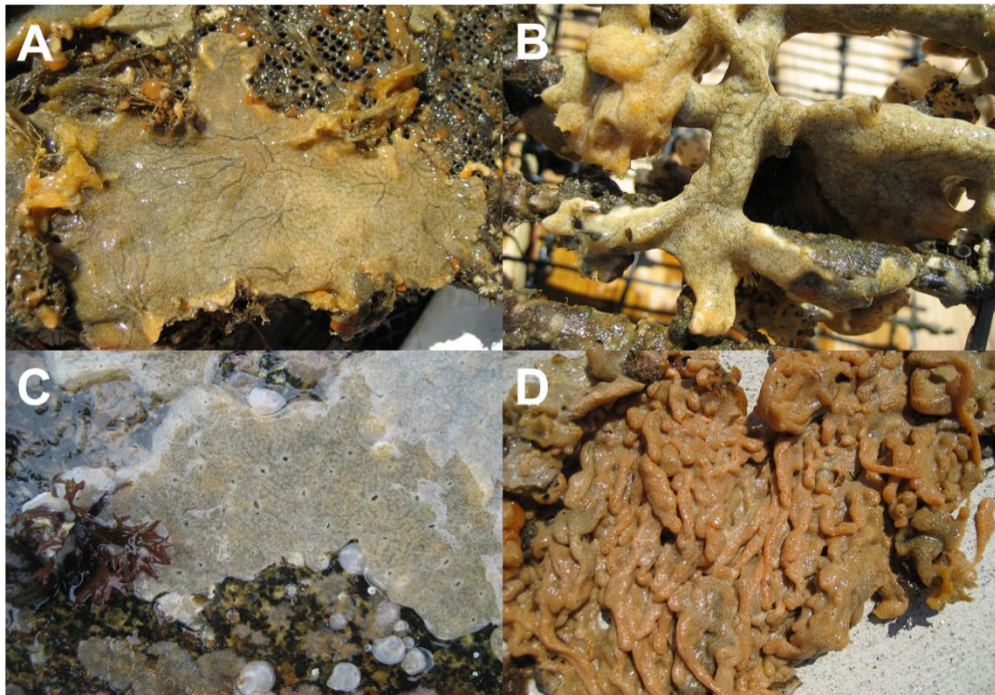
Figure 2.1. Tunic surface patterns of *Didemnum vexillum*. (A) Marine Biological Laboratory dock, Woods Hole, MA, USA, 24 July 2007; (B) Marine Biological Laboratory dock, Woods Hole, MA, USA, 24 July 2007; (C) Rye Harbor State Park intertidal, Rye, MA, USA, 29 July 2007; (D) Massachusetts Maritime Academy floating dock, Bourne, MA, USA, 25 July 2007 (Photo: G. Lambert).

At the colony surface, the zooids form irregular groups each with a 6-lobed oral siphon opening at the surface of the tunic (Figure 2.2). Dark meandering lines are visible around zooid groups where spicules are absent (Figure 2.1A,C). Scattered throughout the colony are the common cloacal cavities where the atrial openings of the zooids discharge digestive waste and gametes at the tunic surface (Berrill, 1935; Van Name, 1945; Kott, 2002). The zooids of *D. vexillum* are small in size, measuring 1–2 mm (Kott, 2002; Daniel and Therriault, 2007; Lambert, 2009) with two body parts, a posterior yellow abdomen containing the digestive and reproductive organs, and the colourless anterior thorax, which contains the branchial sac (Figure 2.2). In the abdomen, the post-pyloric part of the gut is long and flexed ventrally, forming a double loop. There are about nine coils of the vas deferens surrounding the single spherical-to-oval-shaped testis, and a single oocyte at a time maturing in the ovary, which lies almost behind and under the gut loop. The number of coils in the vas deferens and orientation of the internal organs is critical in the accurate identification of *D. vexillum* (Figure 2.2; Kott, 2002; Lambert, 2009). Crescent-shaped, dense, white, lateral organs of the thorax filled with new spicules are visible in newly settled and developing individual oozoids (Valentine *et al*., 2009), as well as in the zooids of mature colonies. The branchial sac of each individual zooid has four rows of stigmata; the first row contains 8 or 9 stigmata per side (Kott, 2002).
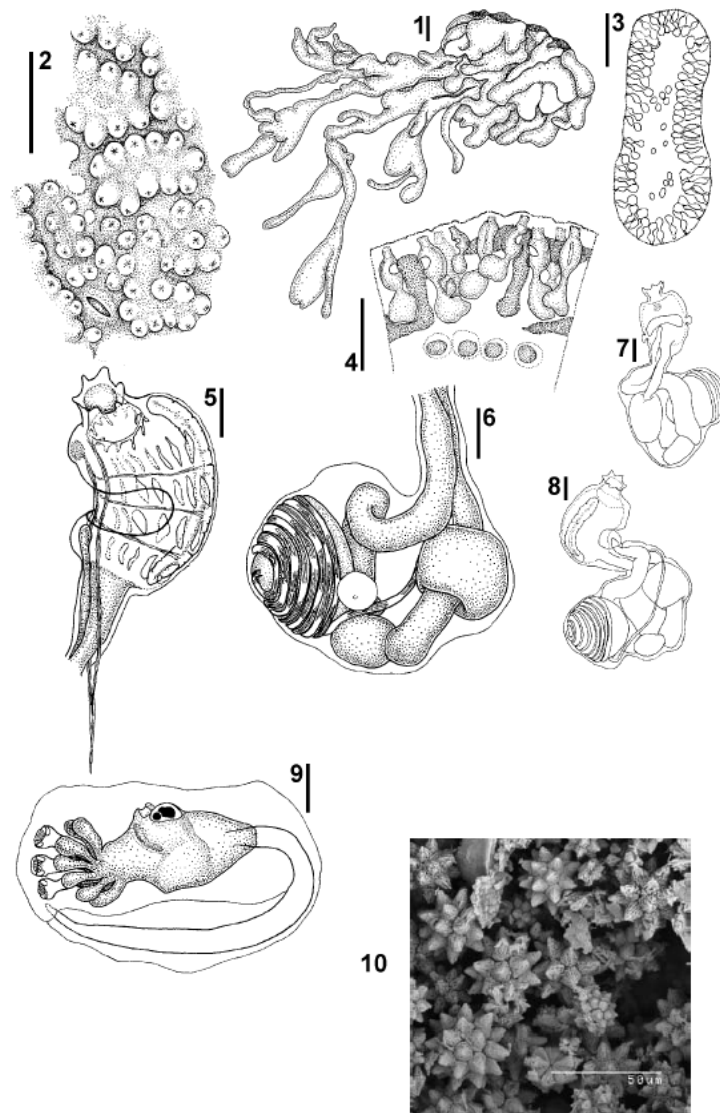
Figure 2.2. (1) Outline of part of a colony; (2) colony surface showing branchial apertures; (3) cross section of colony lobe showing zooid layer at surface and embryos in test core; (4) vertical section of part of colony showing zooids, embryos, and common cloacal canals (darkly shaded); (5) thorax; (6) abdomen; (7) whole zooid, dorsal view; (8) whole zooid, ventro-lateral view; (9) unhatched larva in tunic; (10) spicules. Scale bars: 1) 1 cm; 2 and 3) 2.0 mm; 4) 1.0 mm; 5–10) 0.1 mm (Source: Kott, 2002).

Larval characteristics also play a crucial role in the accurate identification of *D. vexillum* (Lambert, 2009). Larvae can often be found within the tunic beneath the zooids during the reproductive season. Key features of *D. vexillum* larvae include 6 pairs of lateral ampullae and three adhesive papillae (Figures 2.2 and 2.3).

Figure 2.3. Brooded unhatched *D. vexillum* larva, Whangamata, New Zealand. Scale bar: 200 μm (Photo: G. Lambert).

3 Biology/ecology

3.1 Natural history

3.1.1 Habitat

D. vexillum is a successful fouling organism because of its ability to grow on many nat-ural and artificial structures (see review in Daniel and Therriault, 2007) and can thrive in both coastal and offshore environments in depths ranging from <1 to 65 m (Valentine *et al.*, 2007a,b; Kleeman, 2009). In coastal habitats, *D. vexillum* is found clinging to the undersides of wharves, boats, and other artificial substrates, in addition to natural sur-faces, such as rocks or living organisms, usually in locations where colonies are pro-tected from wave action, predation, sedimentation, and deposited fecal matter (Bullard *et al.*, 2007; Coutts and Forrest, 2007; Osman and Whitlatch, 2007). *D. vexillum* can en-gineer habitats by utilizing resources and space and is a well-known nuisance, due to its ability to rapidly foul harbour facilities, vessel hulls, and aquaculture equipment (Blum *et al.*, 2007; Valentine *et al.*, 2009). In offshore environments, such as Georges Bank in the Northwest Atlantic, huge masses of colonies have been observed by un-derwater ROVs, covering more than 230 km^2 on benthic substrates (Valentine *et al.,* 2007a). However, shifting sandy substrates appear to limit colony formation in this area.

3.1.2 Feeding

Only the adult stages of ascidians are active filter feeders, whereas larval stages (which have a very short duration) obtain energy from an egg yolk (Cloney, 1978; Tyree, 2001). Like all other ascidians, *D. vexillum* is a ciliary-mucus feeder that relies primarily on phytoplankton for nutrients, but it also feeds on small particulate matter and sus-pended bacteria (Millar, 1971; Lambert, 2005). Factors such as the amount of food in the water, particle size, filtering capacity (which increases with the size of the colony), and time spent filtering all affect feeding rates (Millar, 1971; Tyree, 2001). For these reasons, actual filtering capacity for colonial ascidians is difficult to determine and re-mains unknown (Daniel and Therriault, 2007).

3.1.3 Environmental tolerances

Like other invasive species, *D. vexillum* can tolerate wide ranges of environmental pa-rameters, including temperature and salinity (Lambert, 2005; Valentine *et al.*, 2007b, 2009). Like most ascidians, *D. vexillum* colonies are rarely found in salinities less than 25 psu (Millar, 1971; Lambert, 2005), and colony die-offs have been observed at salini-ties below 20 psu (Bullard and Whitlatch, 2009). In fact, Bullard and Whitlatch (2009) showed that optimal growth of *D. vexillum* colonies occurs at higher salinities (26–30 psu) compared to medium and low salinities (i.e. 15–28 psu, 10–26 psu). Valentine *et al.* (2007b) demonstrated that *D. vexillum* colonies can survive temperatures ranging from –2 to 24°C, and fluctuations of up to 11°C in one day. While survival is not an issue within this wide range, *D. vexillum* thrives best within a narrower temperature range (14–20°C; Valentine *et al.*, 2009), which may vary among colonies from different locations and exposed to different climates (Fletcher *et al.*, 2013a). For example, colonies grow more rapidly in water temperatures between 15 and 20°C than those grown in temperatures >21°C (McCarthy *et al.*, 2007). Furthermore, recruitment generally occurs between 14 and 20°C, but continues into cooler temperatures towards the end of the season. Colonies have been observed to initiate both development and spawning at the start of the season (Millar, 1971; Valentine *et al.*, 2009), making the lower temperature limit at the end of the spawning and larval-release season unclear. However, some data

suggest that recruitment ceases between 9 and 11°C (Kleeman, 2009; Valentine *et al.*, 2009).

3.1.4   Predation

Colonies of *D. vexillum* thrive best when attached to shaded undersides of suspended objects, like boat hulls or floats. However, colonies can be preyed upon by chiton (Kleeman, 2009), sea urchins, and other echinoderms (Bullard *et al.*, 2007; Lambert, 2009). Littorinid snails have been seen feeding on both dying, which they prefer, and live colonies (Valentine *et al.*, 2007b; Carman *et al.*, 2009; Lambert, 2009). Some research has studied the potential use of predation by organisms, such as the green sea urchin, to reduce localized populations (i.e. on suspended shellfish aquaculture), but predation rates are unlikely to be high enough to reduce populations or prevent further spread (Epelbaum *et al.*, 2009).

3.2   Reproduction

3.2.1   Asexual reproduction

D. vexillum reproduces both sexually and asexually. Asexual reproduction occurs in two ways. The first is by budding of each zooid. Didemnid budding, or "pyloric budding", is the formation of two buds near the middle or narrowing of the zooid body between thorax and abdomen. The anterior-most bud arises from the region of the oesophagus and forms a new abdomen; the posterior-most bud, which arises from the anterior of the abdomen, forms a new thorax (Van Name, 1945; Millar, 1971; Monniot *et al.*, 1991). The new buds that are formed, called blastozooids, are genetically identical to their parent zooid (Monniot *et al.*, 1991). Asexual reproduction by budding expands the colony size from the first recruit (the oozooid that forms from the settlement and metamorphosis of the tadpole larva). The second type of asexual reproduction involves the breaking off of pieces of the colony which float away, settle, and re-establish a sister colony from the fragments (Lambert, 2005; Valentine *et al.*, 2009). The establishment of colonies can occur from very small fragments (Morris and Carman, 2012). At temperatures between 6 and 10°C, ca. 77% of *D. vexillum* fragments reattached to both plastic and eelgrass substrates in laboratory experiments (Carman *et al.*, 2014).

3.2.2   Sexual reproduction

Sexual reproduction, unlike asexual reproduction, produces larvae to colonize new sites. Like all Didemnidae, *D. vexillum* is hermaphroditic and ovoviviparous. Eggs in the ovary develop one at a time, with an average of 1–20 eggs produced per zooid (Berrill, 1950; Kott, 2001; Lambert, 2005), and development of mature eggs is linked to the presence of conspecific sperm in the environment, as is the case for *Diplosoma listerianum* and probably most or all other didemnids (Bishop *et al.*, 2000a). Spawning appears to be linked to the period with maximum food availability (Lambert, 2005); the exact time of year and temperature vary with geographic location. During spawning, sperm are released from the atrial aperture of the zooid and leave the colony through the common cloacal openings. Sperm then enter the oral siphon of a different zooid in another colony (and possibly the cloacal openings) and eggs are then fertilized, probably before being released from the ovary into the tunic (Millar, 1971; Monniot *et al.*, 1991; Bishop *et al.,* 2000a; Kott, 2001). This form of broadcast spawning, where only sperm is released, is known as spermcast mating; it is likely that sperm may be stored and utilized on a first-in-first-out manner (Bishop *et al.*, 2000b; Bishop and Pemberton, 2006). Larval brooding, which can take several weeks, occurs within the tunic and re-

sults in the release of free-swimming larvae (1.4 mm in length; Lambert, 2009). Research has shown that light exposure plays a large role in both spawning and the release of ascidian larvae, including *D. vexillum* (Svane and Havenhand, 1993; Fletcher and Forrest, 2011). Larvae are released from common cloacal apertures (Fletcher and Forrest, 2011) in response to light stimulation and often at dawn in nature (Olson, 1983; Svane and Young, 1989). Didemnid tadpole larvae are equipped with two sensory organs in their trunk called the statocyte and the ocellus. Upon release from the colony, these organs cause larvae to be positively phototactic (Grave, 1937; Berrill, 1955; Monniot *et al.*, 1991) and negatively geotactic and swim towards the light (Millar, 1971; Cloney, 1982).

When larvae are ready to settle, they become positively geotactic and negatively phototactic and seek shaded areas, such as undersides of docks (Berrill, 1955; Millar, 1971; Olson, 1983; Monniot *et al.*, 1991), rather than well-lit or completely dark environments (Fletcher and Forrest, 2011). Research has shown that larvae may attach to a substrate and initiate metamorphosis only minutes after release, but most larvae will successfully settle and begin metamorphosis within 24 h (Fletcher and Forrest, 2011). Like many ascidian larvae, *D. vexillum* may begin metamorphosis before attachment to a substrate, which may have implications for increased dispersal, as it extends the possible distance travelled before attachment (Millar, 1971; Feng *et al.*, 2010; Fletcher and Forrest, 2011; Reid *et al.*, 2016).

Metamorphosis transforms non-feeding mobile tadpole larvae into sessile feeding juveniles (Cloney, 1982). Cloney (1982) has explained in detail the process of metamorphosis. Following metamorphosis, new *D. vexillum* recruits have a colourless tunic, free of spicules, a pale yellow digestive system, and a black statocyte and ocellus. Just days after settlement, the lateral organs of the thorax become white with the production of calcium carbonate spicules, which then move out into the tunic (Valentine *et al.*, 2009). Further colony expansion occurs through asexual budding, which can occur within two weeks of settlement, but is dependent on environmental conditions (Fletcher and Forrest, 2011).

3.2.3 Reproduction and growth

The success of the species as an invader is, in part, due to its impressive capacity for growth and reproduction (Kott, 2002; Bullard and Whitlatch, 2009; Lambert, 2009;). *D. vexillum* colonies in New England exhibited 6- to 11-fold increase in size in only 15 d (Valentine *et al.*, 2007b); however, it is difficult to determine the age of a colony because of regular degeneration and regrowth of colonies during cold and warm seasons respectively (Millar, 1971; Tyree, 2001; Valentine *et al.*, 2009). Like many other aspects of the life history of *D. vexillum*, colony growth and lifespan are greatly affected by season and changes in temperature, habitat, and other environmental variables (Millar, 1971; Lambert, 2005; Valentine *et al.*, 2007b; Fletcher *et al.*, 2013a). For example, growth rates slow or even stop during unfavourable conditions in colder months, while rapid colony expansion occurs during nutrient-rich and warmer months (Valentine *et al.*, 2007b). Temperatures <0°C cause colonies to regress, but not necessarily die, while the maximum temperature for survival is likely >25°C, although temperatures >23°C may have adverse effects on a colony (McCarthy *et al.*, 2007). During the warm season, highly variable temperatures likely inhibit the reproductive process and successful colonization (Valentine *et al.*, 2009).

In New England, May–July is a regrowth period when surviving remnants of overwintered colonies begin asexual budding and expansion; in July–September during the

warmest seawater conditions (e.g. ca. 20°C), colonies experience rapid growth. The degree to which colonies degrade in cooler seasons influences the length of time required to regenerate, reproduce sexually, brood, and release larvae during the warm season (Valentine *et al*., 2009). Larvae are released at the end of their developmental period, as temperatures increase, but not when a specific temperature is reached. Recruitment occurs between 14 and 20°C, and is dependent on local environmental conditions (Valentine *et al*., 2009). While growth may continue beyond December in New England, a decline coincides with decreases in temperature (McCarthy *et al*., 2007; Valentine *et al*., 2007b). However, *D. vexillum* demonstrates a greater affinity or ability to grow in cooler temperatures, compared to other invasive ascidians, such as *Botryolloides violaceus* and *Botryllus schlosseri* (McCarthy *et al*., 2007). Dijkstra *et al*. (2007) also found that *D. vexillum* in the Gulf of Maine in 2003 and 2005 had the highest percent coverage in autumn and winter, while percent coverage of *B. violaceus* was greatest in summer and autumn. Times of the year for each stage of growth can vary between geographic locations (e.g. Valentine *et al*., 2007b; Tagliapietra *et al*., 2012; Fletcher *et al*., 2013a). Evidence suggests that larvae appear at different temperatures among climatically different locations and similar temperatures at climatically similar sites (Valentine *et al*., 2009). However, regardless of location, the release of brooded larvae consistently occurs in water temperatures between 14 and 20°C (Valentine *et al*., 2009). The time it takes for a newly settled larva to begin budding to form a colony is also dependent upon environmental conditions. Fletcher and Forrest (2011) found that the majority of recruits had undergone asexual budding to become 2-zooid colonies within two weeks after settlement and metamorphosis at 18–20°C. After four weeks, they had multiplied into 4- to 6-zooid colonies.

Changes in habitat type can also influence growth cycles of *D. vexillum*. For example, colonies exhibited faster growth in open coastal habitats than in a protected marina, likely because of less competition with co-occurring species in the open habitat (Osman and Whitlatch, 2007). Further studies by Valentine *et al*. (2007b) show greater seasonal fluctuations in colony growth in a coastal tide-pool location than in subtidal habitats. In the more stable temperature regimes of deeper subtidal habitats, colonies degenerated less during the cold season, which led to a longer recruitment season than in shallow coastal locations (Valentine *et al*., 2009). While deep offshore colonies can exhibit more growth throughout the year, due to smaller temperature fluctuations, Bullard and Whitlatch (2009) compared growth at depths of 1, 2.5, and 4 m and found highest growth rates at 1 m. However, these differences may be due to differences in food availability at shallower depths, rather than changes in temperature.

While *D. vexillum* can withstand wide variations in salinity, this, like other environmental parameters, also affects colony growth. A controlled experiment carried out in the natural environment by Bullard and Whitlatch (2009) showed that higher salinity in the range of 26–30 psu (normal seawater salinity) results in higher growth than in 15–28 psu or 10–26 psu. In fact, colonies exposed to lower salinities experienced dieoffs during this experiment.

4 Distribution

4.1 Native range

Deciphering the native and invasive distribution of an invasive ascidian is vital to effectively managing and controlling the species (Stefaniak *et al.*, 2012). *D. vexillum* likely originated in Japan (Figure 4.1; Lambert, 2009; Stefaniak *et al.*, 2009). The description of *D. vexillum* matches that of specimens identified as *D. pardum* (Nishikawa, 1990), the earliest of which was a museum sample collected in 1926 from Mutsu Bay in northern Japan (Lambert, 2009), where it is still common today (Lambert, 2009), as well as numerous other sites in Japan. Stefaniak *et al*. (2012) utilized the DNA sequence of two genes, cytochrome c oxidase subunit 1 (*co1;* mitochondrial) and THO complex subunit (tho2; nuclear) to determine the portion of the current distribution that was native. They demonstrated that Japan is the most genetically diverse region for *D. vexillum* (a typical test for determining endemicity), although there is possibly a wider native distribution. While initial reports suggested that *D. vexillum* was spread with the importation of oysters and spat from Japan prior to the 1960s, this hypothesis was rejected because there were no reports of sudden appearance of this species prior to the 1970s (Lambert, 2009). It is more plausible that introductions occurred through shipping (either from fouling on boat hulls or in sea chests), while secondary spread within regions likely resulted from recreational boating activities (Lambert, 2009).

4.2 Invasive range

The invasive distribution includes New Zealand, the Netherlands, France, Ireland, United Kingdom, Spain, Italy, and both the west and east coasts of the United States and Canada (Figure 4.1). The invasive range is described by country and chronicles the first detection in the region (e.g. earliest European report is from the Netherlands). This does not always imply spread, but highlights the relatively recent introduction of this species in several regions and the potentially high risk for its spread to neighbouring areas or countries, if current biosecurity practices are not considered and controlled.

4.2.1 New Zealand

In New Zealand, *D. vexillum* was first detected in October 2001 on the North Island near Tauranga Harbour and Whangamata Harbour (Figure 4.2A; Kott, 2002; Kleeman, 2009; Lambert, 2009). This area is used largely by recreational boaters, and the colonies at the time dominated the community on 112 of 130 mooring posts and on some of the infrequently-used anchored boats (Kott, 2002). The description provided by Kott (2002) of these colonies would later be declared as the official species description of *D. vexillum* by Lambert (2009). Two months later, it was discovered in Shakespeare Bay, ca. 500 km south of its original detection site, after the movement of a heavily fouled barge (Coutts and Forrest, 2007). *D. vexillum* was subsequently detected on the seabed below the barge (Coutts and Forrest, 2007).

4.2.2 The Netherlands

The earliest European report of *D. vexillum* was in the Netherlands in 1991 by Ates (1998), who listed it as a range extension of *D. lahillei* in the Dutch Delta (Figure 4.2B; Gittenberger, 2007; Lambert, 2009; Gittenberger *et al*., 2014). Ates (1998) reported the species as "extremely dominant" in the Zijpe region (Eastern Scheldt estuary). By 2014, the species was confirmed in the Dutch Wadden Sea off Terschelling, Oudeschild, Texel, and Vlieland, as well as on oyster reefs near Terschelling and Texel (Gittenberger *et al*., 2014).
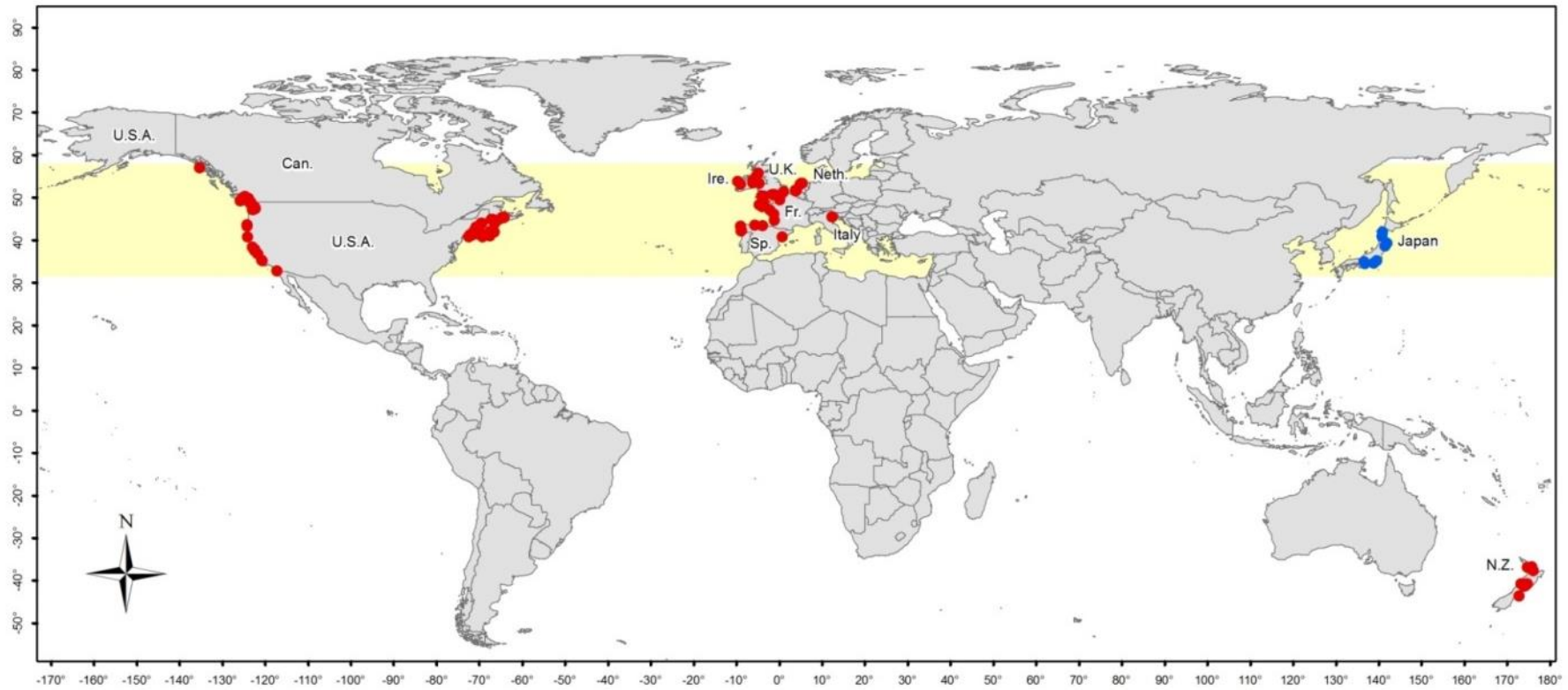
Figure 4.1. Map showing global native (blue) and invasive (red) geographic range of *Didemnum vexillum*. Potential Northern Hemisphere distribution range limits (in yellow) are based on current distribution.

4.2.3 France

The first record of *D. vexillum* in France may have been as early as 1968 in the Glénan archipelago (Lafargue, 1968), but was only confirmed in December 1998 in the port of Le Havre. Colonies were then found in 2002 on floating docks, at two additional harbours in northwest France (Figure 4.2B; Perros Guirec and Camaret-sur-Mer). In both instances, the species was originally identified as *D. lahillei* (Lambert, 2009). By 2005, the species had spread to new ports around the French Atlantic coast, including Brest and Concarneau (Stefaniak *et al.*, 2009), and has remained prevalent (Valentine, 2003; Breton, 2005, 2014; Goulletquer, 2016). Due to regional shellfish stock movements, further spreading of the species is likely (FranceAgriMer, 2013). As the species is reported to be present in neighbouring countries in the Mediterranean (the Ebro delta in Spain and along the Italian Adriatic coast), its spread to the French Mediterranean coast is highly likely. Its environmental tolerance makes it possible for it to extend to the entire French coastline.

4.2.4 Ireland

The first record of *D. vexillum* in Ireland was in Malahide Estuary, north of Dublin on the east coast of the island in 2005 (Figure 4.2B; Minchin and Sides, 2006). Colonies were found overgrowing chains, ropes, pontoons, boat hulls, and fouling organisms, such as sabellid worms and mussels. Due to the extensive fouling by this species, it was assumed that the arrival was not recent. Some years later during a heavy rainfall event, all tunicates attached to the marina pontoons expired. In 2006, it was reported farther north in Carlingford Lough (Minchin, 2007), and additional surveys found *D. vexillum* on the west coast in 2007 in Clew Bay and Galway Bay associated with mussel longline cultivation and on oyster bags held on trestles. Along one shore, the tunicate was found to cover the sides of boulders and stones at low water spring tides. In Strangford Lough in Northern Ireland, it was found attached to the hull of an unused lightship serving as a yacht-club base. Both carpet and pendulous colonies were found (Minchin and Nunn, 2013).

4.2.5 United Kingdom

An established population of *D. vexillum* was found in September 2008 in Holyhead Harbour, north Wales (Figure 4.2B). Follow-up rapid assessment surveys in Wales showed that this was an isolated invasion (Griffith *et al.*, 2009). Following several waves of eradication attempts within the harbour in 2009 and 2010, colonies re-established onto freshly treated surfaces (Holt and Cordingley, 2011). After further eradication attempts during the winters of 2010, 2011, and 2012, during which all surfaces were treated, no additional colonies were observed. In addition to the eradication, development of a decontamination berth was undertaken. Initial trials looked promising, but parts of the equipment were damaged during a winter storm. A self-cleaning rotating pontoon float was also developed (R. Holt, pers. comm.). Since then, populations have been found extending along the southern English coast (Plymouth, Kingswear, Lymington, Cowes, and Gosport) and farther east and on the north coast of Kent (Hitchin, 2012; Bishop *et al.*, 2015). In 2010, *D. vexillum* was found in the Clyde at Largs Yacht Haven on the southwest coast of Scotland. In a follow-up survey, the species was found in three additional locations in close proximity to the yacht haven, including Fairlie Quay Jetty, Fairlie moorings, and Clydeport Jetty (Beveridge *et al.*, 2011), but was not found in northern Scotland (Nall *et al.*, 2015). To date, this is the most northerly record for *D. vexillum* in Europe.

4.2.6 Spain

D. vexillum was first reported in 2008 in northern and northwestern Spain (Figure 4.2B; Santander, Baiona, Moaña, Corme-Porto, and Gijón; Nagar *et al.*, 2010). The range of *D. vexillum* in Spain expanded to the Mediterranean coastline in May 2012 on cultivated oysters in the Ebro Delta (Ordóñez *et al.*, 2015).

4.2.7 Italy

The first reported *D. vexillum* population was in 2012 in the Venetian Lagoon (Figure 4.2B; Tagliapietra *et al.*, 2012) and was later verified in five other locations inside the same lagoon (Venetian Arsenal, Certosa marina, San Nicolò, Sant'Andrea beacon tower, and Marina di Lio Grando) overgrowing various invertebrates and other ascidians, in addition to docks, pilings, and pontoons (Ordóñez *et al.*, 2015).

4.2.8 Germany

Germany has a monitoring programme dedicated to locating new non-indigenous species, with several sampling stations in ports. To date, no *D. vexillum* has been detected, although it occurs in nearby countries.

4.2.9 United States

In the United States, established populations have been reported from both the west (Figure 4.2C) and east (Figure 4.2D) coasts. The first observed population on the northeast coast was in the late 1980s (although its identification was not confirmed until 2000) and on the west coast during the 1990s (Bullard *et al.*, 2007). Rapid population expansion has led this species to become a dominant member of the subtidal community on both coasts (Bullard *et al.*, 2007). During surveys conducted between 1998 and 2005 by Bullard *et al.*, (2007), west coast populations ranged from several sites in Puget Sound, Washington and from Humboldt Bay to San Diego Bay in California (Figure 4.2C; Lambert, 2009). East coast populations extended from Eastport, Maine to Shinnecock Bay, New York (Figure 4.2D). Deeper subtidal populations also occur off the northeast coast, including Georges, Stellwagen, and Tillies banks, with coverage reaching 50–90% on Georges Bank (Bullard *et al.*, 2007), marking the first detection of this species in an offshore habitat (Langyel *et al.*, 2009). On Georges Bank, the species has been observed growing in continuous mats over an area covering 230 km^2, with coverage up to 90% and at depths between 45 and 65 m (Bullard *et al.*, 2007; Valentine *et al.*, 2007a). The northern-most distribution of *D. vexillum* in the United States is in coastal waters of Alaska, where it was first discovered in Whiting Harbor near Sitka on an oyster farm. This was the only site out of the 10 surveyed in the region where *D. vexillum* was detected. (Cohen *et al.*, 2011; McCann *et al.*, 2013).

4.2.10 Canada

D. vexillum has invaded both the west and east coasts of Canada (Figure 4.2C, D). The species is present in British Columbia on the west coast and Nova Scotia on the east coast. Established populations were first discovered in British Columbia in 2003, where colonies were found fouling mussel cages in Okeover Inlet; shortly thereafter, colonies were also detected in Deep Bay and Lemmens Inlet (Figure 4.2C). The species has since been detected in numerous locations on the east and west sides of the Strait of Georgia, including various inlets and harbours and along the south and west coast of Vancouver Island (Daniel and Therriault, 2007; Therriault and Herborg, 2007; Lambert, 2009). Coincidently, as of 2009, the infestations are on or near Pacific oyster (*Crassostrea gigas*)

farms, which were originally imported from Japan (Lambert, 2009). Probably, however, the current infestations on oyster farms have resulted from movement of contaminated oysters from spawning and settlement bays in British Columbia to grow-out areas.

Although several rapid assessment surveys targeted at *D. vexillum* were conducted in southwest New Brunswick near Eastport, Maine in 2010 and 2012 (Martin *et al.,* 2010; Sephton and Vercaemer, 2015) and southwest Nova Scotia in 2013 (Sephton and Vercaemer, 2015), no *D. vexillum* was detected at survey locations. *D. vexillum* was discovered by a recreational diver in October 2013 (although it may have been present as early as 2011) in Parrsboro, Nova Scotia, attached to rock substrate (Figure 4.2.D). This marked the northern-most population of this species on the east coast of North America (Moore *et al.,* 2014). Rapid response surveys were conducted in the Minas Basin in the Bay of Fundy off Parrsboro in April 2014, and further sampling took place in May–August of the same year, during a scallop stock assessment in the Bay of Fundy and Scotian shelf (German Bank, northern Browns Bank, and eastern Georges Bank; Vercaemer *et al.,* 2015). Extensive coverage of *D. vexillum* was found in the Minas Basin and Minas Channel and additional sites in the Bay of Fundy off Digby Gut and Yarmouth (Moore *et al.,* 2014; Vercaemer *et al.,* 2015). The introduction into the Bay of Fundy is likely from populations along the east coast of the United States, probably introduced to this new area through coastal vessel activity.
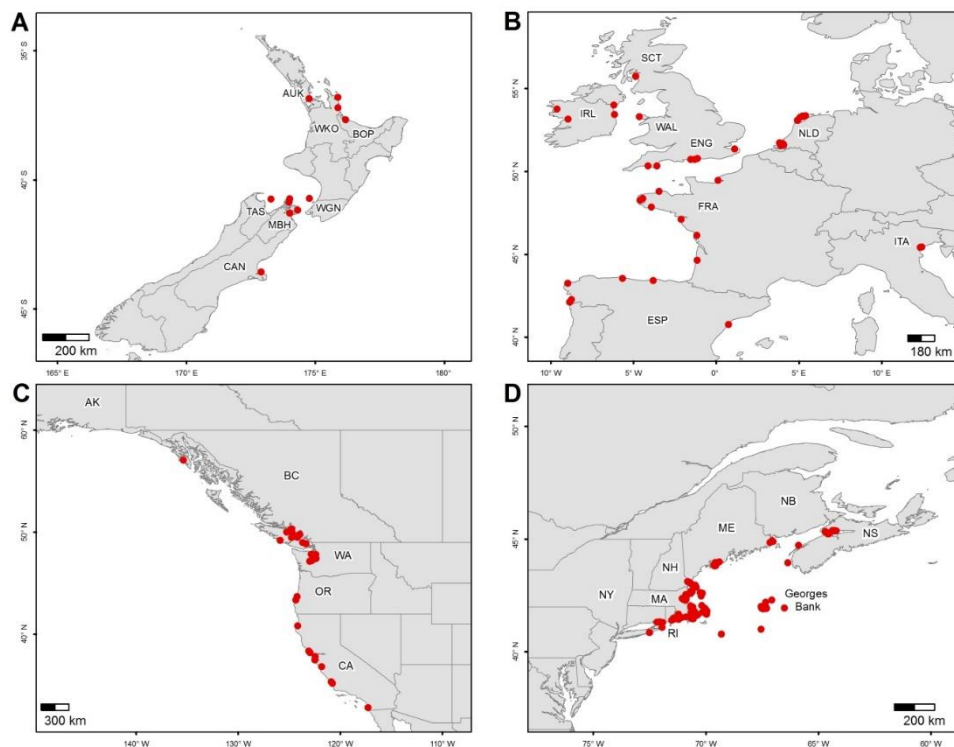

Figure 4.2. Map showing invasive (red circles mark a station) geographic range of *Didemnum vexillum* by region. (A) New Zealand; (B) Europe; (C) North America west coast; (D) North America east coast.

5   Impacts

Invasive biofouling species have created a range of complications for marine commercial activities and are considered one of the primary issues facing the marine aquaculture industry (Lambert, 2007). Shellfish aquaculture is particularly susceptible because operations create an array of multifaceted artificial substrates and are often positioned in protected embayments with significant food resources, creating optimal conditions for fouling organisms (Osman and Whitlatch, 1999; McKindsey *et al*., 2007). Solitary ascidians, such as *Styela clava* (clubbed tunicate) and *Ciona intestinalis* (vase tunicate) have had considerable impacts on shellfish aquaculture, increasing costs for production and processing (e.g. Carver *et al*., 2003; Thompson and MacNair, 2004; Ramsay *et al*., 2008) and negatively affecting meat yields and growth rates because of increased competition for resources (Daigle and Herbinger, 2009).

In New Zealand, the recent introduction of *D. vexillum* has threatened the green-lipped mussel (*Perna canaliculus*) industry in the Marlborough Sound region. Early income loss for the green mussel industry had been estimated to be more than NZD 800 000 over five years (Sinner and Coutts, 2003). Experiments by Fletcher *et al*. (2013a) demonstrated that early life stages of green-lipped mussels (i.e. 20–40 mm) are most vulnerable to fouling by *D. vexillum*, and impacts are mostly restricted to fouling-related displacement of spat, rather than to reduced growth and condition. Elsewhere, studies in northeast United States have shown that overgrowth by *D. vexillum* can lead to decreased growth rates (Auker, 2010). On the west coast of Canada, Pacific oysters fouled by *D. vexillum* were shown to have a lower condition index than oysters that underwent chemical or mechanical treatments to reduce fouling (Switzer *et al*., 2011). These impacts can also be the result of decreased water flow to shellfish, limiting access and creating competition for food resources.

Beyond artificial substrates, *D. vexillum* can readily foul natural substrates (Valentine, 2003; Valentine *et al*., 2007a). In addition to pebbles, cobble, and boulders, it can rapidly overgrow and out-compete species, such as other tunicates, hydroids, seaweeds, sponges, and various bivalves (Valentine *et al*., 2007a,b; Lengyel *et al*., 2009). In northeast United States, *D. vexillum* has been observed growing on eelgrass (Carman and Grunden, 2010; Carman *et al*., 2016), which may lead to reduced growth due to reductions in light transmission, as observed in eelgrass fouled by other invasive colonial ascidians (Wong and Vercaemer, 2012). Overgrowth by *D. vexillum* can decrease swimming ability in sea scallops (*Placopecten magellanicus*), which may limit their ability to escape predation and access food-rich habitats, which may ultimately affect growth and survival (Dijkstra and Nolan, 2011). Also, *D. vexillum* may affect recruitment of bay scallops (*Argopecten irradians*), as scallop larvae have been observed to avoid settlement on *D. vexillum* colonies (Morris *et al*., 2009).

Unlike other fouling invasive ascidians, *D. vexillum* can alter habitat complexity by forming extensive mats over cobble–pebble substrates (Mercer *et al*., 2009). Mercer *et al*. (2009) and McCann *et al*. (2013) found no substantial differences in benthic diversity between infested and non-infested areas; only subtle changes in community structure were observed, which included more deposit-feeders and infauna in fouled samples, possibly a result of decreasing foraging ability by larger predators. However, Lengyel *et al*. (2009) demonstrated that *D. vexillum* can significantly increase abundance of polychaete species. It has been speculated that such an altered benthic habitat community structure may negatively affect prey availability for benthic fish species (Lengyel *et al*., 2009). It might also have an impact on Atlantic herring (*Clupea harengus*) spawning

grounds where eggs are laid on gravel, which can be localized in shallow coastal waters.

6   Prospects for further invasions

Factors leading to further spread of invasive ascidians include fragmentation of established colonies that result in local population intensification, combinations of anthropogenic and larval dispersal (local range expansions), and human-mediated transport (regional scales or greater; Wasson *et al.*, 2001). Tadpole larvae of colonial ascidians are generally motile for only a few hours before settling, which tends to retain populations close to the site of origin, but leads to local intensification and usually limits further natural spread of the species (Berrill, 1950; Lambert, 2002). However, Fletcher *et al.* (2013b) consistently detected larval recruitment of *D. vexillum* 250 m away from the source, and models suggest that dispersal >1 km is theoretically possible. *D. vexillum* colonies that form long tendrils are susceptible to fragmentation from wave action and mechanical forces from vessels or equipment and can survive for extended durations of time (up to 30 days) to disperse and establish new populations (Figure 6.1; Bullard *et al.*, 2007; Reinhardt *et al.*, 2012). Furthermore, the colonization of seagrasses or macroalgae by *D. vexillum* can lead to rafting colonies, as seagrass shoots or algal fronds break and have the potential to travel farther than individual larvae (Worcester, 1994; Carman and Grunden, 2010; Fletcher *et al.*, 2013b). While non-feeding tadpole larvae remain in the plankton for a very limited time and thus are not a factor for anthropogenic transport in ballast water, this may be a significant vector for the transport of colony fragments.

Artificial substrates, such as those in marinas, can provide a competitive advantage to non-indigenous fouling species over native species since native species may not be as evolutionarily adapted to colonize as wide an array of novel surfaces (Tyrrell and Byers, 2007). Closely spaced networks of harbours and other artificial substrates can act as dispersal strongholds and "stepping stones" for introduced ascidians (Wasson *et al.*, 2001; López-Legentil *et al.*, 2015).

As globalization accelerates, the risk of continued spread of non-indigenous species worldwide also increases (Ruiz *et al.*, 1997, 2000; Cohen and Carlton, 1998). As ascidian larvae are non-feeding and generally short-lived, new introductions and spread of invasive ascidians, including *D. vexillum*, are expected primarily through human-mediated transport (e.g. fouled vessels and aquaculture gear; Figure 6.1; Kleeman, 2009; Lacoursière-Roussel *et al.*, 2012; Roche *et al.*, 2015). For example, recreational boating is considered a high-risk vector for primary introduction and secondary spread of non-indigenous ascidians, in part because it is a largely unregulated vector (Wasson *et al.*, 2001; Clarke-Murray *et al.*, 2011; Simard *et al.*, 2016). The IMO (2011) has provided biofouling guidelines for commercial and recreational vessels, which could be strong management tools to avoid the future spread of this species, particularly when used as a basis for biosecurity protocols. Since TBT (tributylin) was banned, anti-fouling coatings are less effective at limiting transport of non-indigenous species. Research continues on finding effective alternative antifouling coatings and treatments for vessels and gear. Enclosed seas, such as the Mediterranean, where yachting and recreational boating are increasing (Occhipinti-Ambrogi *et al.*, 2016) are particularly exposed to the rapid spread of this ascidian. The October 2015 sighting in Crete (Greece) of putative colonies of *D. vexillum* (Aylin Ulman, pers. comm.) may confirm the eastward progression of this species. However, in-water encapsulation treatment methods provide novel attempts to limit transfer of fouling non-indigenous species on vessels at marine transportation hubs (Roche *et al.*, 2015).
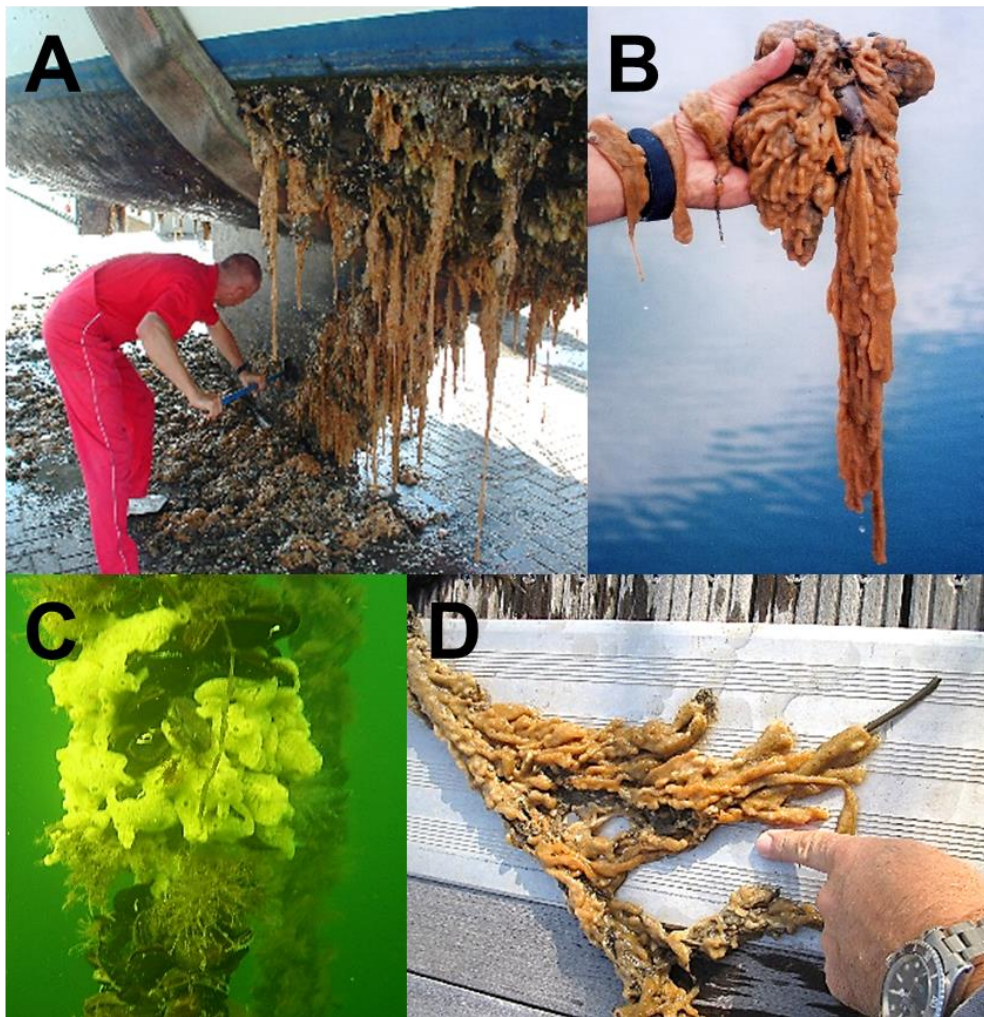
Figure 6.1. Growth patterns of *Didemnum vexillum*. (A) Pendulous growth on a yacht hull in 2006 (Photo: Damien Offer, Manager of Malahide Marina, Ireland); (B) Pendulous growth removed from a yacht hull in Malahide Marina, Ireland, June 2006 (Photo: Dan Minchin); (C) *D. vexillum* attached to cultivated mussels in Clew Bay, Ireland October 2008 (Photo: Fergal Guilfoyle); (D) *D. vexillum* overgrowing sabellid worms in Malahide Marina, October 2015 (Photo: Dan Minchin).

As management of long-established invaders is increasingly being questioned (Simberloff, 2014), prevention through the restriction of introduction pathways is the universally preferred management option. However, there are inherent challenges because of the high number and complexity of pathways combined with the openness of marine environments. The development of marine biosecurity strategies is critical for effective prevention, as well as preparedness and response to invasions. Without such rapid-response plans and the legal authority to implement them, the time to decide "what to do" limits the rapidness of any response (Kleeman, 2009; McKenzie *et al*. 2016). Furthermore, the risk of reinoculation from nearby sources, especially those where several countries share geographic boundaries (e.g. UK) is greater without widespread biosecurity protocols (Kleeman, 2009; Sambrook *et al.*, 2014). With recent uncontrolled novel introductions and range expansions of *D. vexillum* in locations such as, but not limited to, Atlantic Canada (Vercaemer *et al.*, 2015), Alaska (McCann *et al.*, 2013), regions of Europe (e.g. Ates, 1998; Minchin and Sides, 2006, Griffith *et al.*, 2009), and New Zealand (Forrest and Hopkins, 2013), further introductions and local population intensification are likely. For example, consequences of climate change, such as

the disappearance of summer Arctic sea ice, can lead to increased opportunities for shipping, mineral exploration, and other human developments and may greatly increase invasion prospects in the Arctic (Ruiz and Hewitt, 2009).

7   Prospects for control or management where introductions occur

Prospects for control and management of invasive species have gained an increasingly high profile with the introduction of numerous policies that address management of invasive species (IMO, 2004, 2011; Hewitt and Campbell, 2007). Thus far, examples showcasing the eradication of an established marine invasive species are rare (e.g. Anderson, 2005), but it is increasingly recognized that control or management efforts are worthwhile strategies, although the probability of success depends primarily on compliance with biosecurity protocols. Lessons learned from recent attempts to manage invasive ascidians (e.g. Coutts and Forrest, 2007; Kleeman, 2009; Rolheiser *et al.*, 2012; McKenzie *et al.*, 2016) and development of biosecurity measures, such as those in Australia requiring records of international vessel movement in and out of marinas, regular cleaning, and application of antifouling coatings (Sambrook *et al.*, 2014) provide frameworks for future management of *D. vexillum*. Attempts to eradicate *D. vexillum* have proven unsuccessful in the long term because of, but not limited to, lack of sustained efforts, affordable control tools, stakeholder support, vector management, delayed responses, spatial distribution of species, and site topography (Coutts and Forrest, 2007; Sambrook *et al.*, 2014). Regarding vector management, the IMO Ballast Water Management Convention and IMO Biofouling Guidelines could be strong management tools to avoid the future spread of this species (IMO, 2004, 2011).

A variety of methods have been tested to remove or control *D. vexillum*, including exposing the colonies to different chemical solutions, such as acetic acid, lime, chlorine, bleach, brine, freshwater, or desiccation (air drying). Most control options have been tested as potential solutions to mitigate growing concerns about invasive tunicate impacts in shellfish aquaculture. With this in mind, solutions must effectively remove biofouling organisms, but have limited or acceptable impacts on shellfish or other non-target organism survival and the environment (Locke *et al.*, 2009). The use of lime solutions (3–4%) for 4–5 min effectively removed *D. vexillum* from Pacific oysters (*Crassostrea gigas*) without adverse impacts on oyster survival, growth, or condition, but multiple applications are suggested to attain 100% mortality in *D. vexillum* (Switzer *et al.*, 2011; Rolheiser *et al.*, 2012). Although the use of 5% acetic acid can be effective against *D. vexillum* even after 1 min, 5% acetic acid can also dramatically reduce the survival of oysters (Piola *et al.*, 2010; Rolheiser *et al.*, 2012; McCann *et al.*, 2013). Furthermore, studies in New Zealand indicated that acetic acid, sodium hydroxide, and bleach are all effective at reducing fouling of *D. vexillum* on green-lipped mussel seed, but only 0.5% bleach resulted in 100% tunicate mortality with limited impact on seed mussels (Denny, 2008). The use of brine and freshwater did not reduce fouling after short (<10 min) exposures (Rolheiser *et al.*, 2012), but longer-term exposures (4 h) effectively removed *D. vexillum* (McCann *et al.*, 2013). A recent study designed and tested decontamination berths, which are based on the concept that a vessel can be positioned in an enclosed berth, seawater can be pumped out, and a chemical solution pumped in to treat non-indigenous species (Roche *et al.*, 2015). This study suggests that treatments with acetic acid and sodium hypochlorite were equally effective at reducing fouling by *D. vexillum*, and the development of decontamination berths (or similar devices) can be useful to contain the spread of other non-indigenous species (Roche *et al.*, 2015). This method may be particularly effective in killing organisms that have settled in sea chests, which are under the waterline and inaccessible except during dry-docking. Mechanical cleaning has proven effective (Switzer *et al.*, 2011), but biological controls testing susceptibility of *D. vexillum* or other ascidians to various predators (e.g. urchin, sea star, crab, gastropod, periwinkles, and nudibranchs) are unlikely to limit biofouling by these species (Carman *et al.*, 2009; Epaulbaum *et al.*, 2009; Switzer *et al.*, 2011).

Eradication of *D. vexillum* was attempted in Shakespeare Bay, New Zealand, in 2003, following its discovery and concerns for its likely spread to shellfish aquaculture sites in the region. The tunicate spread from a barge to the seabed beneath where it was moored and to nearby vessels and artificial structures (Coutts and Forrest, 2007). Coutts and Forrest (2007) documented the short-term removal success of *D. vexillum* by taking out and cleaning vessels, encapsulating pilings, barge hulls, and an artificial rock wall with plastic sheeting, and smothering the seabed with uncontaminated dredge spoil (Pannell and Coutts, 2007). After considerable biomass of *D. vexillum* re-established and spread, sustained regional control efforts occurred in 2006–2008 (Forrest and Hopkins, 2013). These sustained efforts had increased benefits in reducing risks of further vector reinfection and spread, but the termination of control measures due to very high costs again led to rapid biomass accumulation of *D. vexillum* (Forrest and Hopkins, 2013). Most recently, focus has shifted to biosecurity measures in an attempt to prevent further spread.

Following the discovery of *D. vexillum* in Holyhead Marina, north Wales in 2008, it was determined that eradication would be viable because of its limited natural dispersal potential and the consensus that the invasion was detected early (Kleeman, 2009; Sambrook *et al.*, 2014). Based on previous knowledge gained from activities in New Zealand, wrapping, chemical application, and removal methods were scheduled when water temperatures were between 9 and 11°C. This timing was chosen because *D. vexillum* had been observed to cease recruitment at these temperatures (Valentine *et al.*, 2009). However, this eradication attempt did not succeed partly because larvae continued to settle on panels in the marina until water temperatures fell below 9°C, which occurred 6 weeks after initiation of eradication activities (Sambrook *et al.*, 2014). The difficulty in securing funding led to delays in a second eradication attempt until 2012, and although results were promising, the continued appearance of small colonies led to focusing on long-term containment (Sambrook *et al.*, 2014).

8 Summary and conclusions

Didemnum vexillum has demonstrated the ability to be a highly invasive colonial tunicate with the capacity to reproduce rapidly, outcompete native species, deteriorate environmental integrity, and cause significant economic harm. Identification of *D. vexillum* Kott (2002) is challenging due to the vast number of species within the genus *Didemnum*. Increased taxonomic training in ascidian morphology is an important tool in early detection. In addition to traditional identification, ongoing work to develop genetic molecular tools may aid future detection of invasive ascidians for early detection and rapid response activities.

D. vexillum can engineer habitats by utilizing resources and is a well-known nuisance due to its ability to rapidly foul harbour facilities, vessel hulls, and aquaculture equipment. In offshore environments, colonies have been observed growing on benthic rocky substrates. Although *D. vexillum* has predators (e.g. chiton, sea urchins, other echinoderms, and littorine snails), predation is unlikely to be high enough to reduce populations or prevent further spread. The species' success as an invader is partly due to its impressive capacity for growth and reproduction. *D. vexillum* colonies in New England exhibited a six- to 11-fold increase in size in only 15 d. Like many other aspects of *D. vexillum,* life history, colony growth, and lifespan are greatly affected by season, changes in temperature, and habitat, among other environmental variables. The consequences of climate change, such as the disappearance of summer Arctic sea ice, can lead to increased opportunities for shipping, mineral exploration, and other human developments, and may greatly increase invasion prospects in the Arctic and expand current temperature-limited ranges for this species.

D. vexillum likely originated in Japan; however, the invaded distribution now includes New Zealand, the Netherlands, France, Ireland, United Kingdom, Spain, Italy, and both the west and east coasts of the United States and Canada. Impacts include aquaculture (e.g. bivalve spat settlement displacement, decreased growth rates, lower condition index), overgrowth, and out-competition of species (e.g. seaweeds, sponges, bivalves), and reduced growth of overgrown eelgrass. Unlike other fouling invasive ascidians, *D. vexillum* can alter habitat complexity by forming extensive mats over cobble–pebble substrates, which may negatively affect prey availability for benthic fish species and Atlantic herring spawning grounds.

Factors leading to further spread of these invasive ascidians include fragmentation of established colonies resulting in local population intensification, combinations of anthropogenic and larval dispersal (local range expansions), and human-mediated transport, particularly as biofouling on vessel hulls (both commercial and recreational). As ascidian larvae are non-feeding and generally short-lived, new introductions and spread of invasive ascidians, including *D. vexillum*, are expected primarily through human-mediated transport (e.g. fouled vessels and aquaculture gear). For example, recreational boating is considered a high-risk vector for primary introduction and secondary spread of non-indigenous ascidians, partly because vessel biofouling (commercial and recreational) is a largely unregulated vector.

Attempts to eradicate *D. vexillum* have proven unsuccessful in the long term, because of, but not limited to, lack of sustained efforts, affordable control tools, stakeholder support, vector management, delayed responses, spatial distribution of species, and site topography. A variety of methods has been tested to remove or control *D. vexillum*, including exposing the colonies to different chemical solutions, such as acetic acid, lime, chlorine, bleach, brine, freshwater, or desiccation (air drying). Most control options have been tested as potential solutions to mitigate growing concerns of invasive

tunicates in shellfish aquaculture. With this in mind, solutions must effectively remove biofouling organisms, but have limited or acceptable impacts on shellfish or other non-target organism survival and the environment. A recent study designed and tested decontamination vessel berths, which are based on the concept that a vessel can be positioned in an enclosed berth, seawater can be pumped out, and a chemical solution pumped in to treat non-indigenous species.

Prevention through the restriction of introduction pathways is the universally preferred management option for invasive species. However, there are inherent challenges because of the high number and complexity of pathways combined with the openness of marine environments. The IMO Ballast Water Management Convention (2004) and the IMO Biofouling Guidelines (2011) could be strong management tools to avoid the future spread of this species.

The development of marine biosecurity strategies and the legal frameworks to implement them are critical for effective prevention, as well as preparedness and response to invasions. Furthermore, the risk of reinoculation from nearby sources, including those where several countries share geographic boundaries, is greater without widespread, regional, biosecurity protocols.

9   References

Anderson, L. W. J. 2005. California's reaction to *Caulerpa taxifolia*: a model for invasive species rapid response. Biological Invasions, 7: 1003–1016.

Ates, R. 1998. De druipzakpijp, *Didemnum lahillei* Hartmeyer, 1909 in Zeeland [The sea squirt *Didemnum lahillei* Hartmeyer, 1909 in Zeeland] Het Zeepaard, 58(4): 101–110.

Auker, L. A. 2010. The effects of *Didemnum vexillum* overgrowth on *Mytilus edulis* biology and ecology. PhD Thesis, University of New Hampshire, Durham, New Hampshire, USA. 152 pp.

Berrill, N. J. 1935. Studies in tunicate development. IV. Asexual reproduction. Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences, 255: 327–379.

Berrill, N. J. 1950. The Tunicata, with an account of the British species. The Ray Society, London. 354 pp.

Berrill, N. J. 1955. The origin of vertebrates. Clarendon Press, Oxford. 257 pp.

Bishop, J. D., Manriquez, P. H., and Hughes, R. N. 2000a. Water-borne sperm trigger vitellogenic egg growth in two sessile marine invertebrates. Proceedings of the Royal Society of London B, 267: 1165–1169.

Bishop, J. D., Pemberton, A. J., and Noble, L. R. 2000b. Sperm precedence in a novel context: mating in a sessile marine invertebrate with dispersing sperm. Proceedings of the Royal Society London B, 267: 1107–1113.

Bishop, J. D., and Pemberton, A. J. 2006. The third way: spermcast mating in sessile marine invertebrates. Integrative and Comparative Biology, 46: 398–406.

Bishop, J. D., Wood, C. A., Yunnie, A. L. E., and Griffiths, Y. C. 2015. Unheralded arrivals: non-native invertebrates in Marinas on the English coast. Aquatic Invasions, 10(3): 249–264.

Blum, J. C., Chang, A. L., Liljesthröm, M., Schenk, M., Steinberg, M. K., and Ruiz, G. M. 2007. The non-native solitary ascidian *Ciona intestinalis* (L.) depresses species richness. Journal of Experimental Marine Biology and Ecology, 342: 5–14, doi: 10.1016/j.jembe.2006.10.010.

Breton, G. 2005. Le Port du Havre (Manche Orientale, France) et ses peuplements: un exemple de domaine paralique en climat tempéré. Bulletin de la Société zoologique de France, 130(4): 381–423.

Breton, G. 2014. Espèces introduites ou invasives des ports du Havre, d'Antifer et de Rouen (Normandie, Fr.). Hydroécologie appliquée, 18: 23–65.

Bullard, S. G., Lambert, G., Carman, M. R., Byrnes, J., Whitlatch, R .B., Ruiz, G., Miller, R. J., *et al.* 2007. The colonial ascidian *Didemnum sp*. A: current distribution, basic biology, and potential threat to marine communities of the northeast and west coasts of North America. Journal of Experimental Marine Biology and Ecology, 342: 99–108.

Bullard, S. G., and Whitlatch, R. B. 2009. *In situ* growth of the colonial ascidian *Didemnum vexillum* under different environmental conditions. Aquatic Invasions, 4(1): 275–278.

Carman, M. R., Allen, H. M., and Tyrrell, M. C. 2009. Limited value of the common periwinkle snail *Littorina littorea* as a biological control for the invasive tunicate *Didemnum vexillum*. Aquatic Invasions, 4(1): 291–294.

Carman, M. R., and Grunden, D. W. 2010. First occurrence of the invasive tunicate *Didemnum vexillum* in eelgrass habitat. Aquatic Invasions, 5(1): 23–29.

Carman, M. R., Grunden, D. W., and Ewart, D. 2014. Coldwater reattachment of colonial tunicate *Didemnum vexillum* fragments to natural (eelgrass) and artificial (plastic) substrates in New England. Aquatic Invasions, 9: 105–110.

Carman, M. R., Colarusso, P. D., Nelson, E. P., Grunden, D. W., Wong, M. C., McKenzie, C. H., Matheson, K., *et al*. 2016. Distribution and diversity of tunicates utilizing eelgrass as substrate in the western North Atlantic between 39° and 47° north latitude (New Jersey to Newfoundland). Management of Biological Invasions, 7(1): 51–57.

Carver, C. E., Chisholm, A., and Mallet, A. L. 2003. Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production. Journal of Shellfish Research, 22(3): 621–631.

Clarke-Murray, C., Pakhomov, E. A., and Therriault, T. W. 2011. Recreational boating: a large unregulated vector transporting marine invasive species. Diversity and Distributions, 17(6): 1161–1172. doi: 10.1111/j.1472-4642.2011.00798.x.

Cloney, R. A. 1978. Ascidian metamorphosis: review and analysis. *In* Settlement and Metamorphosis of Marine Invertebrate Larvae, pp. 255–282. Ed. by F. S. Chia, and M. Rice. Elsevier/North Holland Inc., New York. 302 pp.

Cloney, R. A. 1982. Ascidian larvae and the events of metamorphosis. American Zoologist, 22(4): 817–826.

Cohen, A. N., and Carlton, J. T. 1998. Accelerating invasion rate in a highly invaded estuary. Science, 279(5350): 555–558.

Cohen, C. S., McCann, L., Davis, T., Shaw, L., and Ruiz, G. 2011. Discovery and significance of the colonial tunicate *Didemnum vexillum* in Alaska. Aquatic Invasions, 6: 263–271.

Cordell, J. R., Levy, C., and Toft, J. D. 2013. Ecological implications of invasive tunicates associated with artificial structures in Puget Sound, Washington, USA. Biological Invasions, 15: 1303–1318.

Coutts, A. D., and Forrest, B. M. 2007. Development and application of tools for incursion response: lessons learned from the management of the fouling pest *Didemnum vexillum.* Journal of Experimental Marine Biology and Ecology, 342(1): 154–162.

Daigle, R. M., and Herbinger, C. M. 2009. Ecological interactions between the vase tunicate (*Ciona intestinalis*) and the farmed blue mussel (*Mytilus edulis*) in Nova Scotia, Canada. Aquatic Invasions, 4: 177–187. doi: 10.3391/ai.2009.4.1.18.

Daniel, K. S., and Therriault, T. W. 2007. Biological synopsis of the invasive tunicate *Didemnum* sp. Canadian Manuscript Report of Fisheries and Aquatic Sciences, 2788. 53 pp.

Denny, C. M. 2008. Development of a method to reduce the spread of the ascidian *Didemnum vexillum* with aquaculture transfers. ICES Journal of Marine Science, 65(5): 805–810.

Dijkstra, J., Harris, L. G., and Westerman, E. 2007. Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. Journal of Experimental Marine Biology and Ecology, 342: 61–68.

Dijkstra, J. A., and Nolan, R. 2011. Potential of the invasive colonial ascidian, *Didemnum vexillum,* to limit escape response of the sea scallop, *Placopecten magellanicus*. Aquatic Invasions, 6(4): 451–456. doi: 10.3391/ai.2011.6.4.10.

Epelbaum, A., Pearce, C. M., Baker, D. J., Paulson, A., and Therriault, T. W. 2009. Susceptibility of non-indigenous ascidian species in British Columbia (Canada) to invertebrate predation. Marine Biology, 156(6): 1311–1320.

Feng, D., Ke, C., Lu, C., and Li, S. 2010. The influence of temperature and light on larval pre-settlement metamorphosis: a study of the effects of environmental factors on pre-settlement metamorphosis of solitary ascidian *Styela canopus.* Marine and Freshwater Behaviour and Physiology, 43(1): 11–24. doi: 10.1080/10236240903523204.

Fletcher, L. M., and Forrest, B. M. 2011. Induced spawning and culture techniques for the invasive ascidian *Didemnum vexillum* (Kott, 2002). Aquatic Invasions, 6: 457–464. doi: 10.3391/ai.2011.6.4.11.

Fletcher, L. M., Forrest, B. M., Atalah, J., and Bell, J. J. 2013a. Reproductive seasonality of the invasive ascidian *Didemnum vexillum* in New Zealand and implications for shellfish aquaculture. Aquaculture Environment Interactions, 3: 197–211. doi: 10.3354/aei00063.

Fletcher, L. M., Forrest, B. M., and Bell, J. J. 2013b. Natural dispersal mechanisms and dispersal potential of the invasive ascidian *Didemnum vexillum*. Biological Invasions, 15(3): 627–643.

Forrest, B. M., and Hopkins, G. A. 2013. Population control to mitigate the spread of marine pests: insights from management of the Asian kelp *Undaria pinnatifida* and colonial ascidian *Didemnum vexillum*. Management of Biological Invasions, 4: 317–326.

FranceAgriMer. 2013. Commerce extérieur des produits de la pêche et aquaculture 2012. http://www.franceagrimer.fr/content/download/27524/242632/file/BIL-MER-commerce_ext%C3%A9rieur-A13.pdf.

Gittenberger, A. 2007. Recent population expansions of non-native ascidians in The Netherlands. Journal of Experimental Marine Biology and Ecology, 342: 122–126.

Gittenberger, A., Rensing, M., Dekker, R., Niemantverdriet, P., Schrieken, N., and Stegenga, H. 2014. Native and Non-native species of the Dutch Wadden Sea in 2014. Gittenberger Marine Research Inventory Strategy Report No. GiMaRIS Report 2015–08.

Goulletquer, P. 2016. Guide des Organismes Exotiques Marins. Editions Belin, Paris. 304 pp.

Grave, C. 1937. Notes on the culture of eight species of ascidians. *In* Culture Methods for Invertebrate Animals, pp. 560–564. Ed. by P. S. Galtsoff, F. E. Lutz, P. S. Welch, and J. G. Needham. Dover Publications, New York. 720 pp.

Griffith, K., Mowat, S., Holt, R. H. F., Ramsay, K., Bishop, J. D. D., Lambert. G., and Jenkins, S. R. 2009. First records in Great Britain of the invasive colonial ascidian *Didemnum vexillum* Kott, 2002. Aquatic Invasions, 4(4): 581–590.

Hewitt, C. L., and Campbell, M. L. 2007. Mechanisms for the prevention of marine bioinvasions for better biosecurity. Marine Pollution Bulletin, 55(7): 395–401.

Hitchin, B. 2012. New outbreak of *Didemnum vexillum* in North Kent: on stranger shores. Porcupine Marine Natural History Society Newsletter, 31: 43–48.

Holt, R. H. F., and Cordingley, A. P. 2011. Eradication of the non-native carpet ascidian (sea squirt) *Didemnum vexillum* in Holyhead Harbour: Progress, methods and results to spring 2011. CCW Marine Monitoring Report No. 90.

IMO (International Maritime Organization). 2004. International convention for the control and management of ships' ballast water and sediments, 2004. BWM/CONF/36, IMO, London.

IMO (International Maritime Organization). 2011. Development of international measures for minimizing the transfer of invasive aquatic species through biofouling of ships. Sub-committee on bulk liquids and gases, 16th session, Agenda item 5 (BLG16/5).

Kleeman, S. N. 2009. *Didemnum vexillum* - Feasibility of eradication and/or control. Bangor, Wales. CCW Science Report, 875: 53.

Kott, P. 2001. The Australian Ascidiacea. Part 4, Aplousobranchia (3) Didemnidae. Memoirs of the Queensland Museum, 47(1): 1–407.

Kott, P. 2002 A complex didemnid ascidian from Whangamata, New Zealand. Journal of the Marine Biological Association, 82: 625–628.

Lacoursière-Roussel, A., Forrest, B. M., Guichard, F., Piola, R. F., and McKindsey, C. W. 2012. Modeling biofouling from boat and source characteristics: a comparative study between Canada and New Zealand. Biological Invasions, 14(11): 2301–2314.

Lafargue, F. 1968. Les peuplements sessiles de l'Archipel de Glénan II les Didemnidae-Systématique-Ecology. Vie et Milieu série A, 19(A): 353–446.

Lambert, G. 2002. Nonindigenous ascidians in tropical waters. Pacific Science, 56(3): 291–298.

Lambert, G. 2005. Ecology and natural history of the protochordates. Canadian Journal of Zoology, 83(1): 34–50. doi: 10.1139/z04-156.

Lambert, G. 2007. Invasive sea squirts: a growing global problem. Journal of Experimental Marine Biology and Ecology, 342(1): 3–4.

Lambert, G. 2009. Adventures of a sea squirt sleuth: unravelling the identity of *Didemnum vexillum*, a global ascidian invader. Aquatic Invasions, 4: 5–28.

Langyel, N. L., Collie, J. S., and Valentine, P. C. 2009. The invasive colonial ascidian *Didemnum vexillum* on Georges Bank – Ecological effects and genetic identification. Aquatic Invasions, 4: 143–152. doi: 10.3391/ai.2009.4.1.15.

Locke, A., Doe, K. G., Fairchild, W. L., Jackman, P. M., and Reese, E. J. 2009. Preliminary evaluation of effects of invasive tunicate management with acetic acid and calcium hydroxide on non-target marine organisms in Prince Edward Island, Canada. Aquatic Invasions, 4(1): 221–236.

López-Legentil, S., Legentil, L. M., Erwin, P. M, and Turon, X. 2015. Harbor networks as introduction gateways: contrasting distribution patterns of native and introduced ascidians. Biological Invasions, 17: 1623–1638. doi: 10.1007/s10530-014-0821-z.

Martin, J. L., LeGresley, M. M., Cooper, J. A., Thorpe, B., Locke, A., Simard, N., Sephton, D., *et al*. 2010. Rapid assessment for *Didemnum vexillum* in southwest New Brunswick. Canadian Technical Report of Fisheries and Aquatic Sciences, 2882. 16 pp.

McCann, L. D., Holzer, K. K., Davidson, I. C., Ashton, G. V., Chapman, M. D., and Ruiz, G. M. 2013. Promoting invasive species control and eradication in the sea: options for managing the tunicate invader *Didemnum vexillum* in Sitka, Alaska. Marine Pollution Bulletin, 77(1): 165–171.

McCarthy, A., Osman, R. W., and Whitlatch, R. B. 2007. Effects of temperature on growth rates of colonial ascidians: A comparison of *Didemnum* sp. to *Botryllus schlosseri* and *Botrylloides violaceus*. Journal of Experimental Marine Biology and Ecology, 342: 172–174.

McKenzie, C. H., Matheson, K., Reid, V., Wells, T., Mouland, D., Green, D., Pilgrim, B., and Perry, G. 2016. The development of a rapid response plan to control the spread of the solitary invasive tunicate, *Ciona intestinalis* (Linnaeus, 1767), in Newfoundland and Labrador, Canada. Management of Biological Invasions, 7(1): 21–32.

McKindsey, C. W., Landry, T., O'Beirn, F. X., and Davies, I. M. 2007. Bivalve aquaculture and exotic species: a review of ecological considerations and management issues. Journal of Shellfish Research, 26(2): 281–294.

Mercer, J. M., Whitlatch, R. B., and Osman, R. W. 2009. Potential effects of the invasive colonial ascidian (*Didemnum vexillum*, Kott, 2002) on pebble-cobble bottom habitats in Long Island Sound, USA. Aquatic Invasions, 4(1): 133–142.

Millar, R. H. 1971. The biology of ascidians. Advances in Marine Biology, 9: 1–100. doi: 10.1016/S0065-2881(08)60341-7.

Minchin, D. 2007. Rapid coastal survey for targeted alien species associated with floating pontoons in Ireland. Aquatic Invasions, 2: 63–70.

Minchin, D., and Nunn, J. D. 2013. Rapid assessment of marinas for invasive alien species in Northern Ireland. Northern Ireland Environment Agency Research and Development Series No. 13/06.

Minchin, D., and Sides, E. 2006. Appearance of a cryptogenic tunicate, a *Didemnum* sp., fouling marina pontoons and leisure craft in Ireland. Aquatic Invasions, 1(3): 143–147.

Monniot, C., Monniot, F., and Laboute, P. 1991. Coral reef ascidians of New Caledonia. Office de la Recherche Scientifique et Technique Outre-Mer, Paris. 247 pp.

Moore, A. M., Vercaemer, B., DiBacco, C., Sephton, D., and Ma, K. C. K. 2014. Invading Nova Scotia: First records of *Didemnum vexillum* Kott, 2002 and four more non-indigenous invertebrates in 2012 and 2013. BioInvasions Records, 3(4): 225–234.

Morris, J. A., and Carman, M. R. 2012. Fragment reattachment, reproductive status, and health indicators of the invasive colonial tunicate *Didemnum vexillum* with implications for dispersal. Biological Invasions, 14: 2133–2140.

Morris, J. A. Jr., Carman, M. R., Hoagland, K. E., Green-Beach, E. R. M., and Karney, R. C. 2009. Impact of the invasive colonial tunicate *Didemnum vexillum* on the recruitment of the bay scallop (*Argopecten irradians irradians*) and implications for recruitment of the sea scallop (*Placopecten magellanicus*) on Georges Bank. Aquatic Invasions, 4(1): 207–211.

Nagar, A. E., Huys, R., and Bishop, J. D. D. 2010. Widespread occurrence of the Southern Hemisphere ascidian *Corella eumyota* Traustedt, 1882 on the Atlantic coast of Iberia. Aquatic Invasions, 5(2): 169–173.

Nall, C. R., Guerin, A. J., and Cook, E. J. 2015. Rapid assessment of marine non-native species in northern Scotland and a synthesis of existing Scottish records. Aquatic Invasions, 10(1): 107–121.

Nishikawa, T. 1990. The ascidians of the Japan Sea. I. Publications of the Seto Marine Biological Laboratory, 34(4–6): 73–148.

Occhipinti-Ambrogi, A., Ferrario, J., and Marchini, A. 2016. Trans-national dispersal of NIS in the Mediterranean Sea: pathways of secondary spread and control strategies. EuroMarine Workshop: Management of bioinvasions in the Mediterranean Sea - the way forward Ischia (Naples, Italy) 4–5 May 2016.
https://www.researchgate.net/publication/303345134_Trans-national_dispersal_of_NIS_in_the_Mediterranean_Sea_pathways_of_secondary_spread_and_control_strategies.

Olson, R. R. 1983. Ascidian-*Prochloron* symbiosis: the role of larval photoadaptations in midday larval release and settlement. Biology Bulletin, 165: 221–240.

Ordóñez, V., Pascual, M., Fernandez-Tejedor, M., Pineda, M.C., Tagliapietra, D., and Turon, X. 2015. Ongoing expansion of the worldwide invader *Didemnum vexillum* (Ascidiacea) in the Mediterranean Sea: High Plasticity of its biological cycle promotes establishment in warm waters. Biological Invasions, 17(7): 2075–2085. doi: 10.1007/s10530-015-0861-z.

Osman, R. W., and Whitlatch, R. B. 1999. Ecological interaction of invading ascidians within epifaunal communities of southern New England. *In* Marine Bioinvasions: Proceedings of the First National Conference, January 24–27, 1999, pp. 164–174. Ed. by J. Pederson. Massachusetts Institute of Technology Sea Grant College Program, Cambridge, MA, USA. 427 pp.

Osman, R. W., and Whitlatch, R. B. 2007. Variation in the ability of *Didemnum sp.* to invade established communities. Journal of Experimental Marine Biology and Ecology, 342: 40–53.

Pannell, A., and Coutts, A. D. M. 2007. Treatment methods used to manage *Didemnum vexillum* in New Zealand. Report prepared for Biosecurity New Zealand. New Zealand Marine Farming Association.

Piola, R. F., Dunmore, R. A., and Forrest, B. M. 2010. Assessing the efficacy of spray delivered 'eco-friendly' chemicals for the control and eradication of marine fouling pests. Biofouling, 26: 187–203.

Ramsay, A., Davidson, J., Landry, T., and Stryhn, H. 2008. The effect of mussel seed density on tunicate settlement and growth for the cultured mussel, *Mytilus edulis*. Aquaculture, 275(1): 194–200.

Reid, V., McKenzie, C. H., Matheson, K., Wells, T., and Couturier, C. 2016. Post-metamorphic attachment by solitary ascidian *Ciona intestinalis* (Linnaeus, 1767) juveniles from Newfoundland and Labrador, Canada. Management of Biological Invasions, 7(1): 67–76.

Reinhardt, J. F., Gallagher, K. L., Stefaniak, L. M., Nolan, R., Shaw, M. T., and Whitlatch, R. B. 2012. Material properties of *Didemnum vexillum* and prediction of tendril fragmentation. Marine Biology, 159(12): 2875–2884.

Roche, R. C., Monnington, J. M., Newstead, R. G., Sambrook, K., Griffith, K., Holt, R. H. F., and Jenkins, S. R. 2015. Recreational vessels as a vector for marine non-natives: developing biosecurity measures and managing risk through an in-water encapsulation system. Hydrobiologia, 750(1): 187–199.

Rolheiser, K. C., Dunham, A., Switzer, S. E., Pearce, C. M., and Therriault, T. W. 2012. Assessment of chemical treatments for controlling *Didemnum vexillum*, other biofouling, and predatory sea stars in Pacific oyster aquaculture. Aquaculture, 364: 53–60.

Ruiz, G. M., Carlton, J. T., Grosholz, E. D., and Hines, A. H. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. American Zoologist, 37(6): 621–632.

Ruiz, G. M., Fofonoff, P. W., Carlton, J. T., Wonham, M. J., and Hines, A. H. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annual Review of Ecology and Systematics, 31: 481–531.

Ruiz, G., and Hewitt, C. L. 2009. Latitudinal patterns of biological invasions in marine ecosystems: a polar perspective. *In* Smithsonian at the Poles Contributions to International Polar Year Science, pp. 347–358. Ed. by I., Krupnik, M. A., Lang, and S. E. Miller. Smithsonian Institution Scholarly Press, Washington, DC. 405 pp.

Sambrook, K., Rohan H. F. H., Sharp, R., Griffith K., Roche R. C., Newstead, R. G., and Wyn, G., *et al.* 2014. Capacity, capability and cross-border challenges associated with marine eradication programmes in Europe: The attempted eradication of an invasive non-native ascidian, *Didemnum vexillum* in Wales, United Kingdom. Marine Policy, 48: 51–58.

Sephton, D., and Vercaemer, B. 2015. Rapid assessment for the pancake batter tunicate, *Didemnum vexillum* (Kott 2012) in southwest New Brunswick (October 2012) and southwest Nova Scotia (September 2013). Canadian Technical Report of Fisheries and Aquatic Sciences, 3111.

Simard, N., Pelletier-Rousseau, M., Clarke Murray, C., McKindsey, C. W., Therriault, T. W., Lacoursiere-Roussel, A., Bernier, R., *et al*. 2016. National risk assessment of recreational boating as a vector for marine nonindigenous species. Canadian Science Advisory Secretariat Research Document. *In press.*

Simberloff, D. 2014. Biological invasions: What's worth fighting and what can be won? Ecological Engineering, 65: 112–121.

Sinner, J., and Coutts, A. D. M. 2003. Benefit-cost analysis of management options for *Didemnum vexillum* in Shakespeare Bay. Cawthron Report, 924. 24 pp.

Stefaniak, L., Lambert, G., Gittenberger, A., Zhang, H., Lin, S., and Whitlatch, R. B. 2009. Genetic conspecificity of the worldwide populations of *Didemnum vexillum* Kott, 2002. Aquatic Invasions, 4: 29–44.

Stefaniak, L., Zhang, H., Gittenberger, A., Smith, K., Holsinger, K., Senjie, L., and Whitlatch, R. B. 2012. Determining the native region of the putatively invasive ascidian *Didemnum vexillum* Kott, 2002. Journal of Experimental Biology and Ecology, 422–423: 64–71.

Stewart-Clark, S. E., Siah, A., Greenwood, S. J., Davidson, J., and Berthe, F. C. J. 2009. Development of 18S rDNA and COI gene primers for identification of invasive tunicate species in water samples. Aquatic Invasions, 4(4): 575–580.

Svane, I. B., and Havenhand, J. N. 1993. Spawning and dispersal in *Ciona intestinalis* (L.). Marine Ecology, 14(1): 53–66.

Svane, I., and Young, C. M. 1989. The ecology and behaviour of ascidian larvae. Oceanography of Marine Biology: An Annual Review, 27: 45–90.

Switzer, S. E., Therriault, T. W., Dunham, A., and Pearce, C. M. 2011. Assessing potential control options for the invasive tunicate *Didemnum vexillum* in shellfish aquaculture. Aquaculture, 318(1): 145–153.

Tagliapietra, D., Keppel, E., Sigovini, M., and Lambert, G. 2012. First record of the colonial ascidian *Didemnum vexillum* Kott, 2002 in the Mediterranean: Lagoon of Venice (Italy). BioInvasions Records, 1(4): 247–254.

Therriault, T. W., and Herborg, L-M. 2007. Risk assessment for two solitary and three colonial tunicates in both Atlantic and Pacific Canadian waters. DFO Canadian Science Advisor Secretariat. Research Document, 2007/063.

Thompson, R., and MacNair, N. 2004. An overview of the clubbed tunicate (*Styela clava*) in Prince Edward Island. PEI Department of Agriculture, Fisheries, Aquaculture and Forestry Report, 234. 29 pp.

Tyree, S. 2001. The Ascidians (Sea Squirts): Their Biology, Physiology and Natural Filtration Integration. D.E. Publishing, Rancho Cucamonga, California, USA. 368 pp.

Tyrrell, M. C., and Byers, J. E. 2007. Do artificial substrates favor nonindigenous fouling species over native species? Journal of Experimental Marine Biology and Ecology, 342(1): 54–60.

Valentine, P. C. 2003. USGS national geologic studies of benthic habitats, marine nuisance species, genus *Didemnum*: U.S. Geological Survey data available on the web, http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/index.htm. Accessed 01/15/2016.

Valentine, P. C., Collie, J. S., Reid, R. N., Asch, R. G., Guida, V. G., and Blackwood, D. S. 2007a. The occurrence of the colonial ascidian *Didemnum* sp. on Georges Bank gravel habitat - Ecological observations and potential effects on groundfish and scallop fisheries. Journal of Experimental Marine Biology and Ecology, 342: 179–181.

Valentine, P. C., Carman, M. R., Blackwood, D. S., and Heffron, E. J. 2007b. Ecological observations on the colonial ascidian *Didemnum* sp. in a New England tide pool habitat. Journal of Experimental Marine Biology and Ecology, 342(1): 109–121.

Valentine, P. C., Carman, M. R., Dijkstra, J., and Blackwood, D. S. 2009. Larval recruitment of the invasive colonial ascidian *Didemnum vexillum*, seasonal water temperatures in New England coastal and offshore waters, and implications for spread of the species. Aquatic Invasions, 4: 153–168. doi: 10.3391/ai.2009.4.1.16.

Van Name, W. G. 1945. The North and South American ascidians. Bulletin American Museum Natural History, 84: 1–476.

Vercaemer, B., Sephton, D., Clement, P., Harman, A., Stewart-Clark, S., and DiBacco, C. 2015. Distribution of the non-indigenous colonial ascidian *Didemnum vexillum* (Kott, 2002) in the Bay of Fundy and on offshore banks, eastern Canada. Management of Biological Invasions, 6: 385–394.

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

Wong, M. C., and Vercaemer, B. 2012. Effects of invasive colonial tunicates and a native sponge on the growth, survival, and light attenuation of eelgrass (*Zostera marina*). Aquatic Invasions, 7(3): 315–326.

Worcester, S. E. 1994. Adult rafting versus larval swimming: dispersal and recruitment of a botryllid ascidian on eelgrass. Marine Biology, 121(2): 309–317.

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