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Research article

First records in Great Britain of the invasive colonial ascidian *Didemnum vexillum* Kott, 2002

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Abstract

The colonial ascidian *Didemnum vexillum* was observed overgrowing fouling organisms present on structures within Holyhead Marina in North Wales in September 2008. Despite the examination of 9 other marinas in Wales during December 2008 and February 2009 no further populations were found. During December 2008 the extent of *D. vexillum* within Holyhead Marina and the surrounding harbour area was examined by diving. *D. vexillum* formed dispersed colonies throughout the marina, with <1% to 10% cover, but was not observed at any location within Holyhead harbour outside of the marina. Levels of infestation appeared to be low indicating a recent possible arrival. *D. vexillum* was found on the hulls of two vessels berthed within Holyhead marina but these vessels were long-term residents and not believed to be the source of infection. However, it is expected that hull fouling on recreational boats may become important vectors in the dispersal of *D. vexillum* to other regions around Britain. Although the species has been known in northern Europe since the 1990s, this is the first recorded established population of *D. vexillum* in Great Britain. In September 2008 a single colony of *D. vexillum* was collected in a marina in Plymouth and a colony, appearing to be *D. vexillum*, was photographed at the Darthaven Marina in 2005. The species was confirmed at this site in July 2009 by the collection of a single colony.

Key words: *Didemnum vexillum*; colonial ascidian; invasive species; marinas; Great Britain; hull-fouling; recreational vessels

Introduction

Since the 1970s the colonial ascidian *Didemnum vexillum* Kott, 2002 has been expanding its worldwide distribution in temperate regions and has attracted attention as a nuisance species due to its ability to spread rapidly and foul ships' hulls, aquaculture facilities and maritime structures and to occupy extensive areas of offshore seabed habitat (Bullard et al. 2007a; Lambert 2009). The current distribution range includes Japan, NE and NW U.S., British Columbia, northern Europe (France, The Netherlands), New Zealand and, more recently, Ireland (Minchin and Sides 2006; Bullard et al. 2007a; Lambert 2009: U.S. Geological Survey 2009). There has been uncertainty surrounding the native origin of *D. vexillum*, but recent evidence suggests that it may be native to Japan (Lambert 2009; Stefaniak et al. 2009).

Didemnum vexillum colonises a variety of firm substrates and is particularly prevalent on coastal structures such as docks, pilings, marina pontoons and aquaculture equipment. It also colonises natural seabed habitat including rocks, cobbles and gravel, but is unable to establish colonies on mud, mobile sand, or other unstable substrates (Coutts 2002; Valentine et al. 2007a). In New Zealand *D. vexillum* has become a serious pest at aquaculture facilities as the fouling of equipment and stock increases maintenance and processing time, and can lead to poor health and high rates of mortality of stock (Coutts 2005; Denny 2008). The impact of *D. vexillum* can be profound on native species due to its ability to rapidly outgrow and displace

other organisms, e.g. bivalves, sponges, hydroids, and ascidians (Bullard et al. 2007a; Lengyel et al. 2009). Few organisms seem capable of settling on this tunicate's surface and it is subject to little or no predation with the exception of anecdotal information on seastars and gastropods (Lambert 2009). The discovery of *D. vexillum* covering at least 230 km^2 of pebble gravel habitat on Georges Bank raised concerns about the impacts that this species could have on economically and environmentally important off-shore habitats (Valentine et al. 2007b). *D. vexillum* has a unique ability to form extensive mats over cobble-pebble substrates, essentially binding the small pebbles and cobbles together, and altering the habitat complexity of the sea floor (Valentine et al. 2007b; Mercer et al. 2009).

Didemnum vexillum is capable of both sexual and asexual reproduction and can spread rapidly owing to its high reproductive and population growth rates. The larval phase of *Didemnum* spp. is typically short and the non-feeding larvae produced by sexual reproduction will generally swim for only a few hours before attaching to a suitable substrate (Olson 1983). Valentine et al. (2009) suggest that the release of larvae and subsequent recruitment occurs at water temperatures between 14 and 20°C and depends on local conditions. Colony growth by asexual budding is initiated once water temperature exceeds 8-12°C and can be rapid once conditions are favourable (Valentine et al. 2007b). For example, colony fragments in the tidepools at Sandwich, Massachusetts, USA grew in area 11 to 19-fold in 30 days (Valentine et al. 2007b). *D. vexillum* colonies can range in morphology from undulating and encrusting mats to long pendulous growth forms (Minchin and Sides 2006; Lambert 2009). The long fragile tendrils often break off and these fragments are capable of reattaching to a suitable substrate and forming a new colony (Bullard et al. 2007a, b). It is likely that *D. vexillum* has spread to new locations either via hull or sea chest fouling, or transport of fragments in ballast water, with subsequent local spreading by fouled recreational craft and barges, drifting and reattachment of dislodged fragments, and movements of fouled aquaculture stock and gear (Lambert 2009).

Although *D. vexillum* has been known in northern Europe since 1991 (Gittenberger 2007), there have been no records of it occurring in Great Britain (Arenas et al. 2006). We report the first known established population of *D. vexillum* in Great Britain and the additional occurrence of single colonies in south-west England, and document surveys carried out to assess the current status of this invasive species in Wales.

Material and Methods

Initial visual surveys of non-native species in North Wales

Five marina sites in North Wales were selected and surveyed for the presence of non-native species during August and September 2008 (Figure 1, Sites 1-3, 5, 6). A target list of 11 non-native marine species known from marina environments of Britain or Europe was used to facilitate the survey. All recognised non-native species were recorded and a semi-quantitative assessment of abundance made. Each marina site was visited at low tide and examined for 2 hours to record the presence of non-native species. The visible surfaces of pontoons and pilings were inspected, and any submerged artificial substrates such as hanging ropes, fenders and buoys were pulled out of the water and examined. The possible introduction of *D. vexillum* into Holyhead Marina (Figure 1, Site 1) was recognised when a colonial tunicate was observed smothering native and non-native ascidians present on the marina structures. Pictures of the species were taken in situ and samples were collected to confirm its identity.

Following confirmation of the presence of *D. vexillum* in Holyhead Marina, North Wales, the other marina sites were re-examined in greater detail and a wider survey of marinas throughout Wales was initiated. A more thorough survey of the marina and harbour area of Holyhead was also undertaken.

Expanded visual survey for Didemnum vexillum in Wales

Nine marina sites throughout Wales were surveyed for the presence of *D. vexillum* (Sites 2-10, Figure 1 and see Annex 1). Sites 2-6 in North Wales were investigated in December 2008 and January 2009 and sites 7-10, in Mid and South Wales, were examined between the 9th and 13th of February 2009.

At least two people inspected each marina site to determine the presence of *D. vexillum*. All subtidal substrata that were visible from the pontoons were examined including pontoon sides, ropes, buoys and fenders. An underwater

colour video camera system (Videotech VT-21111 with attached Sunray HID light) on the end of a flexible pole was used to survey surfaces that could not be observed from the pontoons, such as the underside of pontoons, marina walls and pilings to 3 m below sea surface. All surveys were timed to coincide with low water to optimise the area of marina structures that could be searched. The presence or absence of *Didemnum vexillum* was recorded and notes were made concerning other dominant flora and fauna observed, in particular the presence of other ascidians.

Dive survey for Didemnum vexillum in Holyhead Marina and Harbour

Holyhead Marina is located in the south-west corner of Holyhead Harbour and has been operational since 2001. It is situated within a fully marine environment, sheltered from the Irish Sea by a large breakwater and has an entrance to the north-east that allows access at all states of the tide. The maximum depth at the entrance of the marina is ~ 10m and the tidal range is ~ 6m.

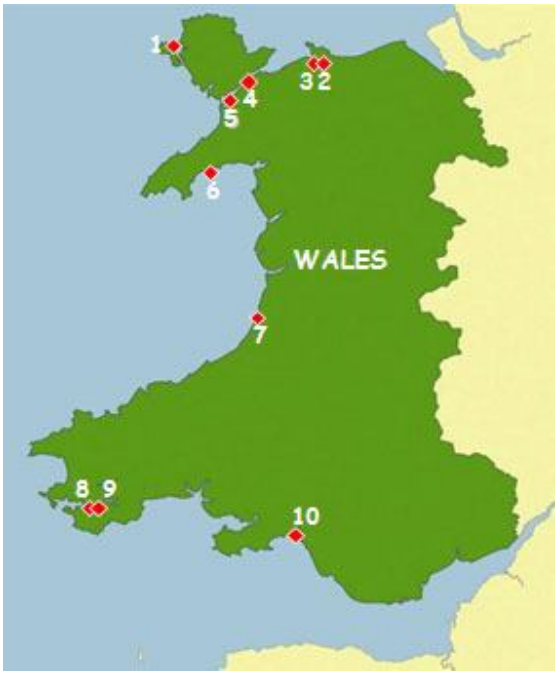

Figure 1. Distribution of marina sampling sites around Wales in 2008 and 2009, see also Annex 1 for location coordinates and survey results

Figure 2. Aerial view of Holyhead Harbour; ◆ indicates dive survey location

Two divers were deployed in Holyhead Marina (Site 1, Figure 2) on 9 and 10 December 2008. The divers' purpose was to record the presence and approximate abundance of *D. vexillum* by visually estimating percent cover of the species from a wide variety of substrates, including: the sides of pontoons (depth 0-2 metres), undersides of pontoons (depth 1.5-2m), pontoon mooring chains and ropes (depth 2-5m), concrete pontoon mooring blocks (depth 3-5m), hulls of vessels in the marina, seabed below the marina (depth 3-6m), and rocky substrata under the walkway bridge at the entrance to the marina. A small section of the marina, currently under construction, and an area of rocky substrata were left unsurveyed due to time limitations.

Artificial structures throughout the harbour were examined on 20 and 21 January 2009 (sites 2-21, Figure 2). To the east of the marina there are approximately 140 yacht mooring buoys, 8 of these buoys (sites 2-9 in Figure 2) were examined by hauling the mooring components aboard the stern deck of the research vessel at low water. This allowed inspection for the presence of *D. vexillum* on each mooring's buoy, the surface to seabed rope attached to the steel cable and approximately 3-4 m of steel cable mooring.

Divers were deployed in pairs to examine other structures around the harbour including the large-vessel mooring buoys (sites 10-13, Figure 2). These have vertical heavy chains attached to mooring blocks and/or ground-chains. Structures in Holyhead Port, in the east of the harbour were also examined, including: the steel piles driven into the seabed that form the 'legs' of the jetties (sites 14-18, Figure 2): the floating fish dock (site 20, Figure 2): and the cylindrical piles of the ferry terminals (sites 19, 21, Figure 2) that are approximately 2-3 m in diameter, and extend to water depths of 4 to 10 m range of approximately 4-10 m, much of which is potential *D. vexillum* habitat.

Sample collection in Holyhead Marina and Harbour

Divers retrieved *D. vexillum* specimens by hand from a range of different locations and substrata. Those easiest to collect were found overgrowing the abundant solitary ascidians such as *Ciona intestinalis* (Linnaeus, 1767), *Ascidiella aspersa* (Müller, 1776) and *Styela clava* (Herdman, 1882) on the marina pontoon mooring chains and the permanently submerged sides of the pontoons. In such cases the 'host' ascidians and *D. vexillum* were collected intact together. Thin sheets of *D. vexillum* were removed from the marina pontoon sides using a flat-bladed knife. Where there was doubt on the identity of a colonial ascidian, samples were collected for subsequent analysis.

All specimens were labelled on site with their location in the harbour (e.g. berth number in the marina or pile position on a particular terminal) and substratum. Samples were narcotised after collection by dissolving menthol crystals in seawater within each specimen bag and then preserved in 95% ethanol for laboratory analysis and for taxonomic identification.

Photographs of *D. vexillum* were taken in situ using a Nikon D70s digital camera with a 60 mm macro lens in a Seacam underwater housing with twin Seacam Seaflash 250 strobes. Several video sequences were recorded using a high-definition Sony HDV 1080i video camera in a Light and Motion Blue-fin underwater housing lit by twin Sunray HID video lights.

Results

Identification of Didemnum vexillum

During the course of the initial marina survey in North Wales, samples collected from Holyhead marina suspected to be *D. vexillum* were examined and found to contain late stage unhatched larvae in the tunic (Figure 3). The larvae were recognised as being characteristic of *D. vexillum* with six pairs of lateral ampullae and three adhesive papillae. Adult colonies of *D. vexillum* (Figure 4) were recognised by the yellowish cream colouration, spicule-free bands between zooid groups, and colony form that ranged from thin to thick and encrusting to lobed (Lambert 2009).

Marina surveys – Wales

D. vexillum was recorded only at Holyhead Marina from the 10 marinas surveyed in Wales (Sites 1-10; Annex 1). Native (e.g. *Ciona intestinalis*) and non-native ascidians (*Styela clava*) were recorded within some marinas including Pwllheli, Port Dinorwic, Milford Haven and Neyland (Annex 1). More details of the species recorded from marinas surveyed during August-September 2008 (Sites 1-3, 5, 6) are available in Griffith (2008).

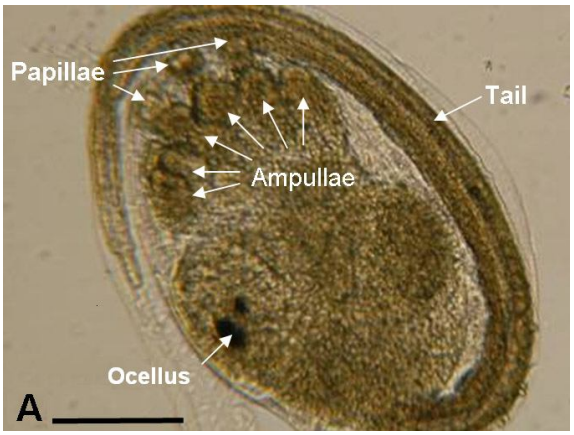

Figure 3. Brooded unhatched *D. vexillum* larva removed from a colony sampled from Holyhead marina in December 2008. The larva has diagnostic features of *D. vexillum* including: six pairs of lateral ampullae and three adhesive papillae. Scale bar A: 300µm (Image: Rohan Holt, CCW)

Figure 4. *Didemnum vexillum* colony overgrowing solitary ascidians *Ciona intestinalis* on marina mooring chains (Image: Rohan Holt, CCW)

Distribution of Didemnum vexillum in Holyhead Marina and Harbour

D. vexillum colonies were distributed throughout most of the area of the pontoons (Figure 5) where it covers from <1% to 10% of the substrates surveyed. Although there was no previous quantitative analysis of *D. vexillum* in the marina it was evident the species had experienced 'die-back' over the winter. Based on observations made from the surface, the colonies appeared reduced in size on 9 and 10 December 2008 compared to when *D. vexillum* was first identified in summer 2008. The colonies also exhibited signs of degeneration as they were easily peeled from substrates and had thickened margins, as described by Valentine et al. (2007a).

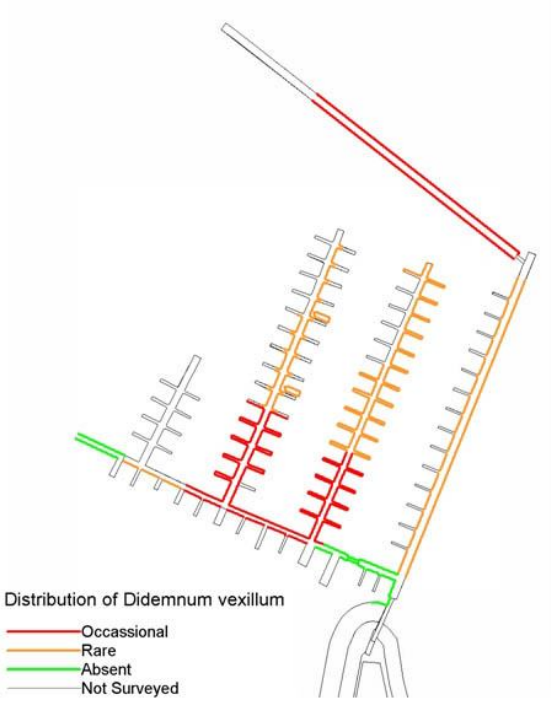

Figure 5. Distribution of *D. vexillum* within Holyhead marina where 'rare'=1-5% cover and 'occasional' =5-10%

Figure 6. *Didemnum vexillum* colonies overgrowing fouling communities on the hull and prop shaft of a recreational vessel (Image: Harry Goudge, Marine Ecological Solutions)

Of the different habitats surveyed, *D. vexillum* formed encrusting mats on the flat pontoon sides and perimeter of the undersides of the pontoons. It was also found on the pontoon mooring chains and, in particular, on the tests of the solitary ascidians *Styela clava*, *Ascidiella aspersa* and *Ciona intestinalis* growing on the shallower parts of the mooring chains (Figure 4). The 'natural' seabed under the pontoons was composed of fine

silty mud and did not support *D. vexillum*. It was not present on the deeper sections of mooring chain that are immersed in mud at low water of spring tides. *D. vexillum* was not found on the rocky sea defences below the entrance walkway nor on any of the concrete mooring blocks that lie in the mud.

At the time of the survey the marina was half full (approximately 130 boats out of a possible 250 boats were present). Of these, only two were found to have hulls fouled with *D. vexillum*. A number of small (<20cm^2) *D. vexillum* colonies were found as secondary fouling upon other species that had colonised the hull. The hulls of both these vessels were heavily fouled with large sugar kelp plants *Saccharina latissima* (Linneaus) C.E. Lane, C. Mayes, Druchl and G.W. Saunders and other algae. Mature mussels *Mytilus edulis* (Linnaeus, 1758), feather stars *Antedon bifida* (Pennant, 1777), large solitary ascidians and other encrusting fauna such as barnacles and bryozoans were also present. *Didemnum vexillum* was found encrusting parts of the propeller shaft and the rudder on one of the yachts and also colonising the mussels and the tube of a peacock worm *Sabella pavonina* (Savigny, 1820) on the motor vessel's hull (Figure 6). The density of flora and fauna colonising both vessels indicated that it was unlikely that either had moved within at least the last 12 months.

Didemnum vexillum was not found anywhere in the harbour outside the marina area (see Figure 2 for overview of sample sites). There was a high abundance of native and non-native species of ascidians present on virtually all the structures surveyed. Colonial ascidians collected from the mooring chains, piles and harbour walls from all the locations outside the marina were identified as *Lissoclinum perforatum* (Giard, 1872) or other native species.

Other records of Didemnum vexillum

A single colony of *D. vexillum* was found on an experimental settlement rack suspended below a pontoon in Plymouth Yacht Haven marina, Plymouth, Devon, UK on 22nd September 2008. Inspection of nine similar racks on the same day revealed no other colonies. The colony was removed and fragments were brought into culture at the Marine Biological Association in Plymouth. Additional pieces were preserved, and the remainder of the colony was destroyed. Subsequent visits to Plymouth Yacht Haven in October 2008 and May 2009, during which 10 to 15 settlement racks were accessed on each occasion, did not reveal any additional colonies of *D. vexillum*. A photograph taken by Judith Oakley in the Dart estuary at Darthaven Marina, Kingswear, Devon on 29 June 2005 shows a colony of *D. vexillum* encrusting a mussel. No colonies were noted during two subsequent visits to Darthaven Marina; on the first of these occasions, an almost complete die-off of sessile fauna had occurred, apparently in response to a period of markedly reduced salinity following heavy rainfall. However a large colony of *D. vexillum* showing the onset of pendulous growth was found in the Darthaven Marina on 27 of July 2009.

Discussion

The discovery of *D. vexillum* in Holyhead Marina, North Wales is the first recorded established occurrence of the species in the UK. The widespread but fairly sparse and patchy distribution of *D. vexillum* within the marina (Figure 5) indicates the levels of infestation are fairly low, which could be interpreted as evidence of it being a relatively recent arrival. Colonies were not present as "massive pendulous growth forms" such as those observed in Malahide marina in Ireland (Minchin and Sides 2006) and in Whangamata harbour in New Zealand (Coutts 2002). At present, *D. vexillum* is confined to Holyhead Marina and was not found at any location in the wider harbour area or on the commercial terminals in Holyhead Harbour.

By December 2008 *D. vexillum* colonies appeared to have regressed since the initial discovery in summer 2008. This phenomenon has also been reported in other areas, during winter periods (Valentine et al. 2007a). Water temperature conditions within Holyhead Marina range between 5°C and 22°C throughout the year (Kleeman 2009), so it is expected that the regressed colonies will increase in size during the spring and summer when water temperatures reach above 8-12°C and become favourable for asexual growth (Valentine et al. 2007a). Larval dispersal and recruitment is likely to occur when water temperatures exceed 14°C (Valentine et al. 2009) and may result in spread of *D. vexillum* from the marina into the wider harbour area. Judging from the vigorous growths of other native and non-native ascidians on virtually all of the structures surveyed in Holyhead Harbour,

there is considerable scope for further colonisation by *D. vexillum*. As *D. vexillum* larvae are short lived it is unlikely that the species could extend its range out of Holyhead Harbour and along the coast by larval dispersal alone, but there is great risk of it spreading to new locations by hull fouling, drifting and reattachment of dislodged fragments, or movements of fouled aquaculture stock and gear (Lambert 2009). The nature of vessel activity within Holyhead Harbour brings large international shipping and fishing vessels into close proximity to *D. vexillum* colonies and increases the risk of it spreading locally, nationally and internationally.

The spread of *D. vexillum* to other locations in the UK could cause some serious impacts upon fisheries and benthic habitats (Valentine et al. 2007b; Lengyel et al. 2009; Mercer et al. 2009). *D. vexillum* is considered to be an "ecosystem engineer" capable of adversely modifying the habitats it invades (Wallentinus and Nyberg 2007). An assessment of the potential economic and ecological impacts of *D. vexillum* in Welsh waters has been made by Kleeman (2009).

Didemnum vexillum was not detected at any of the other marinas surveyed around Wales. However, it must be recognised that with visual surveys that there is a small possibility that colonies may have been present at a site, but were not observed. Populations of other ascidian species were present at some of the other marinas surveyed (Annex 1) and were in greatest abundance at Milford Haven, Neyland, Port Dinorwic and Pwllheli marinas. These sites are probably those which would also be suitable for *D. vexillum* and should be monitored regularly for its arrival.

The presence of *D. vexillum* in Holyhead Marina and its absence in the wider harbour area suggests it was introduced into the region on the hulls of one or more infected recreational vessels. If the species had been introduced by another vector, it seems unlikely that it would be confined to just the marina. Two recreational vessels within the marina were found to have their hulls colonised by *D. vexillum* and once contact was made with the boat owners we were assured that the vessels had not moved from the marina in over a year. It is likely that these vessels became infected during their period of inactivity while residing within the marina, rather than being the actual source of infection, as the colonies present were patchy and $<20cm^2$ in size. Recreational vessel movements between Holyhead and Ireland are frequent (Royal Yachting Association 2005) so it is possible that the introduction into Holyhead originated from one or more vessels infected in eastern Ireland at the Malahide or Carlingford Lough marinas where *D. vexillum* was discovered in 2005 and 2006 (Minchin and Sides 2006). *D. vexillum* has since spread to the West coast of Ireland (D. Minchin pers. comm.). The introduction of *D. vexillum* may have originated from a visiting vessel from The Netherlands or France, as the species has been present in these regions since the 1990's (Gittenberger 2007). There is also a possibility that the source was equipment (e.g. barges) used during the construction within Holyhead Marina.

Recreational boats may not be a significant vector for the dispersal of non-native species over great distances but they may be an important vector for successful introductions on a local scale. For example, the major vector for the introduction and spread of the invasive kelp *Undaria pinnatifida* (Harvey) Suringar along the English south coast is thought to be small boats, principally leisure craft (Farrell and Fletcher 2006). Lambert (2006) suggests that the recent rapid expansion of *D. vexillum* populations into many marinas in Washington, U.S.A. is probably due to recreational boat traffic, which may be a highly significant vector once a species has made a successful transoceanic transplantation (Wasson et al. 2001; Minchin et al. 2006). Recreational yachts usually travel short distances and there is a high probability that the donor and recipient region will have similar climatic conditions (Gollasch 2002); therefore the non-native fouling would be likely to survive in the recipient region. There are approximately 120 major marinas and 40 commercial harbours throughout the UK containing water of suitable salinity and temperature for *D. vexillum* to survive (Featherstone and Lambie 2007). North Wales has been identified as a region of major recreational vessel activity (Royal Yachting Association 2005) with frequent visitors from Scotland, the Isle of Man, South Wales and Ireland. Recreational craft based within North Wales also visit these same regions so there is great potential for *D. vexillum* to be transported to other parts of the U.K. on the hulls of infected vessels.

The degree of fouling on boats within a marina can often be quite extensive. In Scotland, 59% of yachts surveyed in a recent study were found to have significant levels of macrofouling attached

to their hulls (Ashton et al. 2006). In North Wales, using the same methodology, Griffith (2008) showed similarly high levels of fouling. Hull fouling is as yet an unmanaged vector in the UK, and represents a significant risk for the introduction and secondary spread of non-native species (Minchin and Gollasch 2003; Hewitt et al. 2004). In New Zealand, hull cleaning guidelines have been introduced (http://www.biodiversity.govt.nz/seas/biosecurity). Australia has also implemented successful control procedures at marinas and has protocols in place for the management of future non-native species (Bax et al. 2002). Similar hull-fouling management procedures may be required within the UK to prevent the spread of *D. vexillum*, and other non-native species, by recreational vessels.

As a new invader to the region the full magnitude of the effect of this species on local ecosystems has yet to be determined. We suggest that future research be focused on the spread and impact of *D. vexillum*. Studies into the growth rates and reproduction of *D. vexillum* within the marina would be beneficial, as this would provide baseline information on the likely survival and persistence of this invasive tunicate if it were introduced to other locations in the UK. Research into the effects of *D. vexillum* on local benthic organisms would enable us to make predictions about its potential impacts on ecosystems. Regular monitoring of selected sites around the UK would allow for early detection of the arrival of *D. vexillum*, potentially allowing practices to be put in place to limit further spread.

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Annex 1. Location of marina survey sites in Wales 2008-2009 with records of fouling species present and presence or absence of *Didemnum vexillum*

Survey site (Figure 1)	Location	Geographic coordinates		Date of survey	Fouling Species Present	*Didemnum vexillum*
		Latitude (N)	Longitude (W)			
1	Holyhead marina	53°19.173'	04°38.488'	10.12.08	Moderate-high species abundance and richness of filter feeding invertebrates and algae. *Mytilus edulis*, *Ascidiella aspersa*, *Ciona intestinalis*, *Botryllus schlosseri*, *Botrylloides leachi*, *Styela clava*, *Botrylloides violaceus*. Fringing kelp	Present
2	Deganwy marina	53°17.480'	03°49.673'	11.12.08	*Mytilus edulis* and filamentous algae, no ascidians recorded.	Absent
3	Conwy marina	53°17.458'	03°50.286'	11.12.08	*Mytilus edulis* and filamentous algae, no ascidians recorded	Absent
4	Port Dinorwic marina	53°11.178'	04°12.582'	12.12.08	*Mytilus edulis*, filamentous algae, *Ascidiella aspersa*, *Botryllus schlosseri*, *Botrylloides leachi*	Absent
5	Victoria Dock - Caernarfon	53°08.562'	04°16.669'	30.01.09	*Mytilus edulis*, filamentous algae, occasional ascidian species (*Ascidiella aspersa*, *Botryllus schlosseri*)	Absent
6	Pwllheli marina	52°53.191'	04°24.389'	16.12.08	*Mytilus edulis*, filamentous algae, *Ascidiella aspersa*, *Ciona intestinalis*, *Botryllus schlosseri*, *Botrylloides leachi*	Absent
7	Aberystwyth marina	52°24.595'	04°05.202'	09.02.09	Filamentous algae, no ascidian species recorded	Absent
8	Milford Haven marina	51°42.699'	05°02.270'	11.02.09 12.02.09	*Mytilus edulis*, filamentous algae, *Ascidella aspersa*, *Ciona intestinalis*, *Botryllus schlosseri, Botrylloides leachi*, *Styela clava*	Absent
9	Neyland marina	51°42.632'	04°56.544'	12.02.09	*Mytilus edulis*, filamentous algae, *Ascidiella aspersa*, *Ciona intestinalis*, *Botryllus schlosseri*, *Botrylloides leachi*, *Styela clava*, *Botrylloides violaceus*	Absent
10	Swansea marinas	51°36.984'	03°56.048'	10.02.09	Barnacles, filamentous algae, no ascidian species recorded	Absent