RISK ASSESSMENT SUMMARY SHEET

Carpet Sea-squirt (*Didemnum vexillum*)

- A marine colonial sea squirt that grows over hard surfaces forming large, leathery matts 2-5mm thick or pendulous growths up to 1m long.
- First detected in Holyhead, Wales in 2008 but was probably already well established in parts of England at this point.
- Highly invasive elsewhere in the world, for example it has overgrown 230km^2 of Georges Bank off the coast of Boston, USA, including fish spawning gravel habitat.
- In GB, the primary concern is potential impact on aquaculture and important seabed habitat as well as contributing to vessel biofouling.


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History in GB

Our understanding of the introduction and spread of this species in GB is limited by its challenging identification and taxonomy. It was first reported from Wales in 2008, although there is evidence of its presence in Devon, England from 2005. It was probably well established, but unrecorded, before this point. There are now well-established populations in southern England and populations in Wales and Scotland. Investigations into its identity continue, with potentially two forms present in GB. An unsuccessful eradication attempt was made in Wales after its initial detection.

Native Distribution

Due to incomplete historical records and misidentifications, the native range of this species is not conclusively known. It is thought to be the temperate north-western Pacific, potentially in waters around Japan.

Distribution in UK and Ireland

source: NBN Atlas 2024, modified (Milford Haven record added) by NNSS

Impacts

Economic: (moderate, medium confidence)

- While not quantified, this species has caused huge economic losses in aquaculture in other countries – particularly on mussel lines and cages, but potentially to other species too.
- In GB, currently not causing much economic loss but there is potential for loss of production and increased management costs to shellfish farming and lays if this species spreads.

Environmental: (major, medium confidence)

- In other countries this species has substantially altered large areas of seabed habitat by smothering native biota.
- While evidence is limited in GB, there is potential for it to overgrow important habitat, including biogenic reef, and disrupt ecosystem function.

Social: (minimal, high confidence)

- None significant.

Introduction pathway

Via vessel hulls, contaminated marina equipment and on shellfish brood stock and farming equipment.

Spread pathway

Natural: (high, medium confidence) – larvae have a short dispersal phase that lasts up to 36 hours or can travel as fragmented colonies for up to three weeks that can re-attach when they settle on a suitable substratum.

Human: (major, high confidence) – via leisure craft, movements of shellfish seed stock and equipment, and the movement of contaminated marina and shipping/marine industry equipment.

Summary

	Response	Confidence
Entry	VERY LIKELY	VERY HIGH
Establishment	VERY LIKELY	VERY HIGH
Spread	MODERATE	MEDIUM
Impact	MAJOR	MEDIUM
Overall risk	HIGH	MEDIUM

GB NON-NATIVE ORGANISM RISK ASSESSMENT SCHEME

Name of organism: Carpet sea-squirt, *Didemnum vexillum*
Author: Rohan Holt, CloudBase Productions Ltd
Risk Assessment Area: Great Britain (England, Scotland and Wales and associated islands)
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Placed on NNSS website: *to be completed*

What is the principal reason for performing the Risk Assessment?

The GB Committee for non-native species is considering whether to add this species to the list of Special Concern. This assessment will form part of the evidence used to inform the Committee's decision. This species was selected because it is likely to pose a risk to native species and other interests in British waters and the government is considering what can be done to limit its spread and impact. This assessment updates an earlier assessment for this species published in 2011.

GB NON-NATIVE ORGANISM RISK ASSESSMENT SCHEME 2
SECTION A – Organism Information 3
SECTION B – Detailed assessment 7
PROBABILITY OF ENTRY 7
PROBABILITY OF ESTABLISHMENT 36
PROBABILITY OF SPREAD 39
PROBABILITY OF IMPACT 44
RISK SUMMARIES 57
ADDITIONAL QUESTIONS - CLIMATE CHANGE 60
ADDITIONAL QUESTIONS - RESEARCH 62
REFERENCES: 63

SECTION A – Organism Information	
Stage 1. Organism Information	**RESPONSE and COMMENT**
1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	No. *Didemnum vexillum* (Kott 2002) is the accepted species name, although it has a highly convoluted identification history including a number of misidentifications and two new species descriptions (Turon *et al.* 2020). Further investigation of previously reported *Didemnum vexillum* populations using molecular and morphological analysis may potentially uncover the presence of new species (Turon *et al.* 2020). Species of *Didemnum* with similar external morphological characteristics occur around the world and include *D. albidum*, *D. areolatum*, *D. maculosum*, *D. misakiense*, *D. pacificum*, *D. pardum*. Kott (2002) named the invasive species found in New Hampshire as *D. vestum* and the New Zealand species as *D. vexillum* but later DNA analysis (Lambert, 2009; Stefaniak, 2009) found that these two entities plus that found in Japan were all *D. vexillum*. The 2011 *D. vexillum* risk assessment lists another five entities that have similar characteristics but most were demonstrated by the above authors to be *D. vexillum*. *D. pseudovexillum* is a putative new taxon, indistinguishable from *D. vexillum* in external aspects and coexists with it in the same habitat, although its larvae and spicules are morphologically different (Turon *et al*., 2020). *D. pseudovexillum* has not yet been recorded in the British Isles although possibly overlooked as a separate entity and may be found amongst the current *D. vexillum* populations in the risk assessment area (Bishop pers com).
2. If not a single taxonomic entity, can it be redefined? (if necessary use the response box to re-define the organism and carry on)	Yes Positive identification of the species requires a combination of external and internal morphological characteristics, supported by genetic analysis.
3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment)	Yes A risk assessment for *Didemnum vexillum* in Great Britain was published on 22/03/2011 (GB NNS 2011) and concluded the following risk summaries:

	• Entry – very likely, low confidence • Establishment – very likely, high confidence • Spread – rapid, medium confidence • Impact – massive, low confidence • Overall – high risk, low confidence
4. If there is an earlier risk assessment is it still entirely valid, or only partly valid?	Partly valid. The 2011 assessment commenced shortly after the first record of this species was confirmed in GB. Since then, a considerable amount of new information has been gathered that could affect the results of the assessment. As such, and given the GB Committee's interest in this species, it was considered necessary to update the assessment. The new risk assessment uses the updated GBNNRA template. Distribution, extent and genetic variation, etc. has been updated in this version.
5. Where is the organism native?	Temperate north-western Pacific, with Japan thought to be located within the native range (Stefaniak *et al.*, 2012). Due to incomplete historical records and the many misidentifications of the species, the native range is not conclusively known.
6. What is the global distribution of the organism (excluding the risk assessment area)?	Northeast Pacific - British Columbia to Southern California (Cohen *et al*., 2001; Lambert, 2009), Northeast of the USA (Bullard *et al*., 2007; Dijkstra *et al*., 2007) New Zealand (Coutts, 2002; Coutts and Forrest, 2007) Netherlands (Gittenberger, 2007) Northwestern France (Lambert, 2009) Mediterranean (Ordonez *et al*., 2015). The CABI website also suggests there have been records in Italy, Germany, Norway https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.107996
7. What is the distribution of the organism in the risk assessment area?	England: • S Devon (Darthaven Marina (J. Bishop *pers. comm*.), Dartmouth, Plymouth) • Hampshire (Solent (Marlin.ac.uk))

	• East Sussex (Brighton (Gibson-Hall *et al*., 2018 in NBN)) • North Kent (NBN Atlas) Scotland: • Clyde (Marlin.ac.uk) • Argyll and Bute (Loch Fyne and Loch Creran – Begg *et al*., 2016) Wales: • Holyhead (Griffith *et al*., 2009; Prentice *et al*., 2021). • Milford Haven (NRW pers comm). Adjacent to risk assessment area: • Channel Isles: Jersey (*pers. comm.* Alastair Christie) • N Ireland: Carlingford Lough, Strangford Lough (Gibson-Hall *et al*., 2018 in NBN), (https://invasivespeciesireland.com/) • Ireland: Galway Bay, Clew Bay, Dunmanus Bay, Malahide Marina (Minchin & Sides 2006; Prentice *et al.,* 2021; Gibson-Hall *et al.,* 2018 in NBN).
8. Is the organism known to be invasive (i.e., to threaten organisms, habitats or ecosystems) anywhere in the world?	Yes *D. vexillum* is known for being highly invasive in temperate marine habitats throughout the world. There are examples of it occurring on natural substrates e.g., colonies covering 230 km^2 overgrowing fish spawning gravel habitat on Georges Bank off the coast of Boston, USA, (Valentine *et al.,* 2007) and on artificial substrates – often associated with mariculture and marinas where it inhibits water flow through netting and cages, and smothers filter-feeders such as mussels (Coutts & Forrest, 2007; Bishop, 2010; Osman & Whitlatch, 2007; Simkanin *et al*., 2012). However, it does not consistently exhibit invasive traits everywhere it occurs. Whether this is due to environmental conditions being sub-optimal, competition from native biota, genetic limitations or a combination of several factors is being investigated (Prentice *et al*., 2021).

9. Describe any known socio-economic benefits of the organism in the risk assessment area.	None known

SECTION B – Detailed assessment			
PROBABILITY OF ENTRY			
Important instructions: • Entry is the introduction of an organism into the risk assessment area. Not to be confused with spread, the movement of an organism within the risk assessment area. • For organisms which are already present in the risk assessment area, only complete the entry section for current active pathways of entry or if relevant potential future pathways. The entry section need not be completed for organisms which have entered in the past and have no current pathways of entry.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
1.1. How many active pathways are relevant to the potential entry of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	many	high	Seven pathways are considered in the assessment below. Introduction of the species into the risk assessment area and spread within the risk assessment area might involve similar pathways - although 'entry' most likely requires crossing the Irish Sea (possibly via the Isle of Man), North Sea or English Channel. Proximity of nearest neighbour *D. vexillum* colonies would suggest the most likely route of entry, with shortest time of transfer, would be across the Irish Sea from Ireland and N Ireland to Wales, NW England and W Scotland or from N France or Netherlands to the south and south-east coast of England.
1.2. List relevant pathways through which the organism could enter. Where possible give detail about the specific origins and end points of the pathways.			i. Hull / sea chest fouling – most likely on poorly maintained or not antifouled vessels from N Europe or Ireland / N Ireland – **likely, high confidence.**

For each pathway answer questions 1.3 to 1.10 (copy and paste additional rows at the end of this section as necessary).			ii. Contaminant associated with shellfish. *D vexillum* has been located at shellfish culture sites in GB. The arrival of this species has been linked to movement of contaminated stock or equipment from outside GB (e.g., oyster / mussel culture) from Europe, Ireland, N Ireland **– likely, medium confidence.** iii. Marine industries – oil, gas, renewables, dredging – **moderately likely, medium confidence**. iv. Movement of marina infrastructure (e.g., pontoons) and barges from contaminated locations across the Irish Sea or English Channel/N Sea – **unlikely, low confidence.** v. Flotsam, damaged or abandoned free-floating boating, mariculture or marina equipment contaminated with *D. vexillum* carried by currents and wind to GB from Ireland, N Ireland, Europe – **unlikely, medium confidence.** vi. Contaminants associated with finfish culture - contaminated equipment and water from outside GB also possible (e.g., fish cages from Europe, Ireland, N Ireland) **– unlikely, medium confidence.** vii. Natural dispersal (from outside GB) **– unlikely, medium confidence.**

Pathway name:	**i. Hull or sea-chest fouling**		
i.1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	accidental	high	Entry via this pathway is accidental. *D. vexillum* colonies have been reported fouling the hulls of recreational vessels (Griffith *et al*. 2009) and in harbours and marinas in the risk assessment areas (Griffith *et al*. 2009). Accidental introduction via contaminated vessels is therefore likely.
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	likely	medium	Vessel activity from areas with *D. vexillum* (NBNatlas.org) into the risk assessment area is high, concentrated around popular leisure and commercial routes in the Irish Sea (Dewey *et al*. 2020) and English Channel (Tidbury *et al*. 2016; MMO, 2014). *D. vexillum* larvae can swim for several hours and cover distances of several kilometres especially if aided by currents (Fletcher et al., 2012). Colonies of *D. vexillum* can also disperse by fragmentation and zooids are capable of reproduction while suspended in the water column. Fragments can survive for up to three weeks and can reattach to biotic, natural, and artificial surfaces (Carman *et al*., 2014). Both motility of larvae and survival of fragments are concerns for attempts to control or eradicate this species (Morris & Carman, 2012) which will survive and re-attach even at low (6^{o}C) temperatures (Carman *et al.*, 2014) and once established in an area such as a marina, will disperse locally and colonise vessel hulls. Fractionation through hull-scraping in situ to remove biofouling can therefore exacerbate the rate of spread on vessel hulls. *D. vexillum* will colonise boat hulls that are not antifouled and remain static in an infested marina, especially during the warmer months when larval

			production is at a peak (Fletcher *et al.*, 2013a., Holt & Cordingley, 2011., Jenkins pers.com.). Contaminated leisure vessels are therefore likely to act as vectors for *D. vexillum* at its peak reproductive potential capable of spreading into new areas via Irish Sea, North Sea or Channel crossings.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	high	*D. vexillum* is a temperate water species tolerant of a wide range of physical parameters which enables it to withstand transitions across different climate regimes. It can survive temperature ranges of -2ºC to +24ºC and salinity of approximately 19-35 PSU (Bullard *et al*., 2007, Ordonez *et al*., 2015), and is widely reported to thrive on floating structures that receive a plentiful supply of clean seawater (e.g., Coutts & Forrest, 2007). Survival on the hulls of vessels during a sea voyage is therefore highly likely, although it is less likely to survive if the final destination is a low salinity estuary or riverine harbour (Bullard *et al*., 2007). It is also able to grow and survive in moderate turbidity (Carman, 2007) and withstand short intervals of exposure to air and immersion in intertidal mud (Holt & Cordingley, 2011) – typical conditions experienced by a vessel moored or beached in the outer reaches of an estuary. Once established *D. vexillum* colonies will attach to boat hulls and will likely continue to grow during a voyage (especially during warmer months) although long pendulous growth forms, occasionally found on pontoon floats and boat hulls, were found to be weakly attached (Holt and Cordingley, 2011) and would likely become detached by the turbulence of being underway. Larvae will also be produced at this time. Larvae have a dispersal phase of approximately 36 hours (Fletcher *et al.*, 2013a) and are unlikely to survive in sea chests and ballast water of large commercial vessels for viable dispersal and subsequent settlement beyond this time but viable larvae shed into the water column could potentially reach hard substrates at any stage during a voyage. If a colonised vessel moved into the risk assessment area (e.g., from one of the colonised marinas in N Ireland or Ireland) at slow speed (e.g., sailing)

			then the colonies are likely to remain on the hull, for example, during an Irish Sea, North Sea or Channel crossing. A similar scenario was observed in Holyhead (Holt & Cordingley, 2011) where infrequently used leisure craft were found to be heavily colonised.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	likely	high	There is currently no common global standard for cleaning ships' hulls and no EC or UK regulations regarding the cleaning of vessels and equipment before returning from outside the risk assessment area. Local management practices, however, vary considerably between locations – both origins and destinations. If marinas have a clean-hull policy, e.g., Holyhead marina had the requirement for heavily fouled vessels to be removed from the water, jet-washed clean and treated with antifouling paint during the initial D. vex incursion (funded by CCW / Welsh Government), *D. vexillum* is less likely to be transferred between vessels but such policies are not enforced and are rarely practiced adequately. Other regions within the risk assessment area have systematic monitoring programmes to check for the arrival of non-native species e.g. Orkney Island council perform annual surveys (Kakkonen, 2019) but these do not reduce the risk of a new arrival becoming established. Organisations such as The Yacht Harbour Association (TYHA), the Green Blue and the Royal Yachting Association (RYA) all promote 'clean marina' measures that include washdown facilities with means to capture and filter biological and antifouling waste (although there is limited evidence of take up of best practice). The foremost method employed globally to reduce recreational vessel biofouling is vessel haul-out followed by scrubbing or jet washing and the application of antifouling paint (Roche *et al*., 2014). However, antifouling paints require regular application to remain effective and even in vessels where this is practiced, there can be difficulties in accessing niche areas of a vessel (e.g. propeller shafts, water-inlets, anchor wells, keel bottom edge) that mean that these untreated, or poorly treated,

			areas show a high frequency of non-native species occurrence (Minchin & Gollasch, 2003; Murray *et al*., 2011).
1.7. How likely is the organism to enter the risk assessment area undetected?	likely	very high	New arrivals from outside the risk assessment area are likely to arrive undetected as there is no formal inspection procedure in place to detect NNS on vessel hulls in GB. Small colonies are difficult to find amongst other biota on fouled hulls and even larger colonies are very difficult to identify positively and discriminate from other native didemnids. Organisations including The Green Blue, Natural Resources Wales, MarLIN and the Northern Ireland Environment Agency have used poster and web campaigns to promote awareness and aid identification of this species which is also included in the Marine Conservation Society's Seasearch list of important non-natives for divers to watch out for.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	high	Fragmentation can occur at any time of year and even at low temperatures experienced during the winter, fragments are able to survive and re-attach to the substrate (Carman *et al*., 2014) although winter growth rates are slower than at peak times in mid to late summer. Other triggers that might stimulate spawning, such as temperature shock or changes in light regimes could occur if a supporting structure is moved into a new location, overturned, or brought into shallow warmer water. Experimental heat shock and light regime changes induce spawning in culture (Fletcher & Forrest, 2011). Contaminated recreational vessels are more likely to move between locations during the calmer spring-autumn period which corresponds with rapid seasonal growth and reproduction.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	likely	high	Marina pontoons and mooring buoys are ideal for development and growth of *D. vexillum* larvae if contaminated vessels are moored along-side. Colonies transported on fouled vessels may readily fragment and disperse into the new

			habitat. Synchronised spawning may also increase the probability of organisms establishing in a suitable habitat.
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	likely	high	The volume of traffic along this pathway is extremely high, and there are large populations at potential donor sites outside the risk assessment area within survivable range. *D. vexillum* has been positively identified on vessel hulls and it is likely that introductions will occur between individual donor and recipient locations multiple times.

Pathway name:	**ii. Contamination associated with shellfish stock or mariculture equipment from outside Great Britain** (e.g., oyster / mussel culture equipment, on stock / seed stock, on boat hulls; from N Europe, Ireland, N Ireland)		
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	accidental	high	Entry along this pathway is accidental. Translocation with juvenile stock for on-growing at a new location. *D. vexillum* may potentially be attached to the shellfish and containers or fragmented colonies amongst the stock, lines, netting and containers used for transporting shellfish.
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	moderately likely	medium	There are currently two known shellfish (oyster) farms within the risk assessment area that have received spat/broodstock contaminated by *D. vexillum*. Loch Creran in Argyll (first recorded in 2016, Cottier-Cook *et al*., 2019; Moore & Harries, 2020) and North Kent (Hitchin, 2012). Current importation of brood stock from outside the risk assessment area is limited. For example, in 2012 there were 109 imports into GB from Guernsey, Norway and Ireland, although the republic of Ireland received 455 with some arriving from France (Tidbury *et al*., 2016). Imports into the risk assessment area from the Republic of Ireland could therefore include contaminants from France. Without a biosecurity plan in place there is a real risk that imported shellfish broodstock from outside the risk assessment area could be contaminated with *D. vexillum*.
1.5. How likely is the organism to survive during passage along the	very likely	high	Evidently *D. vexillum* has already survived being translocated with shellfish broodstock (oysters) suggesting that salinity and temperature tolerances of

pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.			*D. vexillum* (Bullard & Whitlatch, 2009) are maintained in transit. It is likely that it survives both as small, detached fragments and as small colonies attached directly to the shellfish or hard surfaces accompanying them such as packing crates, shell fragments or other biota. Disturbance and temperature shock can induce release of larvae and if this occurs during transport it may promote release of larvae (Fletcher *et al.*, 2013a) which could settle on the content of the containers. Once at the destination broodstock will be placed in mesh containers for on-growing. Detached fragments may adhere to these (Carman *et al*., 2014) or be washed through onto other available substrates in the vicinity which might include natural intertidal habitats. *D. vexillum* has been found under oyster trestles and nearby in intertidal and subtidal habitats in Loch Creran and North Kent (Cottier-Cook *et al*., 2019; Hitchin, 2012).
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	moderately likely	medium	There is no formal inspection procedure in place to detect NNS on shellfish in GB. Although shellfish must be certified as disease-free before being imported to GB, they are not required to have all encrusting organisms removed, so even if cleaned, small colonies of *D. vexillum* could be present.
1.7. How likely is the organism to enter the risk assessment area undetected?	likely	medium	*D. vexillum* might not be apparent in imported juvenile shellfish stock – particularly if fragmentation has occurred to produce small or practically invisible but still viable colonies that can re-attach (Morris & Carman, 2012) or as larvae that might be released near to the time of arrival (Fletcher *et al.*, 2013a).
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	medium	Shellfish spat settlement and subsequent seeding of lays tends to occur during warmer months and would correspond to period of growth and reproduction in *D. vexillum*. Fragmentation and re-attachment can occur at any time of year (Morris & Carman, 2012).

1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	likely	medium	*D. vexillum* has successfully invaded a wide range of habitats including intertidal and subtidal rock, cobbles and gravel via larval dispersal and fragmentation. It can produce detachable tendril-like projections that can disperse widely and re-attach on suitable substrates at any time of year (Morris & Carman, 2012) and larvae that settle during the warmer summer months (Fletcher *et al*., 2013a). Within enclosed or open aquaculture sites there are ample suitable substrates for *D. vexillum* larvae and fragments to settle on, particularly the shellfish themselves. Prentice *et al*., (2021) linked specific genetic characteristics in *D. vexillum* to variable invasiveness – they provided evidence to suggest that of the two haplotypes studied the one they associated with shellfish culture locations has a greater capability to grow on natural habitats compared to the other which appears confined to artificial substrata in marinas. This link between genetics and invasiveness requires further investigation.
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway.	likely	medium	There is little information on the frequency of aquaculture imports into the risk assessment area, but based on current evidence this pathway has already been the cause of *D. vexillum* incursions in GB on at least two separate occasions. If control measures, such as those detailed in biosecurity plans, are ineffective at detecting and eradicating *D. vexillum* in imported live shellfish stock from outside GB, this pathway is quite likely to lead to further occurrences.

Pathway name:	**iii. Marine industries** (oil, gas, renewables, dredging)		
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	accidental	high	Entry to this pathway is accidental. Installations such as rigs and offshore wind farms that require frequent support from boats kept in contaminated marinas and harbours may provide suitable artificial substrata for *D. vexillum* to colonise. If such structures are then moved into GB waters from outside the risk assessment area, they could accidentally import colonies. The biological mechanisms for entering this pathway are similar to those described for hull and sea chest fouling and for marina infrastructure, barges and hulks (i.e. larval or fragment settlement at the point of origin) but the scale of recipient substrates are potentially much greater with different management strategies and risks of further spread.
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	unlikely	medium	The need for fuel efficiency and speed usually drives commercial shipping to adopt clean-hull policies. However, more static structures, such as rigs, wind or tide turbines, and jack-up barges, that may remain in place long enough to establish *D vexillum* colonies before being relocated, perhaps pose a higher risk. There is no evidence that this has caused an incursion into the risk assessment area yet, although monitoring for invasive species has lagged behind the pace of the rapidly expanding offshore wind energy sector which has at least tripled in the last 5 years in GB (International Energy Agency data – IEA.org). Dredging operations pose the risk that fragments may be incorporated into dredge spoil and /or transferred into the wider environment where it might reach the risk assessment carried by natural water movements. However, there is no evidence that this has occurred, but may mediate a more localised dispersal pathway.

			Although the disposal of dredged materials is regulated in the UK (Gov.UK, 2021; RYA, 2021) and elsewhere, there does not appear to be any regulation on the cleaning of dredging vessels and equipment between projects. Waste intended for disposal at one site could become mixed with a subsequent load and be disposed of accidentally at another location.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	moderately likely	medium	If boat hulls, floating structures etc. are not cleaned and dried this makes the potential for transfer into the risk assessment area more likely. Dredged material from harbours and marinas is usually disposed of in designated deep areas offshore (or on land in some cases) within the risk assessment area. It is unlikely that *D. vexillum* would survive on or being buried by fine sediments in the hold of a dredger although it is known to thrive in deep (>30 – 60 m) water on hard substrates if fragments survive the process of dredging and transportation to a disposal site (Valentine *et al*., 2007; McCarthy *et al*., 2007). Dredge spoil has been used to smother areas contaminated with *D. vexillum* as a means of control (Coutts & Forrest, 2007).
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely	medium	There is currently no international legislation that regulates biofouling on ship's submerged surfaces although some countries have introduced their own domestic legislation to make 'clean hulls' a requirement for entry into their waters. If treated appropriately *D. vexillum* will not survive on structures that are cleaned, dried and anti-fouled. Large vessels require periodic dry-docking every 3-5 years to refresh anti-fouling coatings although in-water 'clean and capture' systems to remove biofouling and retain the dislodged debris are improving in efficiency but can miss small fragments in cryptic spaces (Tamburri *et al*., 2020). In Wales, a Biosecurity Risk Assessment must be completed when applying for a marine licence for dredging and/or disposal, works relating to aquaculture or seaweed farming and any band 3 activity (highest risk applications requiring EIA). Marine licencing conditions that may be imposed include the checking

			and washing of equipment (e.g. seabed equipment and filter screens at the end of a dredging campaign), the requirement for the circulation and exchange of hopper water away from shore, and the removal of hopper sediment prior to entering a different ‘region’ of the UK.
1.7. How likely is the organism to enter the risk assessment area undetected?	very likely	high	Very small colonies are very difficult to detect amongst other fouling biota on artificial structures.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	medium	Movement of structures occurs year-round, but can be limited by poor weather. Spawning and rapid colony development occurs during the warmer months, but fragments can re-attach to suitable substrates throughout most of the year (Fletcher *et al*., 2013a; Morris & Carman, 2012; Carman *et al*., 2014).
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	moderately likely	medium	If contaminated structures are brought into close enough range for larval or fragment transfer (Worcester, 1994; Morris & Carman, 2012; Fletcher *et al*., 2013a) this could lead to *D. vexillum* establishing on other floating structures or in natural habitats especially if of a sufficiently invasive strain (Prentice *et al*., 2021).
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	moderately likely	medium	Arrival of commercial structures, dredgers and potentially contaminated commercial vessels from outside the risk assessment area is likely to occur sporadically but the frequency of arrival from *D. vexillum*’s native or previously invaded range is unknown. If measures are in place to control, inspect and clean them on arrival, they are far less likely to pose a risk importing new populations.

Pathway name:	**iv. Movement of marina infrastructure, pontoons, hulks, and barges** (from contaminated locations across the Irish Sea or English Channel/N Sea)		
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	accidental	high	Entry along this pathway is accidental. Entry of *D. vexillum* into the risk assessment area on heavily bio-fouled structures was accidental in the specific example that probably caused the colonisation of Holyhead Marina (anecdotal evidence pers. com.). In this case large marina pontoons were towed across the Irish Sea from a contaminated marina in the Republic of Ireland directly to Holyhead where they were installed on-site during the construction of the marina. Accidental transport of stowaway colonies on a range of floating towed structures such as disused vessel (hulks), barges, mooring and navigation buoys, large fenders and fuelling equipment (e.g. floating fuelling buoys) is also possible. *D. vexillum* has been found on such structures during surveys and eradication work (Holt & Cordingley, 2011) and can represent a significant risk of invasive species entering the pathway (Cook *et al.*, 2014).
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	unlikely	low	It is unlikely that large numbers of *D. vexillum* arrive via this pathway per year but it has probably been the cause of at least one marina infestation over a much longer timeframe (see 1.3). This pathway requires the translocation of an established infestation on marina or harbour equipment which could result in large numbers of colonies being transferred during a single event. It is more likely that *D. vexillum* colonies are transported within the risk assessment area by this mechanism, for examples between marinas within a large harbour (see section 2.2 probability of spread) rather from outside it. It is not known how often such equipment is brought into the risk assessment area from outside.
1.5. How likely is the organism to survive during passage along	likely	high	If not treated by jet-washing, drying and/or antifouling and equipment is transported either by road and ferry soon after being removed from the water and

the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.			kept wet, or by being towed to its destination by sea, it is likely that colonies will survive for at least 24 hours and indefinitely if on a towed structure. *D. vexillum* is known to survive for short periods of time out of water (less than 2-3 hours) but if kept damp and cool would probably survive for longer (Carman *et al.*, 2009; Valentine *et al.*, 2007). It is therefore more likely to survive road and ferry transport during cool weather in an enclosed/covered vehicle than on an open vehicle during warm weather. Colonies adherent to a towed structure will experience similar conditions to those on vessel hulls and will likely continue to grow during a voyage (especially during warmer months) although long pendulous growth forms and weakly attached colonies would likely become detached by the turbulence of being underway and may cause further spread if they reach and adhere to a suitable surface at any stage in the journey (Morris & Carman, 2012; Carman *et al*., 2014). Larvae will also be produced at this time. Larvae have a dispersal phase of approximately 36 hours (Fletcher *et al.*, 2013a) and viable larvae shed into the water column could potentially reach hard substrates at any stage during a voyage.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	unlikely	medium	If biosecurity plans are followed to treat potentially contaminated structures by cleaning and drying before being moved (Cook *et al.*, 2014), then there is very little chance of *D. vexillum* surviving. However, there is little evidence that biosecurity plans are in place and followed in most cases. The risk of transportation through a non-compliant activity is potentially much higher depending on the physical factors described in 1.5 above.
1.7. How likely is the organism to enter the risk assessment area undetected?	moderately likely	medium	Rapid Assessment Survey protocols used to systematically survey marinas and harbours have detected established *D. vexillum* around the coastline of England (Bishop *et al*., 2015). This can aid assessing risk of transfer to nearby areas but relies on expert identification skills and regular visits to detect early arrivals. It is

			far more likely, therefore, to escape detection in areas that are not included in routine surveys that are outside known ‘hot spots’ such as the marinas in south England. Very small colonies in cryptic spaces would likely escape detection unless detailed inspections of structures were carried out on arriving in the risk assessment area. Early detection on land, before structures are placed back in the water, would prevent establishment at the recipient location, but arrivals by sea are far more likely to spread on arrival.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	medium	The best time of year for relocation of such structures by sea is during the calmer months between spring and autumn when *D. vexillum* is at its optimal temperature range for growth and spread by fragmentation or release of larvae although fragmentation can occur at any time of year and re-attach to the substrate (Carman *et al*., 2014). Large structures such as jack up barges may use marinas and harbours for temporary shelter during the winter and other structures may be relocated by surface transport at any time of year.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very likely	high	Floating artificial structures are ideal for growth and reproduction of *D. vexillum* (Simkanin *et al*., 2012). Further spread by transfer of fragments (Morris & Carman, 2012), or larvae during peak spawning time, is very likely if imported on floating structures that are placed near to other similar floating structures. Transfer between floating onto static or natural substrates seems to occur less frequently. For example, it has been found on pilings in Largs Yacht Haven in Scotland (Beveridge *et al*., 2011) but despite its abundance in Holyhead Harbour since 2008 it has only been found on floating artificial structures in Holyhead marina (Holt, 2018). Forrest *et al*., (2013) suggested that benthic predation potentially limits its spread onto static and natural habitats.

1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	unlikely	low	This pathway includes towing of floating marina structures or barges from outside the risk assessment area. Little is known about the frequency of such events but was potentially the cause of *D. vexillum* arriving in Holyhead from origins in an Irish marina (anecdotal evidence pers. obs.). Similarly, relocation of inadequately cleaned pontoons or barge structures that remain damp, by road and ferry, could introduce *D. vexillum* into the risk assessment area. Control measures written into biosecurity plans, should make entry via this this pathway less likely. This pathway is probably likely to occur infrequently but if conditions for *D. vexillum* are optimal, viable colonies are very likely to be transferred to a new location.

Pathway name:	**v. Flotsam, damaged or abandoned free-floating boating, mariculture or marina equipment** (contaminated with *D. vexillum* carried by currents and wind to GB from Ireland, N Ireland, Europe)		
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	accidental	high	Entry to this pathway is accidental. Storm events, vessel wrecks and accidental loss or breakage of equipment through failure of moorings etc., can result in *D. vexillum* colonies attached to floating objects being swept out to sea. Transport by wind and tidal currents could potentially result in translocation of such objects over great distances. There is no documented evidence of *D. vexillum* being transported by this pathway into the risk assessment area, but there are high-profile examples on a global scale of non-native species reaching new destinations on flotsam. For example, 118 non-native species arrived on a dock section carried by oceanic currents to the west coast of the USA having been washed out to sea from Japan by the 2011 tsunami (Lallensack, 2017). In 2006 an oil production platform, lost at sea off the coast of Brazil, ran aground on Tristan da Cunha carrying 60 non-native species – some of which formed new populations at their destination (Wanless *et al*., 2009).
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	unlikely	medium	The likelihood of floating structures being lost into open sea is high and occurred en-masse in the case of the storm event that wrecked Holyhead Marina in March 2018 when broken pontoons and loose mooring buoys with *D. vexillum* were dispersed widely including westwards across the Irish Sea to Ireland. On this occasion, there was no evidence of a successful translocation– possibly due to sea temperatures being very low at the time of year (Holt, 2018). Similar storm events, for example with a prevailing westerly direction, could potentially blow contaminated floating objects east across the Irish Sea – such events are likely to become more frequent with climate change but there is no evidence that *D. vexillum* has arrived in the risk assessment on flotsam.

1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	moderately likely	medium	Survival depends on *D. vexillum* remaining in conditions within its normal tolerances (Bullard & Whitlatch, 2009) during translocation. Floating debris such as pontoons and mooring buoys are most likely to support rapidly growing colonies at peak reproductive potential in the North Sea and Irish Sea during the summer (Fletcher *et al*., 2013a), particularly when in full salinity clean seawater offshore (Coutts & Forrest, 2007) where indefinite survival of colonies is likely. Colony fragments may be dispersed off flotsam and survive long enough to re-attach to other substrates on arrival in a new location at any time of year (Carman *et al*., 2014) but larval dispersal into a new habitat is more likely in summer and requires suitable substrates to be within range of the short-lived larvae. Survival on arrival in a new location is more likely if the floating object arrives in the vicinity of suitable hard substrates within the temperature and salinity tolerances of *D. vexillum* rather than into estuarine conditions and on sandy and soft sediments.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	likely	medium	Management practices that include cleaning and antifouling of pontoons and mooring equipment etc. could potentially reduce this risk – although this is rarely practiced thoroughly.
1.7. How likely is the organism to enter the risk assessment area undetected?	likely	medium	Depending on the size of the structure, fragmented floating structures, buoys etc. supporting *D. vexillum* are likely to be washed ashore or into suitable habitats undetected whether the colonies are conspicuous or not.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	medium	If this pathway is initiated by severe weather, it is more likely that it will occur in the winter months when *D. vexillum* is less likely to reproduce by larval dispersal (Fletcher *et al*., 2013a) but still potentially able to spread by fractionation (Morris & Carman, 2012; Carman *et al*., 2014). Low temperatures and decrease in salinity has also been associated with seasonal reduction in colony size (Jenkins *et al*., 2010; Holt, 2018) as observed in Holyhead Marina post storm Emma in March 2018 when marina debris, a proportion of which was carrying *D. vexillum*, was scattered widely throughout the Irish Sea, but did not appear to cause further dispersal. However, stormy weather can occur in warmer

			months and is more likely to occur with increasing frequency with climate change.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	moderately likely	medium	This is dependent on the type of substrate where flotsam arrives and the invasiveness of the colonies. The work by Prentice *et al*., (2021) as above, implies that a highly invasive strain would have more capability of settling and becoming established in the intertidal or shallow subtidal on natural substrata. Although *D. vexillum* is not widely established on natural habitats in GB there is ample availability of anthropogenic substrates in the risk assessment area in the many harbours and marinas. More information is required on its ability to inhabit benthic habitats.
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	unlikely	medium	The sequence of events required for a successful arrival from outside the risk assessment area via this pathway are not often repeated over time and are more likely to lead to local spread. However, increased frequency of severe weather, mediated by climate change, would increase the risk of *D. vexillum* arriving via this pathway. Offshore wind energy modelling suggests that there will be a gradual increase in proportion and strength of westerly winds in the Irish Sea and North Sea (Susini *et al*., 2022).

Pathway name:	**vi. Contaminant on finfish culture** (Movement of contaminated equipment from outside Great Britain (e.g., fish cages, feed barges, fish farm boat hulls, with fish stock, from Europe, Ireland, N Ireland). This pathway has many similarities to the shellfish pathway although the fish will not host *D. vexillum*. Translocation to and from stock species to infrastructure is not possible but the cages and other equipment associated with fish farming are suitable habitat for *D. vexillum* and fragments and larvae may be transported in the water used to move fish stock.)		
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	accidental	high	Entry to this pathway is accidental. There is potential for this to occur if contaminated fish cages are translocated 'wet' (e.g., towed by sea to a new location from outside GB or transported by road and ferry without being cleaned and dried) – across the English Channel, North Sea or from Ireland / N Ireland. There is an example in Loch Creran, within the risk assessment area, where finfish pens are located within the same water body as *D. vexillum* originating from a nearby oyster farm (Moore & Harries, 2020). Evidence of cross contamination has not been found. Live fish transported by road and ferry in water tanks may be contaminated with viable larvae or fragments of *D. vexillum*. The larvae have a relatively short free-swimming phase before they settle and metamorphose (potentially onto the container walls) compared to fragments that can survive for up to three weeks and can reattach to biotic, natural, and artificial surfaces (Carman *et al*., 2014).
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year?	unlikely	low	Contamination of fin-fish equipment with *D. vexillum* is possible, particularly if kept in the vicinity of contaminated pontoons or shellfish culture. However, the transportation of such equipment in this state from outside the risk assessment area is unlikely. Transportation of live fish in water tanks, for example fry for on-growing, does occur by road and ferry, although mainly within the risk assessment area. The

Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.			frequency of importation of farmed fish stock is unknown. Imports of live fish from outside the risk assessment area are usually destined for the ornamental / aquarist trade.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	high	Established colonies can survive if attached to permanently submerged or wetted surfaces and are likely to withstand the translocation processes if equipment is not washed and dried and remains cool while in transport. Bullard *et al*. (2007) and Ordonez *et al*. (2015) demonstrate the tolerances of *D. vexillum*, especially short term, to a wide range of temperatures and salinities. Colony fragments are likely to survive in water used to translocate fish stock. They may attach to the container walls or be transferred to fish pens and adhere to netting once the stock has been decanted (Carman *et al*., 2014).
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	unlikely	medium	Biosecurity action plans should be in place for fin-fish culture and best practice dictates that all equipment should be washed and dried and any relocation of fish should be monitored for invasive species (Cook *et al*., 2014). This should effectively eradicate or prevent incursions of *D. vexillum* if present. However, incursions of other species have occurred via this pathway, e.g., skeleton shrimp *Caprella mutica* (Cook *et al*., 2007), and the risk of *D. vexillum* escaping notice still exists (see 1.7 below).
1.7. How likely is the organism to enter the risk assessment area undetected?	likely	medium	*D. vexillum* might not be visible on fishfarm equipment or amongst stock – particularly if fragmentation has occurred to produce small or practically invisible but still viable colonies that can re-attach (Morris & Carman, 2012) or as larvae that might be released near to the time of arrival (Fletcher *et al*., 2013a). It is also very difficult to discriminate between small colonies of *D. vexillum* and native didemnid species which are likely to grow on mariculture equipment. Even with biosecurity plans in force within the risk assessment area it is therefore possible that *D. vexillum* will evade detection and enter the risk assessment area.

1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	medium	No data was found on the movement of fish farm equipment and boats by sea into the risk assessment area, but the logistics required to relocate equipment would suggest that this is more likely to occur during the warmer, calmer months of the year which correspond to *D. vexillum*'s rapid growth and reproductive period. Other movements by road and ferry can happen at any time of year. Fragmentation can occur even at low temperatures experienced during the winter and small fragments are able to survive and re-attach to the substrate at any time (Morris & Carman, 2012; Carman *et al*., 2014). Movement of farmed fish tends to be seasonal, for example salmon parr are usually moved to sea cages during the spring and summer (see bestfishes.org.uk).
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	likely	medium	Prentice *et al*., (2021) linked specific genetic characteristics within *D. vexillum* to variable invasiveness – they provided evidence to suggest that of the two haplotypes studied in the UK the one they associated with shellfish culture locations has a greater capability to grow on natural habitats compared to the other which appears confined to artificial substrates in marinas. This link between genetics and invasiveness requires further investigation. If finfish culture equipment is colonised by the more invasive haplotype (possibly originating from shellfish culture installations at the point of origin) it could both transfer to artificial and natural habitats. Within enclosed or open aquaculture sites, there are ample suitable substrates, especially artificial and floating structures, for *D. vexillum* to settle on, spread by either by transfer of fragments or larvae (Morris & Carman, 2012). Transfer between floating onto static or natural substrates seems to occur less frequently. For example, it has been found on pilings in Largs Yacht Haven in Scotland (Beveridge *et al*., 2011) and on intertidal substrates in Loch Creran (Moore & Harries, 2020) but despite its abundance in Holyhead Harbour since

			2008 it has only been found on floating habitats at this location (Holt, 2018). Forrest *et al*. (2013) found that benthic predation potentially limits its spread onto static and natural habitats.
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway	unlikely	medium	The frequency of transfer of finfish culture equipment and fish stock to locations within the risk assessment area is unknown (but probably less often compared to shellfish culture equipment and stock).

Pathway name:	**vii. Natural dispersal**		
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	accidental	high	Entry to this pathway is by natural means, although may originate from an accidental incursion. Long-distance translocation is more likely to occur as fragmented colonies – perhaps attached to floating seaweed or sea grass.
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	very unlikely	medium	*D. vexillum* produces larvae that have a short dispersal phase that lasts up to 36 hours, or can travel as fragmented colonies or lobes that can re-attach when they settle on a suitable substratum (Worcester, 1994; Morris & Carman, 2012; Fletcher *et al*., 2013a). Colony fragments from potential source populations of *D. vexillum* in the west Irish Sea or south of the English Channel would need to be carried by the prevailing winds and tide, potentially on floating natural substrates, such as seagrass (Carman *et al*., 2014) to the GB coast. For large numbers to arrive on the GB coast it would require a very large source population to be releasing fragments; the current population density, especially on natural substrata, at these locations is relatively small and largely unknown. Prevailing westerly and south-westerly winds and near-surface currents tend to drive floating objects eastwards across the Irish Sea and north eastwards up the English Channel (atlas.marine.ie). Mobile marine animals can also act as vectors. Crustaceans such as spider crabs (*Maja* spp., *Macropodia* spp.) often support live and actively growing sponges, hydroids and colonial ascidians and have been known to support *D. vexillum* (Gittenberger, 2010). Dispersal via such animals is more likely to occur at a local level rather than be responsible for importing from outside the risk assessment area.

1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	unlikely	medium	Survival depends on *D. vexillum* remaining in conditions within its normal tolerances (Bullard & Whitlatch, 2009) during translocation. Floating algae and seagrass might support live colonies at peak reproductive potential in the North Sea and Irish Sea during the summer (Fletcher *et al*., 2013a), particularly when in full salinity clean seawater offshore (Coutts & Forrest, 2007). Colony fragments may be dispersed and survive in the water column for up to three weeks. This may be long enough to re-attach to other substrates on arrival in a new location at any time of year (Morris & Carman, 2012; Carman *et al*. 2014). Larval production from free-living fragments can occur in summer and requires suitable substrates to be within range of the short-lived dispersal phase. Survival of *D. vexillum* on arrival in a new location is more likely if it attaches to suitable hard substrates within its temperature and salinity tolerances rather than settling on sandy and soft sediments in estuarine conditions.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	unlikely	medium	n/a No management practices are in place to mitigate introduction events via natural dispersal
1.7. How likely is the organism to enter the risk assessment area undetected?	very likely	high	There is no consistently established routine mechanism for detection of arrival of this species via natural pathways into natural habitats. Most monitoring for marine INNS occurs in marinas and harbours in and adjacent to Marine Protected Areas (e.g., Bishop *et al*., 2012; Wood *et al*., 2014, 2016) and in the proximity to shellfish culture areas where transfer from artificial substrata to natural habitats has occurred (e.g., Begg *et al*., 2016). Environmental DNA (eDNA) analysis has been utilised to confirm the presence of *D. vexillum* in areas where it has already been found by rapid assessment surveys. The authors plan to scale-up the use of eDNA as a survey tool over larger geographic regions (Matejusova *et al*., 2021). Applied consistently

			throughout the risk assessment area this may provide a very useful tool for detection of otherwise unknown occurrences. Small or practically invisible but still viable colonies may be present on drift algae or seagrass or free-floating in the water. These can re-attach to suitable substrates undetected (Morris & Carman, 2012). Larvae might also be released at the time of arrival near suitable substrates during the summer (Fletcher *et al*., 2013a). It is also very difficult to discriminate between small colonies of *D. vexillum* and native didemnid species which are likely to grow on hard substrates. Even with biosecurity plans in force within the risk assessment area it is therefore possible that *D. vexillum* will evade detection. If sufficiently large, *D. vexillum* colonies can detected by eye. Supporting material to aid identification is available from various boating and angling organisations e.g. The Green Blue, Natural Resources Wales, MarLIN and the Northern Ireland Environment Agency have used poster and web campaigns to promote awareness and aid identification of this species which is also included in the Marine Conservation Society's Seasearch list of important non-natives for divers to watch out for. However, such campaigns tend to focus on marinas and harbours, and chance discoveries and positive identification of *D. vexillum* in natural areas is far less likely.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	medium	*D. vexillum* is probably more likely to enter this pathway when the colonies are large and potentially able to produce viable colony fractions and larvae during the summer months. Natural dispersal from outside GB may be promoted by the prevailing west and south westerly winds to cross the Irish Sea. Prevailing winds and tidal currents would suggest that English Channel and North Sea routes are far less likely by natural dispersal.
1.9. How likely is the organism to be able to transfer from the	unlikely	medium	If colonies survive a sea journey across the Irish Sea small numbers might reach the GB coast in the large open bays on the west coasts of England, Wales and

pathway to a suitable habitat or host?			SW and W Scotland. For successful transfer from the pathway to a suitable habitat larvae or fragments will need to settle on suitable hard substrates. Most *D. vexillum* in the UK and Ireland (and globally) thrives on artificial floating substrates (e.g. Fletcher *et al*., 2013a) and only occasionally occurs on natural substrates (e.g. Hitchin, 2012).
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	unlikely	medium	Artificial pathways are more likely to translocate colonies to the GB coastline than natural dispersal although this is an important mechanism for secondary spread once established within the risk assessment area.

End of individual pathways assessment.			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways (comment on the key issues that lead to this conclusion).	very likely	very high	The high volume of shipping traffic from donor sites in and around the invaded range, and the ability of *D. vexillum* to settle and establish on a wide variety of substrates makes entry via hull fouling likely. The level of confidence is high due to its proven presence on ship/boat hulls and in marinas linked by the high volume of vessels that use the busier routes. Entry via fin-fish aquaculture movements is less likely due to the low volume of imports from outside the risk assessment area but has specifically been linked to existing shellfish (oyster) culture stock movements where *D. vexillum* has arrived in GB on seed stock. Entry via the other pathways is feasible but probably unlikely or moderately likely with low confidence. *D. vexillum* colonies are difficult to identify or resemble native species, fragments may be very small and practically invisible and will colonise cryptic spaces on hulls and equipment and therefore evade detection. Small colonies are therefore likely to remain undetected both prior to accidental export and on arrival at a new location. Whether, on arrival, it is capable of invasive spread seems to vary depending on habitat and inherent 'invasiveness' which may be genetically controlled. If it manages to attach to artificial floating substrata, it is very likely to gain a foothold in a new area. Control measures exist for some or parts of the pathways but are not strictly enforced. Vigilance for new arrivals is sporadic and monitoring potentially labour intensive and impractical at a comprehensive scale and likely to miss early-stage colonies.

PROBABILITY OF ESTABLISHMENT			
Important instructions: • For organisms which are already well established in the risk assessment area, only complete questions 1.15, 1.21 and 1.28 then move onto the spread section. If uncertain, check with the Non-native Species Secretariat.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	widespread	high	*D. vexillum* in GB thrives on artificial (especially floating) structures found in harbours and marinas (including boat hulls) and those associated with shellfish culture. There are over 200 major harbours and marinas in the risk assessment area where water quality (salinity and temperature) is potentially suitable (Dijkstra, 2009; Dijkstra *et al*., 2007; Valentine *et al*., 2007) in addition to many smaller developments such as moorings and private pontoons. *D. vexillum'*s current northwards range in GB includes Loch Creran (Begg *et al*., 2020; Cottier-Cook *et al*., 2019) and it is also known from areas in the Mediterranean (Ordonez *et al*., 2015). Inshore sea surface water temperatures throughout England and Wales, recorded during surveys in marinas and harbours from 2009 to 2020, indicate that water temperatures are generally above 16^{o}C and can reach more than 22^{o}C from July to October (Bishop *et al*., 2015; J. Bishop & C. Wood, unpublished data, 2021). During the winter, *D. vexillum* colonies are known to exhibit regression, but not usually complete death (Valentine *et al*., 2007). In other studies, this regression pattern has been strongly correlated to seasonal fluctuations in temperature and salinity (Gröner *et al*., 2011), but resistance to these fluctuations varies across populations (Valentine 2009; Fletcher & Forrest 2011; Gröner *et al*., 2011). Localised ice formation at the surface combined with reduction in salinity from a nearby river during the winter months in Holyhead marina also resulted in colonies of *D. vexillum* reducing in size

			(Holt & Cordingley, 2011) and in the Netherlands, where temperatures drop below 5^{o}C, die back completely (Gittenberger, 2007). Both the northern and southern limits of this species suggest that it can adapt to grow and reproduce in lower and higher temperatures than suggested by some of the earlier studies and thus all the risk assessment area is potentially suitable. See https://invasions.si.edu/nemesis/species_summary/-334 for a summary of temperature tolerances.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	very likely	high	Total eradication of any species in the marine environment is very difficult – such measures have already proved labour intensive, expensive and ultimately ineffective when tackling a well-established *D. vexillum* infestation outside and within the risk assessment area (e.g., Coutts & Forrest, 2007; Holt & Cordingley, 2011) and at best keep the levels of this species down rather than eradicate it completely. Small, virtually undetectable, colonies survive management measures and may perpetuate populations through re-growth and subsequent larval dispersal. In Holyhead the eradication attempt resulted in undetectable levels for several months, but it returned to pre-eradication abundance after a few years once maintenance measures ceased, but only on floating objects (Holt, 2015). This apparent preference for floating structures was emphasised when several contaminated vessels and pontoons sank during a winter storm. All the colonies shrank and eventually disappeared, possibly because of a change in temperature regime and their intolerance to heavy siltation near the muddy seabed (Holt, 2018; Lambert & Lambert 1998). If detected on arrival at a new location a local eradication attempt could be effective providing larvae dispersal or fragmentation of colonies had not occurred onto new habitats away from their vector. Such circumstances are

			unlikely, as fragmentation and re-settlement occur throughout the year in most reported cases, although shrinking colonies exhibiting winter die-back at low temperatures and salinities are less likely to be capable of reproduction by fragmentation (Kleeman, 2009).
1.28. Estimate the overall likelihood of establishment (mention any key issues in the comment box).	very likely	very high	There are suitable environmental conditions over a widespread area in the risk assessment area both within non-natural harbours and marinas and natural habitats. *D. vexillum* is capable of rapid growth and reproduction by both sexual and asexual reproduction and out-competes native species when and where optimal conditions occur. It has already proved very difficult to control in the marine environment and spread has occurred undetected because of its cryptic nature and problems with identification. However, not all occurrences of *D. vexillum* perpetuate as high-density infestations over time. In some of the contaminated marinas on the south coast of England *D. vexillum* remains at low abundance and has not established large nuisance-level populations (J. Bishop pers. com.) or transferred from anthropogenic structures onto natural habitats (Holt, 2018).

PROBABILITY OF SPREAD			
Important notes: • Spread is defined as the expansion of the geographical distribution of a pest within an area.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
2.1. How important is the expected spread of this organism in the risk assessment area by natural means? (Please list and comment on the mechanisms for natural spread.)	major	medium	*D. vexillum* produces larvae that have a short dispersal phase that lasts up to 36 hours or can travel as fragmented colonies for up to three weeks that can re-attach when they settle on a suitable substratum (Worcester, 1994; Morris & Carman, 2012; Fletcher *et al*., 2013a). Short-distance spread of larvae is very likely although settlement on to natural substrates seems highly variable and related to a combination of genetic 'invasiveness' and suitability of habitat (Prentice *et al*., 2021). Natural translocation over longer distance is more likely to occur as fragmented colonies – perhaps attached to floating seaweed, sea grass or other floating biota or as free-floating spherical colonies (Morris & Carman, 2012). In some locations it has spread rapidly by natural means (e.g. in the Grand Banks) but in others it has not (e.g. Holyhead Marina). The response score of 'major' is given because of the potential for rapid natural spread. It is unclear why there are differences between populations in terms of rate of natural spread, hence medium confidence.
2.2. How important is the expected spread of this organism in the risk assessment area by human assistance? (Please list and comment on the mechanisms for human-assisted spread.)	major	high	Spread within the risk assessment area mainly involves similar anthropogenic vectors as those listed for introduction into the area from outside GB (section 1.2). Leisure craft (e.g., Griffith *et al*., 2009) movements of shellfish seed stock and equipment (e.g., Cottier-Cook *et al*., 2019; Moore & Harries, 2020; Hitchin, 2012) and the movement of contaminated marina and shipping/marine industry equipment (e.g., Holt & Cordingley, 2011) are most likely to spread *D. vexillum*.

2.3. Within the risk assessment area, how difficult would it be to contain the organism?	very difficult	high	*D. vexillum* has two main 'strategies' for dispersal that are very difficult to contain. Larval dispersal, even though short-range, can result in cross contamination of nearby suitable vectors with undetectably small colonies. Fragmented colonies are potentially capable of surviving for longer in the water column and therefore can travel further before re-attaching. Inspection and controlling the movement of all potential vectors would be required to contain this species (Worcester, 1994; Morris & Carman, 2012; Fletcher *et al*., 2013a).

2.4. Based on the answers to questions on the potential for establishment and spread in the risk assessment area, define the area endangered by the organism.	Artificial substrata (particularly floating) in marinas and harbours, shellfish culture and other mariculture infrastructure. Intertidal hard substrata and cohesive gravels. Subtidal hard substrata and cohesive gravel and non-silted sediments.		Artificial substrates in marinas and harbours, particularly those with good quality, full salinity sea water (Coutts & Forrest, 2007) are at high risk of establishment and spread anywhere in the risk assessment area. Water temperature and growth rate are strongly correlated (McCarthy *et al*., 2007), and although potentially optimal in the south and west of the risk assessment area, rarely exceeds upper and lower limits for survival and reproduction anywhere in GB (Bullard *et al*., 2007; Ordonez *et al*., 2015). Natural substrates in the intertidal and subtidal include bedrock, boulders, cobbles and cohesive gravels and the biota that inhabits these. Providing salinity and temperature are suitable, colonisation is therefore possible in most coastal areas of the risk assessment area apart from in low-salinity estuaries and on sediment plains. Sandy and muddy habitats are unsuitable for direct attachment but *D. vexillum* will grow on sea grass *Zostera marina* meadows in wave-sheltered sandy bays, lagoons and outer estuaries as an epiphyte on seagrass blades (Carman & Grunden, 2010). Sea grass meadows have a restricted patchy distribution throughout much of GB (see NBNatlas.org). There may be differences between haplotypes and it does appear to be spreading on natural substates in Milford Haven. There appear to be limiting factors that prevent *D. vexillum* spreading widely on natural substrates in the risk assessment area as evidenced by prolific and widespread growth on anthropogenic structures but relatively little growth in adjacent natural habitats. This has been attributed to a combination of competition for space and predation (Forrest *et al*., 2013) and haplotype-related invasiveness of the different strains present in the UK (Prentice *et al*., 2021).
2.5. What proportion (%) of the area/habitat suitable for	0-10	high	When considering the available surface area in GB marinas that carry *D. vexillum* populations, rarely does the abundance, as percentage cover,

establishment (i.e. those parts of the risk assessment area where the species could establish), if any, has already been colonised by the organism?			exceed a small proportion of the total available (i.e., total submerged or potential for colonisation based on similar colonised habitats elsewhere). If considering the total suitable available area including all marinas, harbours and natural substrata that do not support *D. vexillum* populations then that value is far less as expressed as a percentage of the total. Notwithstanding the points above, an attempt to estimate percentage occupied has been made based on the proportion of 10 km squares in which this species occurs in GB. There are approximately 400 10 km squares along the coastline of GB. *D. vexillum* occurs in approximately 17 of these squares. Assuming there is suitable habitat in all available squares (low confidence), this suggests that *D. vexillum* has occupied approximately 4% of its potential range at this scale. This is comfortably within the 0-10% response category selected.
2.6. What proportion (%) of the area/habitat suitable for establishment, if any, do you expect to have been invaded by the organism five years from now (including any current presence)?	0-10	medium	Given that approximately 4% of 10km squares have been occupied since 2005, it is unlikely that this species would spread to more than 10% of 10 km squares in the next five years.
2.7. What other timeframe (in years) would be appropriate to estimate any significant further spread of the organism in the risk assessment area? (Please comment on why this timeframe is chosen.)	30	low	Based on rate of spread to date (set out in response to question 2.5) it is estimated that this species could spread to beyond 10% of potentially suitable habitat within 30 years. There is considerable uncertainty both in the rate of spread of this species and the extent to which it will occupy habitat, hence low confidence in this response.

2.8. In this timeframe what proportion (%) of the endangered area/habitat (including any currently occupied areas/habitats) is likely to have been invaded by this organism?	10-33	low	As per comment in 2.7.
2.9. Estimate the overall potential for future spread for this organism in the risk assessment area (using the comment box to indicate any key issues).	moderately	low	This estimate is based on the number of new areas found supporting *D. vexillum* since monitoring / raised awareness of this species began and the rate at which colonisation occurs at these locations. The earliest record of this species in GB is from 2005. It is now considered to be present in approximately 4% of potentially suitable 10 km squares in GB. This change over time suggests spread between moderate and rapid (see Leach and Mumford spread tool for GBNNRA, in prep.). Given that it is likely this species was established in GB before 2005 but went undetected, the response of 'moderate' appears most appropriate. There is considerable uncertainty about when the species first established in GB and its current extent, confidence in this response is therefore low.

PROBABILITY OF IMPACT			
Important instructions: • When assessing potential future impacts, climate change should not be taken into account. This is done in later questions at the end of the assessment. • Where one type of impact may affect another (e.g. disease may also cause economic impact) the assessor should try to separate the effects (e.g. in this case note the economic impact of disease in the response and comments of the disease question, but do not include them in the economic section). • Note questions 2.10-2.14 relate to economic impact and 2.15-2.21 to environmental impact. Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area separating known impacts to date (i.e. past and current impacts) from potential future impacts. Key words are in bold for emphasis.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENTS**
2.10. How great is the economic loss caused by the organism within its existing geographic range **excluding the risk assessment area**, including the cost of any current management?	major (depending on the industry or resource impacted)	medium	Infestations of *D. vexillum* cause the aquaculture industry huge economic losses through loss of shellfish and biofouling of the equipment. It has been found in association with mussel longline cultivation in Ireland (Minchin & Nunn, 2013) and in Canada fouling mussel cages (Lambert, 2009) where it blocks water flow and weighs down the equipment. Similarly, *D. vexillum* smothers green mussel, *Perna canaliculus* slowing growth rates or even killing it on rope culture in New Zealand. Attempts to eradicate it cost aquaculture industries over $800k (Dijkstra, 2009). While economic impacts are widely reported in North America and also from Europe and New Zealand, no attempt to quantify this impact was found. Loss in condition of oyster stocks in Canada, again through decreased growth rates caused by smothering, place financial burdens on shellfish production (Switzer *et al*., 2011). Wild stock of scallops *Placopecten magellanicus* growing on subtidal shell gravel banks at Eastport, Maine (Valentine *et al*., 2007) and Georges Bank, USA (Morris *et al*., 2009) were completely smothered by *D. vexillum* causing large mortalities (Valentine *et*

			al., 2007). The respiratory currents of the scallops were fouled by *D. vexillum* which reduced their ability to swim and evade predators (Dijkstra & Nolan, 2011).
2.11. How great is the economic cost of the organism **currently** in the risk assessment area **excluding management** costs (include any past costs in your response)?	minor	high	Excluding the cost of attempted eradication in Holyhead (Holt & Cordingley, 2011; Sambrook *et al*., 2014) and Loch Creran and other management programs, the current economic losses caused by *D. vexillum* are small in the risk assessment area.
2.12. How great is the economic cost of the organism likely to be **in the future** in the risk assessment area **excluding management** costs?	moderate	low	This is difficult to gauge based on the current situation as so far *D. vexillum* is causing little damage to fisheries and natural resources in the risk assessment area and the costs involved are mainly related to management and eradication. The areas at risk of economic impact include shellfish farming, shellfish lays (areas of seabed used for on-growing species such as mussels *Mytilus edulis* and 'ranching' scallops *Pecten maximus*) and the finfish farming industries. Economic loss through damage to wild caught species' habitats such as smothering of fish spawning grounds and natural shellfish beds should also be considered. Loss of value of 'clean' seed stock will impact the mussel farming industry's ability to sell to areas that do not already host *D. vexillum*. It also adds to the list of nuisance species that require removal from ships hulls, cooling water intakes etc. Direct impact on mussels farmed in New Zealand caused by smothering, resulted in loss of growth rates of smaller mussels (Fletcher *et al*., 2013b) and subsequent crop losses (Coutts & Forrest, 2007). Economic impacts on wild fisheries (e.g. benthic fish, scallops, lobsters, mussels, etc.) are expected on Georges Bank (Bullard *et al*., 2007; Valentine *et al*., 2007) where

			hundreds of hectares of seabed are covered by *D. vexillum* which outcompetes scallops *Placopecten magellanicus* for space and reduces the area available for fish spawning (Kaplan *et al*., 2017). In New Zealand *D. vexillum* clogs netting and increases the weight of equipment which incurs cleaning costs and also significantly adds to the cost of antifouling commercial shipping (Coutts & Forrest, 2007). Raised levels of mandatory biosecurity measures to prevent the spread of non-native species would incur significant costs if *D. vexillum* requires removing from hulls and equipment before moving to a new location within or outside the risk assessment area. Attempts to assess potential economic impact have been conducted for Wales and Scotland. In Wales, Kleeman (2009) estimated a cost to the mussel industry of approximately £1.4 million over 10 years, based on a loss in production of approximately 5% if the species were to spread nationally. In Scotland, Hambrey *et al* (2011) estimated potential costs to the mussel and fisheries industries of £1.5 million per annum if *D. vexillum* were to spread nationally. Williams *et al* (2010), updated by Eschen *et al* (2023), noted that *D. vexillum* contributes to hull fouling in GB but may not be a significant additional cost compared to fouling by other species (including native species). The cost of hull fouling by INNS to the aquaculture industry was calculated to be in the region of £100k per annum Eschen *et al* (2023), of which *D. vexillum* is responsible for a small fraction.
2.13. How great are the economic costs **associated with managing** this organism **currently** in the	moderate	high	The management and attempted eradication and subsequent monitoring of *D. vexillum* in Holyhead marina is probably the greatest economic cost for a single outbreak in the risk assessment area. The eradication attempt cost approximately £750,000 (Holt & Cordingley, 2011) and has been followed

risk assessment area (include any past costs in your response)?			up with monitoring every few years since (e.g., Holt 2018; Holt, 2020 and 2021 unpublished field reports) which cost in the region of £1-3k each depending on the number of sites surveyed. Other eradication and control actions, e.g., in Loch Creran (Cottier-Cook *et al*., 2019) were smaller and less labour intensive.
2.14. How great are the economic costs **associated with managing** this organism likely to be **in the future** in the risk assessment area?	moderate	medium	Continued monitoring for *D. vexillum* in the marine environment is likely to be an ongoing requirement – ideally nested amongst programmes to routinely check for a suite of marine INNS. This should target likely and known risk areas throughout the risk assessment area. Eradication may be attempted for small-scale infestations if caught early.
2.15. How important is environmental harm caused by the organism within its existing geographic range **excluding the risk assessment area**?	major	medium	Ecological impacts are reported from North America, Europe and New Zealand. This species can form huge colonies that overgrow other sessile communities, displacing native species and altering large areas of habitat. For example, extreme abundance in Oosterschelde, Netherlands (covering >95% of substratum in some areas) was accompanied by a dramatic decline in brittlestar *Ophiothrix fragilis* and sea-urchin *Psammechinus miliaris*. On the pebble gravel bottom of Georges Bank off Massachusetts (which locally covered the majority of the seabed), this species caused significant alteration in species composition of benthos compared to uncolonized areas. Further examples linked to mechanisms of impact, are set out below (summarised from Tillin *et al* 2020). **Competition** *D. vexillum* is capable of overgrowing natural habitats and outcompetes native sessile biota for space (Dijkstra, 2009), smothers filter feeders and has few predators (Valentine *et al*., 2007) and changes community structure (Whitlatch *et al*., 1995; Bak *et al*., 1996). Shell gravel banks at Eastport, Maine (Valentine *et al*., 2007) and huge areas of Georges Bank, USA (Morris *et al*., 2009) were completely smothered by *D. vexillum* (Valentine *et al*., 2007) which slows or prevents growth of native fauna including commercially important species such as scallops and prevents fish from

			spawning on gravel beds. It has been recorded overgrowing kelp, seagrass (Carman & Grunden, 2010), mussels etc. (Minchin & Nunn, 2013) and seems most invasive in disturbed areas (Dijkstra, 2009). Tillin et al (2020) suggested an EICAT impact rating of major concern (medium confidence) for competition where sessile organisms and algae may be overgrown and smothered and where competition is therefore focussed on space occupation. **Bio-fouling** *D. vexillum* has been found overgrowing a wide variety of abiotic and biotic substrates including on pebble and cobble substrates (Mercer *et al*., 2009), algae (Dijkstra *et al*., 2007), bivalves (Bishop, 2010), seagrass (Carman & Grunden, 2010); aquaculture gear (Morris *et al*., 2009) and on the surface of benthic species (Bishop, 2010; Dijkstra, 2009), often coating other unitary sea squirts. Huge areas of seabed, comprising mixtures of sediment and hard substrate covered by *D. vexillum* have been studied on the Georges Bank (Lengyel *et al*., 2009) – they concluded that scallop dredging induced fragmentation which contributed to further spread. **Indirect impacts through interactions with other species** In areas of rapid growth *D. vexillum* creates a monoculture covering a high proportion of the available substrate. Where this occurs on natural seabed (Dijkstra, 2009) this impacts other species that rely on this habitat for food and refuge. It smothers the filter feeding mechanisms of sessile organisms but also prevents predators such as fish and crustaceans from reaching their prey (Morris *et al*., 2009; Reinhardt *et al*., 2012). It also out-competes other species for space by rapidly overgrowing substrates and by producing acid secretions that naturally anti-foul its own surface (Bullard *et al*., 2013). It also occupies stony and gravel habitats that would otherwise be occupied by commercially important bivalves, lobsters and crabs (Morris *et al*., 2009; Reinhardt *et al*., 2012). **Physical impact on ecosystem.**

			In monoculture *D. vexillum* creates a physical barrier to light, water flow and food particles reaching the seabed underneath sheet-like growths (Dijkstra 2009, Morris *et al*., 2009). Light reduction can potentially reduce photosynthesis in seagrass and algal beds (Carman & Grunden, 2010; Long & Grosholtz, 2015) leading to reduction in growth rates and extent. **Structural impact on ecosystem** Its ability to rapidly spread allows it to overgrow many substrates which would normally support communities of benthic invertebrates. These are excluded from settling, experience a reduced growth rate or are smothered and killed, changing the habitat to a monoculture of *D. vexillum* (Dijkstra, 2009). Its surface has a low pH which prevents other larvae from settling (Morris *et al*., 2009). *D. vexillum* has substantially restructured benthic communities on Georges Bank on the E. Seaboard of N. America (Lengyel *et al*., 2009; Kaplan *et al*., 2018).
2.16. How important is the impact of the organism on biodiversity (e.g. decline in native species, changes in native species communities, hybridisation) **currently** in the risk assessment area (include any past impact in your response)?	moderate	medium - further survey is required to determine rate of spread where it has started to grow on natural substrata.	<u>Evidence of impact in GB is limited; however, the following is provided:</u> **North Kent Coast** The largest and most widespread colonisation of the natural marine environment within the risk assessment area has occurred on the N Kent coast – both on the shores and on the adjacent open seabed (Hitchin, 2012). It has been found on the shore under overhanging, shaded surfaces of large boulders and amongst intertidal algae. Subtidal colonisation off Herne Bay appears to be more extensive (see video - YouTube link <u>N Kent D.vex 2016</u>) covering a wide variety of algae, hydroids, bryozoans and mixed gravelly substrates. Its main impact appears to be smothering of the native biota as described in 2.15 where communities become dominated by *D. vexillum*. However, where this overgrowth of native species has been observed, it is often on rapidly growing and seasonal species, such as kelp, sponges, hydroids and ascidians that colonise and recover quickly. As yet there have been no observations of longer-lived, slow growing species such as sea fans

			(e.g. *Eunicella verrucosa*) and slow-growing sponges being colonised in the risk assessment area. The area of natural habitat covered is at least several hundred metres across (videoed by a diver drifting with the current) and its total extent is unknown but is probably only a very small proportion of the total habitat available for colonisation. The rate of increase in extent is unknown. **Other areas** *D. vexillum* has been found on natural substrates in Loch Creran (Cottier-Cook, 2019) adjacent to oyster trestles where it appears to have spread from. It was found overgrowing patches of algae and amongst boulders on the lower shore. Surveys of subtidal habitats in the area did not locate any other colonies. *D. vexillum* is also known from several sites within the Clyde area, Holyhead marina in N Wales, Milford Haven in S Wales and in several marinas along the South England coast from Brighton, the Solent, Dartmouth and Plymouth. It is known to have invaded natural habitat in the Solent (Taylor *et al* 2022) and Milford Haven (Autumn 2023), where it is mainly found on natural substratum (NRW *pers comm*.). Milford Haven is within a marine SAC (Pembrokeshire Marine SAC, this SAC has 'Reef' as a feature). It is not known to have invaded natural habitats in the other areas.
2.17. How important is the impact of the organism on biodiversity likely to be in the **future** in the risk assessment area?	moderate	moderate	If an aggressively invasive form of *D. vexillum* arrives in its ideal habitat, perhaps those circumstances found on the N Kent coast, it may impact biodiversity over very large areas of seabed particularly in the south and west of the risk assessment area. However, current evidence of its ability to spread on natural habitats in this area is sparse and often conflicting, complicated by genetics and its apparent variable habitat preferences and tolerances and its variable ability to adapt to 'new' conditions. If *D. vexillum* can invasively colonise biogenic reefs such as native oyster beds, horse mussel reefs and

			maerl beds or colonise seagrass beds and kelp forests it could have a major impact on features within Marine Protected Areas.
2.18. How important is alteration of ecosystem function (e.g. habitat change, nutrient cycling, trophic interactions), including losses to ecosystem services, caused by the organism **currently** in the risk assessment area (include any past impact in your response)?	moderate	moderate	Where *D. vexillum* occurs as small colonies amongst other native and non-native species, often found growing on artificial floating substrata, it seems to have minimal impact on ecosystem function. There are examples of larger, invasively growing colonies on similar artificial structures and here it appears that *D. vexillum* forms a monoculture and overgrows all other biota. The more extensive subtidal colonisation off Herne Bay appears (see video YouTube link N Kent D.vex 2016) to cover a wide variety of algae, hydroids, bryozoans and mixed gravelly substrates. Its main impact appears to be patchy and incomplete smothering of the native biota where communities become dominated by *D. vexillum*.
2.19. How important is alteration of ecosystem function (e.g. habitat change, nutrient cycling, trophic interactions), including losses to ecosystem services, caused by the organism likely to be in the risk assessment area in the **future**?	major	moderate	If an aggressively invasive form of *D. vexillum* arrives in its ideal habitat, perhaps similar to those circumstances found on the Kent coast, it may impact ecosystem function over very large areas of seabed as has already occurred off the coast of the USA (Morris *et al*., 2009). Spread of *D. vexillum* to new locations, particularly along the S England coast, seems to be progressing slowly but steadily, although its rate of colonisation of artificial habitat at each new location appears slow and there are no recent cases of it occurring on natural substrates (J. Bishop pers. com.).
2.20. How important is decline in conservation status (e.g. sites of nature conservation value, WFD classification) caused by the organism **currently** in the risk assessment area?	moderate	low	*D. vexillum* is able to colonise priority marine features such as biogenic reefs including mussel and native oyster beds, maerl etc. as well as natural subtidal hard substrata. It can also inhabit natural intertidal rocky shores to a limited extent where it requires shade and moisture to avoid desiccation. *D. vexillum* (or non-native *Didemnum* species) is/are referred to as a ‘high impact’ species, is on the MSFD monitoring list and potentially lowers the conservation value of areas (WFD). The Clyde and Argyll areas, for example, are both regions of ‘many concerns’ (WFD information from

			Marine Scotland Assessment, 2020) – in part because of the presence of *D. vexillum* in marinas in the lower Clyde. There are currently many areas of marine conservation importance that do not support *D. vexillum*.
2.21. How important is decline in conservation status (e.g. sites of nature conservation value, WFD classification) caused by the organism likely to be in the **future** in the risk assessment area?	moderate	medium	Increased spread will likely introduce *D. vexillum* into Marine Protected Areas. If it remains as small colonies dispersed amongst native flora and fauna it will have relatively little impact compared to its aggressively invasive form. Tillen *et al* (2020) reviewed the MPA features that provide suitable habitat for this species. Intertidal underboulder/boulder communities were considered suitable (including seagrass, *Zostera* and blue mussel beds) and a wide range of littoral, infralittoral and circalittoral rock were potentially suitable (see Tillen *et al* 2020 for a full list). They concluded that features most at risk were those close to artificial structures in the UK, with establishment most likely in areas of rock and gravel with established sessile communities as well as biogenic reef. In addition, they noted potential for impact on tidal swept deep gravel, bivalve beds and *Zostera* seagrass habitat. They concluded impact ratings of 'major' concern (high confidence) for bivalve beds, seagrass and *Zostera* habitat
2.22. How important is it that genetic traits of the organism could be carried to other species, modifying their genetic nature and making their economic, environmental or social effects more serious?	minimal	high	Unlikely that traits will be carried to other species. There is little evidence to suggest that *D. vexillum* will hybridise with native ascidians.
2.23. How important is social, human health or other harm (not directly included in	minimal	high	No impacts known– although its surface is acidic (see 2.25).

economic and environmental categories) caused by the organism within its existing geographic range?			
2.24. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	minimal	high	*D. vexillum* appears to have very few predators and has not been found to contribute significantly to the diet of other marine organisms – damaging or otherwise. It is not known to transmit diseases to native fauna. It is known to carry parasites in its gut but these are not harmful to other species.
2.25. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	minimal	low	The surface of *D. vexillum* is slightly acidic – probably as a natural antifoulant to prevent other species settling on it (Bullard *et al*., 2013). Whether this has an impact on other biota or potentially on human health is unknown.
2.26. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?	major	medium	There are no known predators, parasites or pathogens present in the risk assessment area.
2.27. Indicate any parts of the risk assessment area where economic, environmental and social impacts are particularly likely to occur (provide as much detail as possible).	Most of GB with suitable habitat	high	This species is not widely established in the UK on natural habitats and there are key uncertainties in the extent and magnitude of colonisation. Parts of the risk assessment area where economic, environmental and social impacts are likely to occur if *D. vexillum* becomes aggressively invasive would include:

			• any areas of shellfish culture where equipment and stock are at risk of smothering and natural habitats where seed stock is gathered, on-grown or 'ranched' (e.g., mussel, oyster and scallop beds/habitat). • all marina and harbour developments where biofouling in general can cause problems for vessels and equipment. • any areas with suitable habitat for settlement – this ranges from bedrock and boulders through to cohesive gravels and biogenic reef in the intertidal and subtidal. The impact assessments of the MPA features for this pathway range from 'minimal concern' to 'major concern' depending on the area within the habitats that *D. vexillum* is likely to foul. MPA features most at risk are likely to be situated near to artificial structures where *D. vexillum* can potentially disperse from. MPA features such as rock and gravel with an established sessile fauna community will provide suitable attachment substrate (Bishop, 2010; Coutts & Forrest, 2007; Osman & Whitlatch, 2007) as do biogenic reefs with many attachment surfaces (see below). *D. vexillum* has been found to die when exposed to air for > 6 hours (Laing *et al*., 2010) and is therefore unlikely to establish well in the littoral zone. *D. vexillum* has substantially restructured benthic communities on Georges Bank on the E. Seaboard of N. America (Lengyel *et al*., 2009; Kaplan *et al*., 2018). Impacts on MPA features have been assessed from 'minimal concern' to 'major concern' depending on the likelihood that *D. vexillum* could foul small or large areas within the habitats. Habitats where establishment is possible and would potentially cause damage:

			• Bivalve beds: (Oysters and mussels) *D. vexillum* has been recorded fouling bivalves including mussels, oysters and scallops. These give the colonial sea squirt a multi- faceted structure with which to adhere to. Pacific oysters have been fouled by *D. vexillum* on the west coast of Canada (Valentine *et al*., 2007) and mussels in Ireland (Minchin & Nunn, 2013). Impacts on bivalve reefs are assessed as 'Major' with high confidence. • Tidal swept deep gravel (cobbles and pebbles) habitats: These habitats are considered to be vulnerable due to their suitability for *D. vexillum* to colonise. At Georges Bank, USA, large swathes of benthic gravel habitat have been colonized by *D. vexillum*. 230 km^2 has been covered and only the presence of mobile sands has limited further spread (Valentine *et al*., 2007). • Seagrass and *Zostera* habitats: *D. vexillum* has been recorded growing on seagrass habitats in northeast United States (Carman & Grunden, 2010). This may lead to reduced light and therefore growth which has been demonstrated with other invasive tunicate species (Wong & Vercaemer, 2012). Impacts are assessed as 'Major' at high confidence. • Biogenic reefs including mussel and oyster beds, with their multi-faceted nature, provide suitable substratum to adhere to (Dijkstra *et al*., 2007; Carman *et al*., 2009; Valentine *et al*., 2007). There is a lack of evidence suggesting that *D. vexillum* establishes well in the intertidal zone however, it can establish on the shore, preferring downward facing surfaces, such as overhangs or the underside of boulders (Hitchin, 2012) where it can evade desiccation. • Intertidal rocky and underboulder/boulder communities (although estuarine hard substrates are less likely to be colonised)

			• Intertidal *Mytilus edulis* beds on mixed and sandy sediments; and *Modiolus modiolus* beds. • Fragile sponge and anthozoan communities on subtidal rock • Tide-swept channels • Littoral and sublittoral habitats with coarse and mixed sediments • Maerl beds • *Musculus discors* beds • *Ostrea edulis* beds
2.28. Estimate the overall impact of this organism in the risk assessment area (using the comment box to indicate any key issues).	major	moderate	Impacts are geographically highly variable within the risk assessment area. Moderate spread has been observed on artificial substrates confined to marinas and harbours. It has also been associated with shellfish farm equipment and has spread to nearby intertidal and subtidal rocky habitats. Spread from a shellfish farm has become widespread and potentially damaging on natural subtidal habitats, but so far only off the north Kent coast. In other areas within the risk assessment area *D. vexillum* has failed to colonise natural habitats even within close range of artificial substrata that have been colonised for at least 10-15 years.

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Entry	very likely	very high	*D. vexillum* is known to occur and has very likely arrived from sources across the Irish Sea from Northern Ireland and the Republic of Ireland, and from NW Europe across English Channel. The most likely vectors are via vessel hulls, contaminated marina equipment and on shellfish brood stock and farming equipment. All potential pathways involve accidental contamination of surfaces where its cryptic nature, particularly of small colonies, allows it to remain undetected both prior to 'export' and on arrival at a new location. Evidence from genetic material suggests that the most likely pathways are directly between marinas or between shellfish culture sites (Prentice *et al*., 2021). Invasive species control measures exist for some of the pathways but are not comprehensively enforced nor effective against *D. vexillum*. 'Clean hull' policies for new arrivals on vessel hulls in marinas and harbours are rarely practiced consistently and monitoring for its arrival is potentially labour intensive and impractical at a comprehensive scale. Such measures are likely to miss early-stage colonies arriving in new areas. Shellfish must be certified as disease-free before being imported into GB, but they are not required to have all encrusting organisms removed. Even if cleaned, small colonies of *D. vexillum* could be present on the shellfish or as free-living fragments amongst them.
Summarise Establishment	very likely	very high	New arrivals within the risk assessment area are likely to establish on suitable habitats/substrates, particularly in the many marinas around the GB coastline where conditions for rapid growth and reproduction are particularly favourable during the warmer months. *D. vexillum* seems to favour floating surfaces such as boat hulls,

			pontoon and mooring buoy sides and undersides which remain permanently wet but remain in the warmest water near the surface. Ample, suitable habitats in marinas tend to be within short distances of one another – well within swimming distance for the short-lived larvae, and well within range for longer-lived colony fragments. However, not all occurrences of *D. vexillum* perpetuate as high-density infestations over time. In some marinas on the south coast of England *D. vexillum* sightings remain rare over time and do not go on to establish large nuisance-level populations (J. Bishop pers. com.) Establishment into natural habitats is more variable and has so far only been associated with *D. vexillum* from shellfish culture sites rather than marinas. Management of existing populations of *D. vexillum* has only been attempted at a small number of locations and, apart from lowering abundance, eradication measures have been unsuccessful at preventing establishment.
Summarise Spread	moderate	medium	This estimate is based on the number of new areas found supporting *D. vexillum* since monitoring / raised awareness of this species began and the rate at which colonisation occurs at these locations. Approximately one or two newly colonised areas are found each year – some of which may be marinas adjacent to one another, for example on the south coast of England, where multiple pathways for transfer make local spread very likely. Larger 'leaps' of geographical location seem to involve pathways associated with the translocation of shellfish stock such as that in Loch Creran. Its success this far north in Scotland suggests that many locations in-between are suitable for colonisation. Spread over wide areas of natural habitat appear to be rare, apart from off the N Kent coast where large areas of mixed stony and gravelly seabed support many large colonies. Whether every introduction to a new area result in nuisance-levels of growth is unknown, although in some cases post-establishment spread at single locations appears to be slow or even halts.
Summarise Impact	major	medium	Environmental impacts of *D. vexillum* seem to vary considerably within the risk assessment area, ranging from smothering extensive areas of native habitat and biota to

			small colonies living amongst native species with little evidence of impact. Invasive spread on natural substrates could potentially degrade sites of conservation importance within the risk assessment area. Economic impacts result from direct costs of removal and suppression in addition to the costs of monitoring for its arrival and spread. *D. vexillum* is currently found in discrete locations associated with marinas and shellfish farms and rarely on natural substrates, although it has covered a large area of seabed off north Kent where it competes for space with native species. If this ability to invade large areas of seabed become more commonplace it could significantly impact suitable habitat throughout much of the risk assessment area, although this seems more likely to occur in the south and west of the risk assessment area. The rate of transition from artificial to natural habitat appears slow.
Conclusion of the risk assessment	high	medium	Unchecked, *D. vexillum* appears capable of spreading widely in some areas within the risk assessment area where it has the potential to overgrow and smother a range of substrates and habitats. Socio-economic impacts involved with managing or removing the species as a bio-fouling nuisance are likely to steadily increase over time as it becomes more widespread. Its potential to damage natural habitats, especially those of conservation importance, has been seen at a few locations globally, but whether it will impact similarly within the risk assessment area is unknown and so far this has only occurred at one location.

ADDITIONAL QUESTIONS - CLIMATE CHANGE			
3.1. What aspects of climate change, if any, are most likely to affect the risk assessment for this organism?	Sea temperature rise	high	An increase in overall mean sea temperature in the risk assessment area will extend the rapid-growth and reproductive season for *D. vexillum*. Temperatures below 12 -14^{o}C (Valentine *et al*., 2009; Fletcher *et al*., 2013a) halt growth and production of larvae, and colony size is often seen to shrink during the winter (pers. obs in Holyhead Marina). If wintertime temperature-induced 'senescence' no longer occurs, growth and reproduction and therefore spread and colonisation of new areas could occur much more rapidly, leading to increased risk of harmful, nuisance levels of growth. Winter temperatures typically drop to a minimum of around 6-8^{o}C in the north of the risk assessment area and around 10-12^{o}C in the south (depending on prevailing weather patterns). Models of sea temperature change predict an average rise of 1.5^{o}C by 2050 and 3.2^{o}C by 2100 (since records began in 1870-1899) (RCP8.5 model - Genner *et al*., 2017). This would eventually allow *D. vexillum* to continue growing and reproducing throughout the year in some parts of the risk assessment area and allow seasonal growth in the far north. Climate change models also predict an increase in extreme weather events. Damaging storms have already resulted in the destruction of a marina colonised by *D. vexillum* (https://www.bbc.co.uk/news/av/uk-wales-43294517) which potentially distributed colonised floating debris widely around the Irish Sea. In this case there was no evidence of further dispersal, although future extreme weather events, if they coincide with warmer times of year when *D. vexillum* is growing more vigorously, could increase the rate of spread to new locations.
3.2. What is the likely timeframe for such changes?	<50 - 100 years	Low-medium	If by 2050 mean sea temperature has risen by 1.5^{o}C, *D. vexillum* will likely be able to colonise anywhere in the risk assessment area. Climate change models do not consider localised changes driven by weather rather than overall climate. Particularly hot summer weather could potentially accelerate growth rates resulting in the hanging pendulous, easily fragmented, growth form and increased larval production causing rapid spread in some areas (Bullard *et al*., 2007; Lambert, 2009) perhaps facilitating the transfer from artificial to natural habitats.

3.3. What aspects of the risk assessment are most likely to change as a result of climate change?	Entry, establishment, spread and impacts	medium	An increase in average sea temperature will likely increase the invasive capabilities of *D. vexillum* and its overall spread and abundance throughout the area.

ADDITIONAL QUESTIONS - RESEARCH			
4.1. If there is any research that would significantly strengthen confidence in the risk assessment, please summarise this here.	'Genetic invasiveness'	high	The work of Prentice *et al*., (2021) examined the relationship between haplotype genetics and invasiveness. Their work suggested that the haplotype of *D. vexillum* found on both shellfish culture equipment and nearby natural habitats in Ireland and SE England, was more invasive than the haplotype associated with marinas that does not seem able to grow in natural habitats. Understanding whether 'invasiveness' is genetically controlled could improve the strength of the risk assessment – to gauge whether individual populations pose a higher risk than others of invasive spread.
	Habitat resilience	high	Invasiveness is potentially linked to the resilience of a habitat and the communities of species that normally occupy it. A healthy and resilient ecosystem with suites of robust native species is better able to cope with the impacts of invasive species (Environment Agency 2021) which might live amongst but not dominate the native flora and fauna. Dafforn (2015) compared 'traditional concrete' non-natural substrates with eco-engineered non-natural structures (e.g. harbour walls with pools and crevices engineered into them) and found that where native species flourished there was a reduction in non-native species that struggle to out-compete them. *D. vexillum* has substantially restructured benthic communities on Georges Bank on the E. Seaboard of N. America (Lengyel *et al*., 2009; Kaplan *et al*., 2018). It is possible that intense dredging and trawling on Georges Bank has significantly weakened the natural communities' resilience to invasion by *D. vexillum* allowing it to dominate the weakened native communities. The relationship between habitat resilience and non-native species reaching invasive and damaging levels requires further investigation, particularly with respect to *D. vexillum* which exhibits a wide range of invasiveness within the risk assessment area.

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