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# Eradication of the non-native carpet ascidian (sea squirt) *Didemnum vexillum* in Holyhead Harbour: Progress, methods and results to spring 2011

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# 1 CRYNODEB GWEITHREDOL

Yn ystod haf 2008, cafodd y chwistrell fôr estron Didemnum vexillum ei darganfod ym marina Porthladd Caergybi gan Kate Griffith, myfyrwraig MSc o'r Ysgol Gwyddorau Eigion. Ymatebodd Cyngor Cefn Gwlad Cymru drwy gynnal arolwg o hyd a lled a dosbarthiad y chwistrell fôr. Ymddengys ei bod wedi'i chyfyngu i fyw ar y strwythurau pontÿn arnofiol a'r cadwyni sy'n angori'r marina yn ei le. Yn ddiweddarach yn y flwyddyn, gwnaeth astudiaeth ddichonoldeb parthed difa'r rhywogaeth ddefnydd o dystiolaeth a oedd wedi deillio o raglenni difa eraill o amgylch y byd – yn arbennig Seland Newydd – ac, yn y gobaith o gael llwyddiant, rhoddwyd rhaglen ddifa ar waith. Dechreuodd y cynllun peilot ar gyfer difa'r rhywogaeth ym mis Hydref 2009. Defnyddiwyd bagiau a deunyddiau lapio plastig i ynysu, mygu a lladd y chwistrell fôr trwy beri iddynt farweiddio o amgylch y pontynau. Yn ddiweddarach yn y flwyddyn, ar ôl cael caniatâd priodol gan FEPA, cyflymwyd y broses ddifa drwy ychwanegu calsiwm hypoclorit at y bagiau a'r gorchuddion. Gweithiodd y broses hon yn dda i bob golwg. Cafodd y pontynau eu trin fesul swp o hyd at 60 o fflotiau ar y tro, a chafwyd gwared â'r holl fywyd morol fwy neu lai. Cafodd y marina cyfan (mwy na 530 o bontynau a chadwyni angori) a thua 100 o angorfeydd siglo, eu trin drwy gydol y gaeaf, ac erbyn diwedd mis Mai 2010 roeddynt yn glir.

Fel rhan o gamau sicrhau ansawdd y rhaglen ddifa, dangosodd arolygon o'r marina a'r strwythurau eraill ym Mhorthladd Caergybi ddiwedd y gaeaf a dechrau'r gwanwyn nad oedd unrhyw olion o D. vexillum ar ôl ar unrhyw un o strwythurau'r marina. Fodd bynnag, ym mis Mai 2010 daeth arolygon deifio o hyd i chwistrell fôr Didemnid gytrefol yn tyfu ar derfynellau'r fferi a glanfa Tinto Aluminium. Roedd nifer o nodweddion D. vexillum yn perthyn i'r chwistrell fôr hon. Wrth i'r haf fynd yn ei flaen, ac ar ôl darganfod sbesimenau a oedd yn cynhyrchu larfâu, cadarnhawyd mai rhywogaeth frodorol oedd hon.

Wrth gynnal arolygon rheolaidd, tua diwedd mis Awst a dechrau mis Medi 2010 daethpwyd o hyd i ambell gytref fach o D. vexillum yn y marina, a rhoddwyd cynlluniau ar waith i drin y llecynnau bach hyn unwaith eto. Ddechrau mis Hydref 2010, yn union cyn i'r gwaith difa ailddechrau, daeth arolygon pellach o hyd i nifer fawr o gytrefi bach iawn, ynghyd â chytrefi mwy a oedd yn tyfu'n gyflym, dros rannau mwy o lawer o'r marina nag a welwyd yn y gorffennol. Erbyn dechrau mis Ionawr 2011, roedd yn amlwg nad oedd gan y Cyngor Cefn Gwlad mo'r arian na'r amser i ddelio â'r broblem; ac roedd tymheredd y môr yn ddigon isel i rw ystro'r broses o gynhyrchu larfâu, fel nad oedd angen ailgynnal rhaglen ddifa arall. Felly, penderfynwyd y dylid defnyddio'r arian a'r llafur i wella bioddiogelwch a chynnal gwaith monitro. Yn y cyfamser, dylid ceisio dod o hyd i arian ar gyfer cynnal rhaglen ddifa well, ar raddfa fawr, yn ystod gaeaf 2011-2012.

# 1 EXECUTIVE SUMMARY

The non-native sea squirt *Didemnum vexillum* was discovered in the marina in Holyhead Harbour by MSc student Kate Griffith from the School of Ocean Sciences in the summer of 2008. Subsequent surveys in the British Isles located this species in Largs (west Scotland), Plymouth and Dartmouth (south-west England), Solent (south England) and Malahaide and Carlingford Lough in the republic of Ireland. Virtually all instances of this species were found in marinas implicating leisure craft as the prime vectors.

The Countryside Council for Wales responded by surveying its distribution and extent in the wider harbour. It appeared to be confined to living on the floating pontoon structures and chains anchoring the marina in place. Later in the year a feasibility study for its eradication drew evidence from other eradication programmes around the World – particularly from New Zealand – and on the basis of a potential success an eradication programme was initiated. The eradication pilot started in October 2009, using plastic wrappings and bags to isolate, smother and kill the sea squirt by inducing a stagnation reaction around the pontoons. Later in the year, once appropriate FEPA permissions had been obtained, the eradication process was accelerated by adding calcium hypochlorite to the bags and wraps. Although very labour intensive this process apparently worked well; the pontoons were treated in batches of up to 60 floats at a time and cleared of virtually all marine life. The entire marina (over 530 pontoons and associated mooring chains) and around 100 surrounding swinging moorings were treated through the winter and finally cleared by the end of May 2010.

As part of the quality assurance measures during the eradication programme, inspection of the marina and other structures in Holyhead Harbour during late winter and early spring, revealed no trace of *D. vexillum* on any of the structures within the marina. However, in May 2010, diving surveys revealed a colonial didemnid sea squirt, with many of the characteristics of *D. vexillum*, growing on the ferry terminals and Tinto aluminium jetty. Once the summer had progressed sufficiently to find larvae-producing specimens this was confirmed to be a native species – this misidentification issue highlighted the difficulty in identifying *D. vexillum*.

In late August and early September 2010 a few small colonies confirmed to be *D. vexillum* were found in the marina during a routine survey and plans were initiated to re-treat these few small areas. In early October 2010, immediately before the eradication work recommenced, further survey work revealed large numbers of very small colonies and rapidly growing larger colonies over a much larger proportion of the marina than had been detected earlier. By early January 2011 it was evident that CCW had neither the funds nor time remaining while sea temperatures were sufficiently low to suppress larval production to re-run an improved eradication programme. It was therefore decided to re-direct funds and effort towards improving biosecurity and monitoring including the building of a prototype quarantine berth and self-antifouling pontoons. In the meantime CCW will be raising funds for a full-scale and much improved eradication attempt for the winter of 2011-2012.

# 2 INTRODUCTION

*Didemnum vexillum* is an invasive colonial ascidian that has become established world-wide in temperate waters having originated in Japan. It is now regarded as a nuisance species in North America, northern Europe, and New Zealand, following a global expansion since the 1970's (see Lambert, 2009 for review and **Figure 1**). It forms sheet-like colonies on natural and artificial hard substrata as well as benthic organisms including other ascidians and algae and even on *Zostera marina* beds (Carman and Grunden 2010). The serious ecological and economic damage experienced in New Zealand and other temperate regions has led to a large investment in on-going research into the biological tolerances and spread of *D. vexillum* (Biosecurity New Zealand, 2009; USGS, 2009; Gittenberger, 2007, Bullard and Whitlach, 2009) as well as rapid response, monitoring and management following introductions (Coutts and Sinner, 2004; Pannell and Coutts, 2007; Locke and Hanson, 2009).

## 2.1 Background

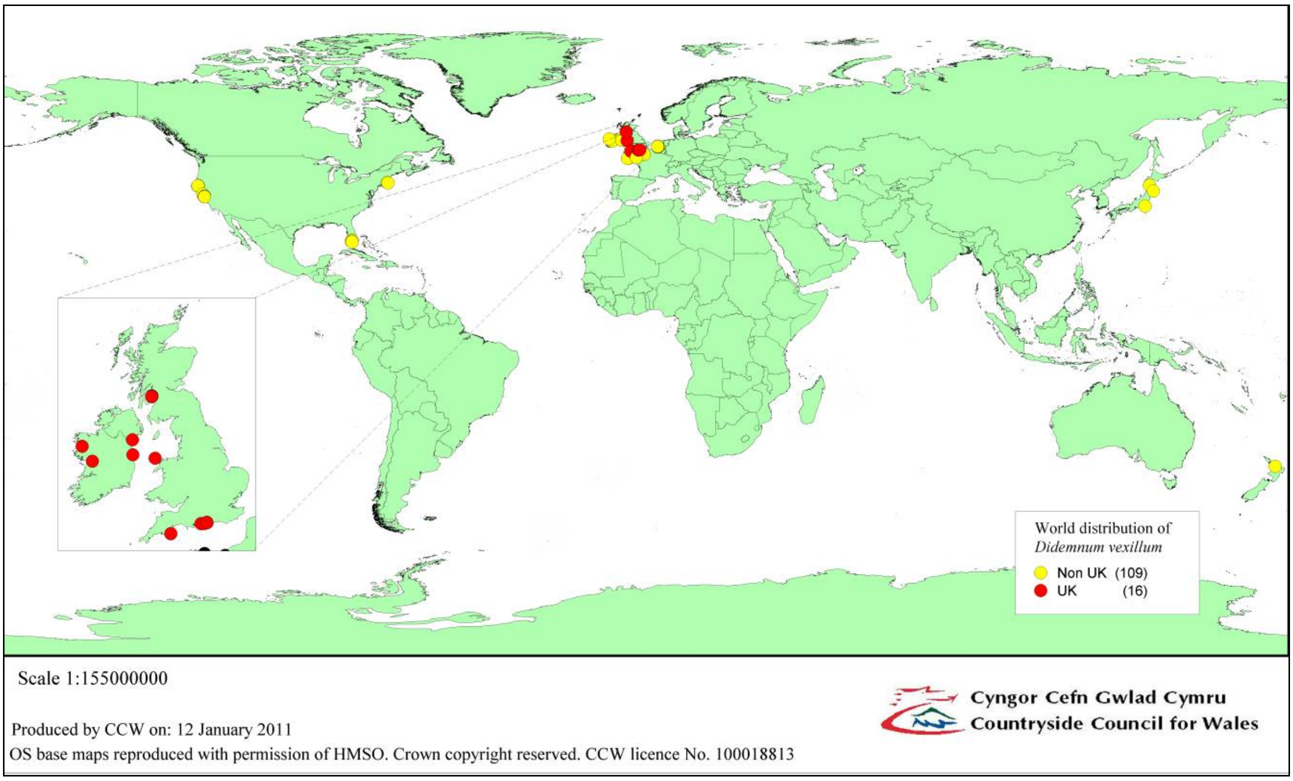



**Figure 1** World distribution of *Didemnum vexillum*

In summer 2008, an MSc student from Bangor University carrying out a survey of non native species in marinas in North Wales found a sea squirt in Holyhead Harbour that looked like the invasive non native species *D. vexillum*. A specimen was sent to a UK expert, who confirmed in November 2008 that it was very likely to be *D. vexillum*. Specimens from early samples were confirmed by Gretchen Lambert (pers. com. University of Washington, USA) as being *D. vexillum* and later samples verified by John Bishop at the Marine Biological Association in Plymouth.

In December 2008, the Countryside Council for Wales conducted a diving survey of Holyhead Marina to ascertain the extent and distribution of *D. vexillum*. The species was found throughout the marina in small dispersed colonies but without an obvious ‘epicentre’ where it might have first colonised, little could be deduced about where or when it was introduced.

In January 2009, a diving survey of the wider Holyhead Harbour area, including international shipping docks and ferry terminals, confirmed that *D. vexillum* was confined to the marina. However, the survey revealed a large area of suitable habitats for *D. vexillum* within the harbour. A rapid survey of other marinas in Wales conducted in 2009 and repeated in 2010 have so far found no other areas of colonisation.

In summer 2009, CCW commissioned a feasibility study for eradicating *D. vexillum* (Kleeman 2009). Kleeman concluded that circumstances within the marina presented a unique opportunity to eradicate *D. vexillum* from Holyhead, due to its relative isolation and phase of infestation (**Figure 2**). With potentially good chances of success CCW, funded directly by WAG, started a three year eradication program. To ensure eradication began within the lag phase of infestation, emergency procurement procedures were used to initiate a pilot eradication.

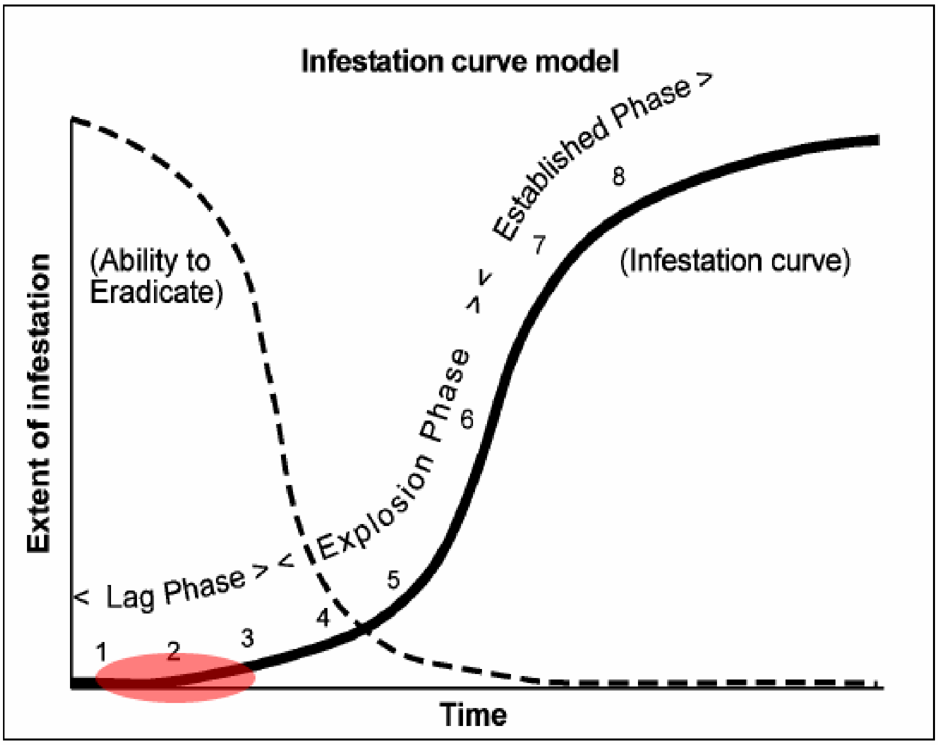



**Figure 2** Infestation Curve model.

On 1st October 2009, CCW’s eradication team started the pilot eradication using methods based on those developed in New Zealand (Coutts 2007) and adapted by CCW and their contractors UK Biosecurity. Initial testing of materials and methods proved successful and a full-scale pilot eradication started in late October 2009.

## 2.2 Irish Sea context

Holyhead Harbour represents an active hub for both commercial and pleasure craft, providing numerous potential vectors to *D. vexillum*. The commercial side of Holyhead Harbour was previously home to Anglesey Aluminium which imported bauxite from around the world; the ‘Tinto’ jetty is now used by cruise ships from around Europe.

The harbour is a primary ferry route to Ireland, with four major terminals each handling up to four vessels per day. However, the risks of commercial shipping carrying this particular species as a hull-fouling organism would seem to be relatively low, as most large commercial vessels are regularly antifouled in the interest of fuel economy. This does not rule out transportation in ballast water or on other commercial, slow-moving vessels such as barges.

Virtually all evidence suggests that leisure craft acted as the vector for *D. vexillum* arriving in Holyhead Marina (Johnson and carlton 1996, Bax *et al.* 2002 and Ashton *et al*. 2006):

- *Didemnum vexillum* has colonised Holyhead Marina and other marinas UK-wide but is not found on ferry terminals or commercial shipping structures even when colonising nearby marina structures.
- Of vessels examined so far, *D. vexillum* has only been found on leisure craft that have been 'neglected' and/or not properly antifouled.
- Vessels that move infrequently are more likely to allow *D. vexillum* to become established on their hulls.
- Colonised sites are on popular leisure craft routes (**Figure 3**) and the nearest marina to Holyhead hosting *D. vexillum* is Malahide on the east coast of Ireland.

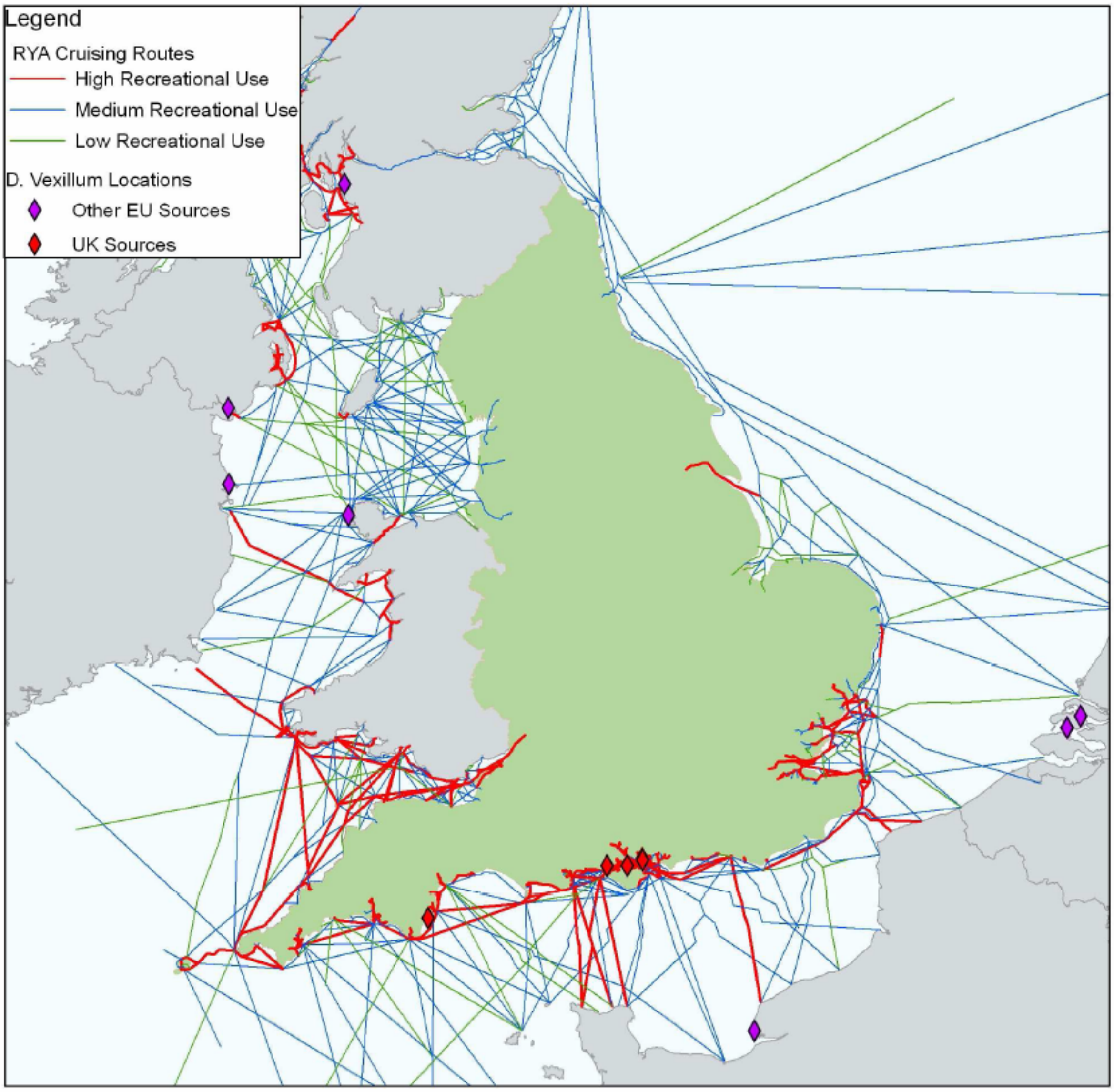



**Figure 3** EU distribution of *D.vexillum* and RYA recreational cursing routes produced by (Laing *et al*. 2010).

## 2.3 Habitats and communities in Holyhead harbour

### *2.3.1 Sensitivity of benthic communities to the eradication process*

The soft mud habitat that immediately surrounds the marina has very little obvious epifauna and flora apart from scavenging hermit crabs *Pagurus bernhardus*, sparse scallops *Pecten maximus*, epipelic diatoms and sea pens *Virgularia mirabilis,* although the latter tend to occur well beyond the boundaries of the marina. Infaunal community composition includes cirratulid polychaetes such as *Chaetozone gibber*, *Aphelochaeta marioni* and *Cauleriella zetlandica* and matches the biotope '*Tubificoides* spp, in variable salinity infralittoral mud' (SS.SMu.SMuVS.AphTubi) described in the Marine Habitat Classification for Britain and Ireland (Version 04.05: Connor *et. al.* 2004). Small outcrops of hard substrata occur on the mud plain and support a few crustose bryozoans, sparse hydroids and silt-tolerant sponges such as *Suberites ficus*. Rocky hard substrata around the perimeter of the harbour, which includes the harbour walls and rip-rap, support suites of species normally found on sheltered intertidal and subtidal rock. Such communities are characterised by fucoid algae, *Laminaria* spp. kelps, smaller red and brown algae and a high abundance of native and non-native sea squirts and encrusting bryozoans.

Any effects on the non-target flora and fauna from the shock treatment chemicals released as part of the eradication process were un-detectable. Once the eradication bags were opened after the treatment was completed any calcium hypochlorite inside the bags is relatively weak and dissociated, therefore virtually instantly dispersed and hugely diluted into the surrounding sea water. Small amounts of detached, chlorine-bleached remains of flora and fauna released from the bags sank to the seabed and were visibly colonized by mats of *Beggiatoa* (a bacterial – fungal association) before disintegrating completely. Whether this particular decomposing material carries any chemical contaminant into the food chain or sediment is unknown although the reaction of calcium hypochlorite with seawater is known to produce a variety of toxic, persistent, organic bromine compounds that can become accumulated up the food chain (El-Hassan and Al-Sulami 2005).

Sensitivity data (Hiscock 2008) for the SS.SMu.SMuVS.AphTubi biotope suggests that there would be little or no detrimental effects resulting from highly diluted 'fall-out' from the eradication bags – neither from the chemicals released nor fine particulate debris. Rocky communities in the area surrounding the marina are already characterized by silt-tolerant and robust species which are unlikely to react adversely to a short-lived increase in turbidity as the bags are being removed.

The effect on mobile species with opportunistic feeding habits is currently unknown. Assuming opportunistic and scavenging species will graze on the contaminated biological material, there is a potential for either bioaccumulation up the food chain or highly localised lethal effects on lower trophic levels.

### *2.3.2 Sensitivity of pontoon species to the eradication process*

The surfaces of a floating structure, such as the pontoons in Holyhead marina, provide a habitat that rarely occurs in nature. With no relative change in water depth with rise and fall of the tide normal phytosociological zonation patterns are absent. Water movement from wave action and tidal currents, particularly on the outward-facing sections of the floating breakwater, result in communities rich in filter feeding species, especially sea squirts and sabellid tube worms, and dense kelps – mainly *Saccharina latissima* on the sheltered areas and *Laminaria digitata* and *Laminaria hyperborea* on the more exposed areas. Similar functional groups of species are found in marinas World-wide (Connell 2002).

The methods used during the eradication kill virtually all species present, apart from the most resilient, such as *Mytilus edulis*, which can close their valves for several days to exclude the harmful effects of the calcium hypochlorite. Opportunistic colonisers rapidly return to the treated pontoons, particularly during the summer, with virtually complete re-colonisation occurring within a few months.

#### *2.3.3 Non-native species in the marina*

During the course of the eradication programme several new records of non-native species were established for the marina in addition to *D. vexillum*. The list below summarises species recorded during the programme (a more comprehensive list of non native species for the whole of Wales, Prepared by P. Brazier and G. Wyn, CCW, is given in **Appendix VII**).

- *Caprella mutica*: Japanese skeleton shrimp. First recorded in UK in Scotland 2000. First record in Holyhead 2010.
- *Bugula neritina*: A bryozoan. First record for Holyhead 2010.
- *Tricellaria inopinata*: A bryozoan. First English record 1998. First record for Holyhead 2011.
- *Schizoporella errata* / *Schizoporella japonica*: An encrusting bryozoan. First record for Holyhead 2011 and probably first UK record. Awaiting confirmation (John Ryland pers. com.).

### 2.4 Aims and objectives

Attempting the eradication of an invasive non-native species requires far more than simply killing it at one of its known locations. The eradication has to be supported by improving biosecurity and maintaining vigilance to prevent spread and re-establishment of the species. The overall aims of this project were therefore:

- Develop eradication techniques for *D. vexillum* and improve biosecurity at Holyhead Marina.
- Set up long-term, Wales-wide marina monitoring for *D. vexillum* and other non-natives.
- Develop monitoring method for detecting re-establishment of *D. vexillum* in Holyhead Marina.
- Develop methods for subtidal monitoring of non-natives in the wider marine environment.

# 3 MATERIALS AND METHODS

### 3.1 The scale of the problem

Holyhead Marina currently has 230 berths (and is undergoing expansion), arranged in a series of herring-bone patterned pontoons sheltered within the arms of a floating breakwater. It is situated in the western corner of Holyhead Harbour (see map in **Appendix IV**) which itself is protected from the north and north-west by the mile-long stone-built breakwater. Unlike many other marinas the floating structures are not held in place by piles driven into the seabed. Instead the marina comprises 530 floating pontoons of varying size and shape (**Figure 4** and **Table 1**) which make up walkways, various sizes of berths and a floating breakwaters - all held into position by 125 chains attached to concrete moorings on the seabed.

Throughout this report the positions of structures within the marina are assigned a letter and number. The letter refers to the main pontoon (A to F) and the number the nearest berth.

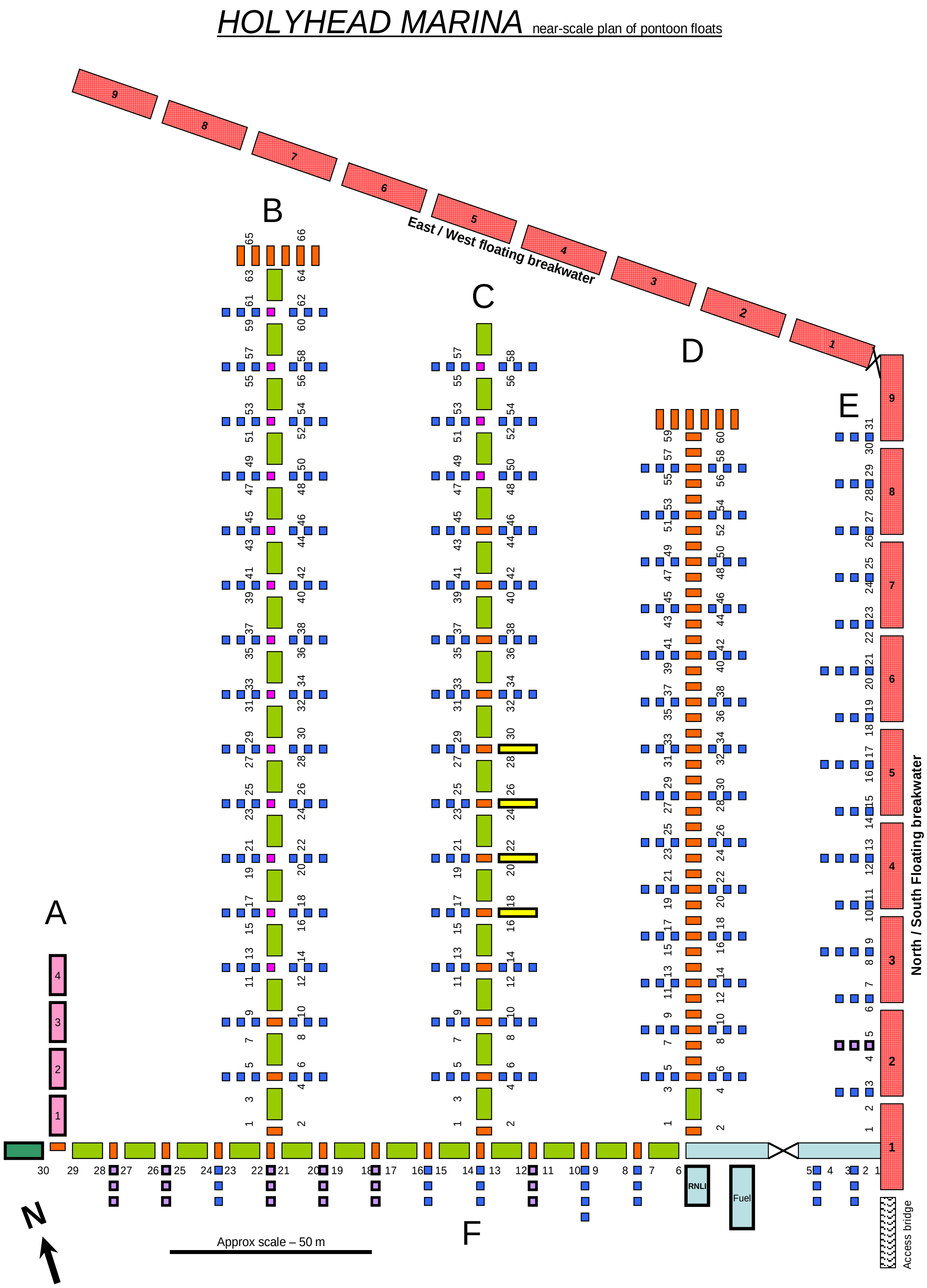



**Figure 4** Holyhead Marina layout colour coded to pontoon types.

**Table 1** Holyhead pontoon types, dimensions and quantities.

| KEY | Pontoon type<br>Size in M | L | W | H | Submerged height | Free Board | Girth | No. of Units | Unit area to be treated $M^2$ | Total area to be treated $M^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | Breakwater | 20.03 | 4 | 1 | 0.7 | 0.33 / 0.18* | | 18 | 113.76 | 2047.68 |
| | Walkway B, C, F | 7.37 | 3.25 | 0.67 | 0.4 | 0.3 | 4.5 | 44 | 32.45 | 1427.8 |
| | Walkway A | 9.58 | ~3.20 | - | ~0.5 | - | - | 6 | 43.44 | 173.76 |
| | Walkway F – west end | 7.45 | 3.13 | 0.72 | 0.42 | 0.3 | 4.4 | 1 | 32.21 | 32.21 |
| | Walkway D / internode / ends | 1.24 | 3.04 | 0.7 | 0.45 | 0.3 | 4.3 | 80 | 7.62 | 609.6 |
| | Walkway F - east end | 20 | 3 | - | ~0.70 | - | - | 2 | 92.2 | 184.4 |
| | Internode | 1.07 | 1.65 | 0.95 | 0.67 | 0.32 | 2.88 | 26 | 5.41 | 140.66 |
| | Finger – solid | 7.42 | 1.04 | 0.95 | 0.7 | 0.3 | 3 | 4 | 19.56 | 78.24 |
| | Finger – standard | 1.23 | 1.02 | 0.8 | 0.55 | 0.25 | 2.46 | 326 | 3.73 | 1204.79 |
| | Finger – type 2 | 1.88 | 1.09 | 0.7 | 0.4 | 0.32 | 2.26 | 21 | 4.43 | 93.03 |
| | Fuel jetty - concrete | 15.01 | 4 | | ~0.70 | | | 1 | 86.65 | 86.65 |
| | RNLI jetty - concrete | 10 | 4.04 | | ~0.70 | | | 1 | 60.06 | 60.06 |
| | **Totals** | | | | | | | **530** | | **6,138.88 m2** |

# Notes:

Height = measurement from base to top of float
Freeboard = measurement from water surface to top of float * and lower edge of wooden batten on breakwater sections
Girth = measurement around narrowest section of each float taking into account space taken by marine growth
L= length
W= Width
H= Height

## 3.2 Eradication materials and methods

The methods used to eradicate *D. vexillum* from the various structures around the marina are adapted from Ashley Coutts' work at the Cawthron Institute in New Zealand (Coutts 2007). In New Zealand they covered pontoons and pilings with various grades of disposable plastic wrapping, similar to that used for wrapping silage bales, or tarpaulins which smothered and killed *D.vexillum*.

The principle behind both Coutts' and CCW's eradication method is to create a watertight barrier between the structures and the surrounding water column to kill *D. vexillum*.

The method works in two ways:

- The wrap contains and isolates *D. vexillum*, preventing larval or fragmented dispersal.
- The contents turn hypoxic, as contained biota utilizes oxygen killing sensitive species. This in turn accelerates the stagnation process which eventually results in totally anoxic conditions - killing *D. vexillum* and everything but the most tolerant species in about 4-8 days.

In Holyhead we considered that the periodically wave-exposed nature of the site would warrant using heavier duty materials and we therefore opted for re-useable bags manufactured from heavy duty reinforced PVC specifically made to fit the various pontoons and walkway floats.

A chemical accelerant, calcium hypochlorite, was used to speed up the eradication process (Denny 2008). This was necessary when sea temperatures drop below about 10˚C or when the bags could only be left on for a short while – for example when bad weather was forecasted, risking waves overtopping the bags and flushing out the contents. Our preference was to use calcium hypochlorite in granular form. This was predominantly for handling and safety reasons as it has the advantages of remaining visible for a while before it dissolves in seawater, doses can be weighed or measured volumetrically and any spillage remains visible on a wet surface but can be flushed away with seawater.

The barrier / stagnation principle was also used to treat chains and swinging moorings by using sheet polythene on rolls. Once cable-tied into position the polythene wraps were left for the duration of the project and not chemically treated ('set and forget' method - see section **3.2.7** below).

### *3.2.1 Bag and wrap design*

All bags were of bespoke design for each type of pontoon float within the marina, with a total of 14 different design variations around three common themes. The larger breakwater and walkway pontoon floats were treated using bags supported by inflatable collars; the medium-sized walkway floats and small finger berth pontoons were treated using draw-string or elasticated bags and the complex-shaped surfaces from which chains originated at the junctions between or at the ends of the breakwater pontoons were treated with wedge-shaped wrappings (**Table 2**).

**Table 2** Bag and fabric types used in Holyhead

| Bag Type | Purpose | | Fabric Type | Colour |
|---|---|---|---|---|
| **Draw-string and Elasticated** | Finger berths – 2 types | | 600 GSM PVC | Red, yellow and grey |
| | 'Internode' (= medium sized junctions between walkways) | | | |
| | Walkway D / Internode / Ends | | | |
| **Inflatable** | Walkway | Walkway on pontoons B, C, F | 500 GSM PVC coated thin polyester | White |
| | | Walkway A | | |
| | | Finger berth – continuous medium sized float | 570 GSM PVC coated thin polyester | Black/ Blue |
| | Terminal wedge | Breakwater | 500 and 570 GSM PVC coated thin polyester | White |
| | Junction wedge | Breakwater – junction wedge | 500 and 570 GSM PVC coated thin polyester | White |
| | | Fuel jetty - concrete | | |
| | | RNLI jetty - concrete | | |
| | | Walkway F - west end | | |
| | | Walkway F - east end | | |
| **'Wedge'** | Terminal wedge | Breakwater- ends | 280 GSM PVC | Green |

### *3.2.2 Draw string and elasticated bags*

The most successful draw-string and elasticated bags were made from 600 GSM (grams per square meter) PVC. The combination of lightweight fabric and loose-fitting design allowed rapid fitting by snorkellers to the smaller pontoon floats - often in less than 4 minutes per unit. Excess water was pumped out using a petrol-driven centrifugal water pump after fitting.

Heavier weight prototypes made from 2-ply PVC fabric exactly tailored to the pontoons' measurements were much more difficult to maneuver and slow to fit (**Figure 5**). Experiments with re-sealable openings (waterproof zips or flap valves) on the undersides of the bags to release excess water displaced by the pontoon float during fitting proved ineffective, using these meant that a diver had to be on hand to close them which slowed the whole process. Openings were subsequently omitted from new bags or left closed during fitting.

**Figure 5** Draw string prototype finger pontoon bag.

Drawstring and elasticated bags were used on a variety of pontoon floats of varying size such as ‘internode’ and ‘finger’ floats (**Figure 6**); however, the method for fitting remained the same on all pontoon types.

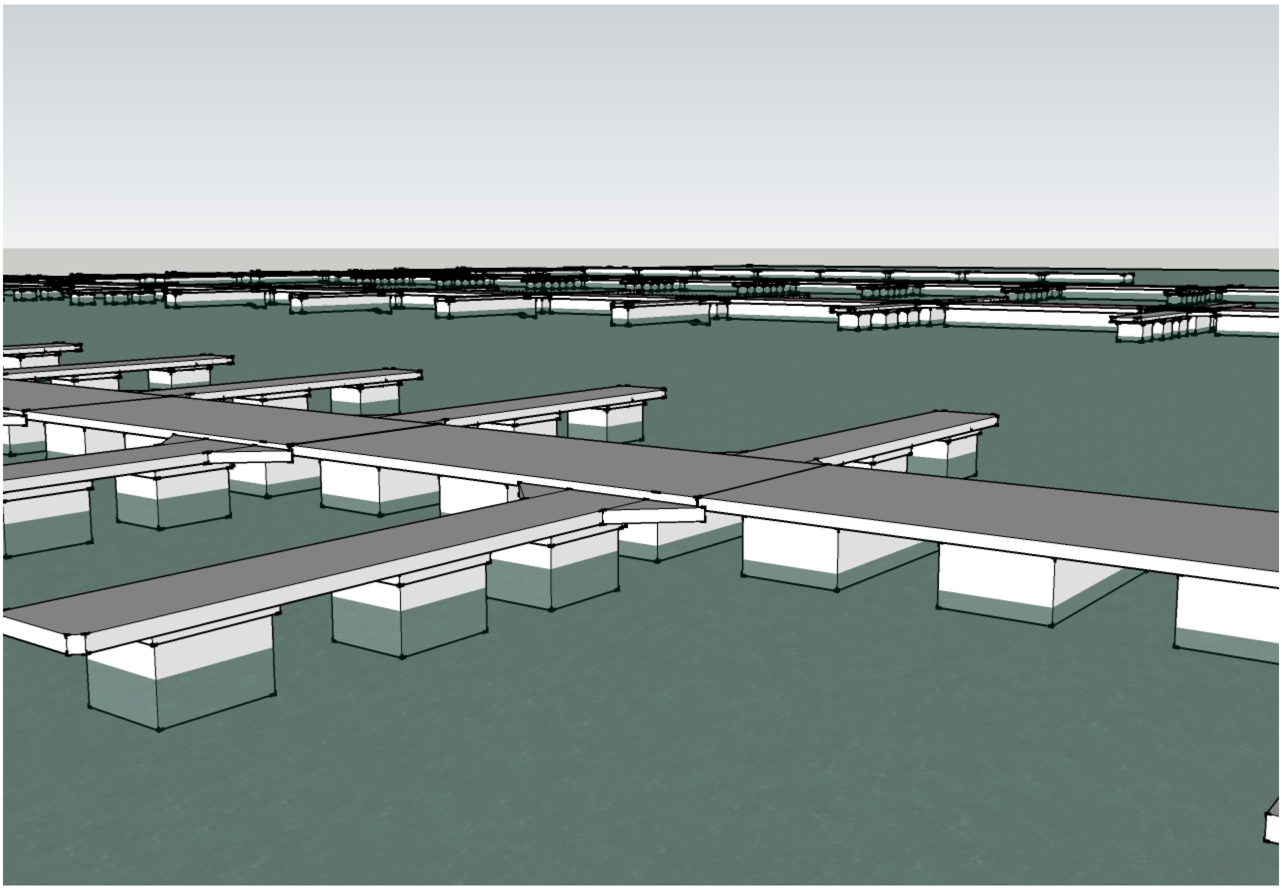


**Figure 6** 3-D Scale drawing of finger and internode floats on pontoon D.

Fitting procedure for drawstring and elasticated bags

For additional instructions, refer to **Figure 7**.

1. A pair of snorkellers orientates and semi-submerges a bag aligned to one side of the pontoon then…

2. …slowly lower the leading edge of the bag under water while keeping the opposite side near the waterline against the pontoon.
3. Once directly below the float the bag is opened fully and brought to the surface around the float's perimeter.
4. The draw strings / elasticated 'bungee' cord is then partially tightened and straps used to prevent the bag sinking back below the waterline.
5. Place the water pump intake, with a strainer attached to the end, into the bag to remove excess water until…
6. …the sides of the bag collapse in and it starts to pump dry.
7. Once the water volume has significantly decreased calcium hypochlorite granules are added (see **Table 4** for required doses).
8. The bag can be removed after 2-3 days with the aid of a boat-hook from the pontoon deck taking care to partially submerge and dilute the content of the bag before attempting to remove it from the water.

If the water temperature is above 15-16˚C the bag can be left for approximately 5-7 days after stage 6 without chemical treatment.

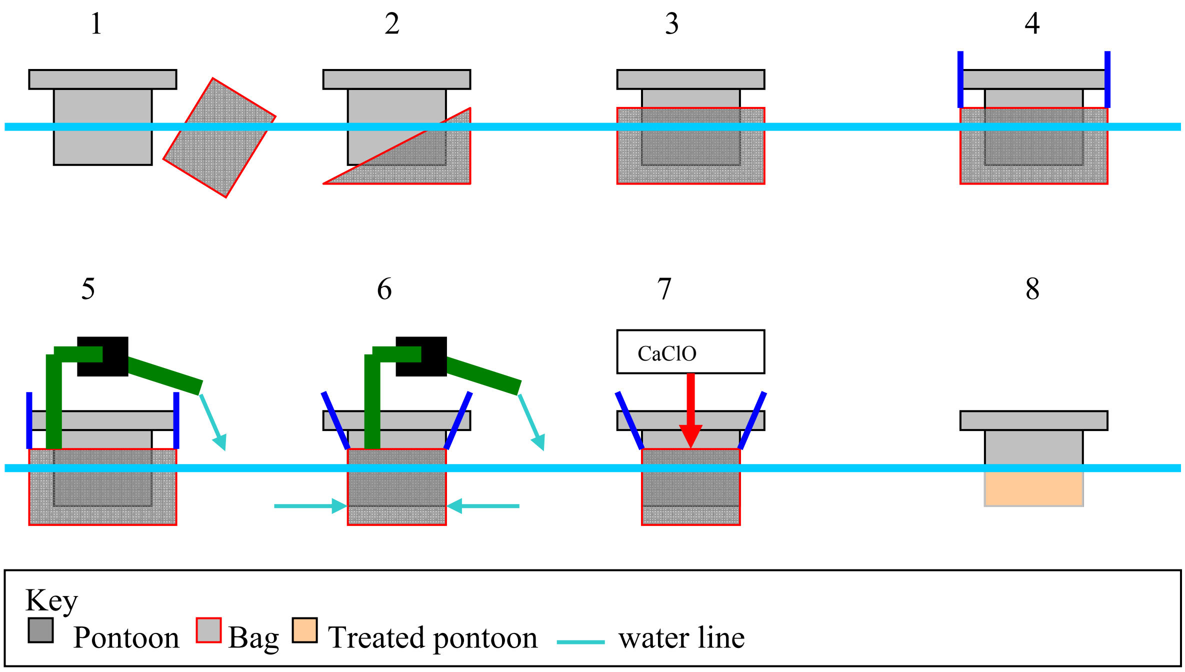



**Figure 7** Fitting procedures for drawstring and elasticated bags.

### *3.2.3 Inflatable collar walkway bags*

Self supporting inflatable bags were designed to treat the longer (7 m up to 20 m) pontoon floats in the marina where attachment points on the pontoon sides were too sparse to quickly provide adequate freeboard (**Figure 8**). A smaller prototype, built to demonstrate the utility of having an inflatable collar, was developed during the method trial and used for wrapping the single solid finger float pontoons (**Table 1** and **Figure 8**).

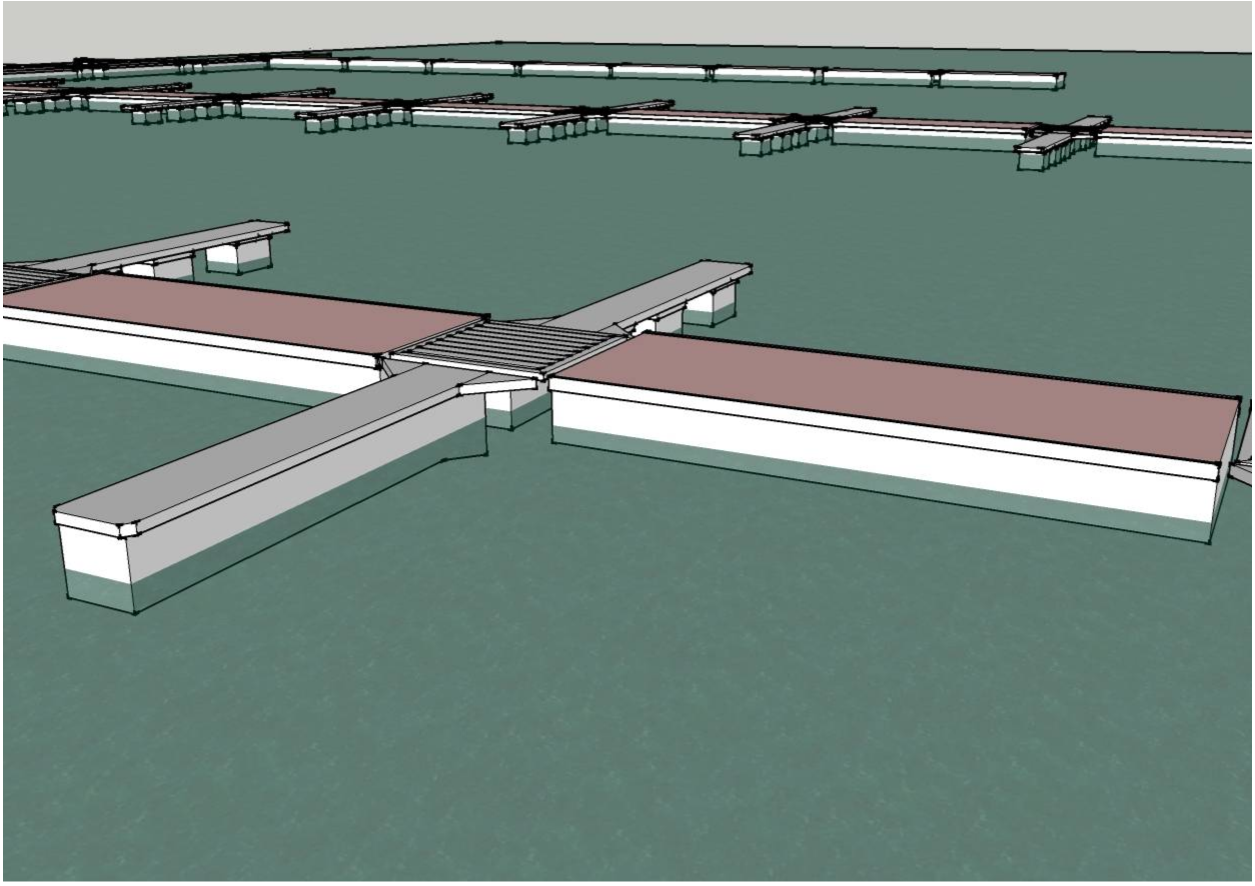


**Figure 8** 3-D Scale drawing of ‘solid’ finger and walkway pontoons

The 7m long walkway bags were made of 500 gsm PVC coated polyester and incorporated sleeves along the long edges for two 570 gsm PVC coated polyester inflatable tubes to be inserted to support the weight of the bag. This eliminated the need for a large number of eyelets and D-rings sown into the fabric thereby reducing manufacturing costs and deployment time especially if a rapid method of inflation (such as using a Nilfisk vacuum cleaner on ‘blow’ or diving cylinder with an inflator hose) is available. The later versions of these bags incorporated sealed/sown-in metal rods along one long side of the bag to promote sinking of the leading edge during deployment from the pontoon deck. Deployment of the prototype versions of these bags required divers, but refinements in design and methods allowed for deployment by snorkellers. The other advantage of the inflatable sections was that they could be towed through the water between different locations in the marina.

Fitting procedure

For additional fitting instructions refer to (**Figure 9**).

If the bag is already in the water step one can be ignored.

1. Unfold the bag and inflate the buoyancy tube on one side, making sure to close the valves on the opposite deflated weighted side correctly to prevent water ingress. Connect 4 m lengths of rope to the eyelets on the corners and centre of both sides. Once the bag is prepared lower it into the water weighted edge first ensuring that the inside faces of the bag are orientated correctly against the pontoon float.
2. A pair of snorkellers, working from each end of the inflated trailing edge, then begin to lift the short sides of the bag out of the water, attaching them to the superstructure with straps/bungee/rope until they reach the opposite deflated long edge. This may mean that they have to work in the narrow gap between two pontoons.

3. Locate the tube inflator valves. Check the valve is set to ‘inflate’, place the inflator hose over valve (above or below water) turn on the air supply and inflate.
4. Once the second tube has been inflated, the bag will support itself and can then be secured into position using the 4 m ropes attached earlier.
5. Pump out excess water with a petrol driven centrifugal pump, ensuring a strainer is used and fixed in position just below the waterline inside the bag. This process takes about 30-40 minutes but can be speeded up by using a second pump.
6. Once pumping is completed and the pump intake hose has been removed, dose the bag with the appropriate amount of calcium hypochlorite spread evenly along each side of the bag (**Table 4**). Treatment will take 2-3 days to complete. A good indication that it has worked is that algae such as *Saccharina latissima* will have bleached white and that any limpets adhering to the pontoon sides near the waterline will have either become loose or detached.
7. To remove the bag, first undo any ropes and lines then deflate the weighted side of the bag allowing it to sink.
8. Allow the bag content to disperse before moving it to the next pontoon or removing it from the water.

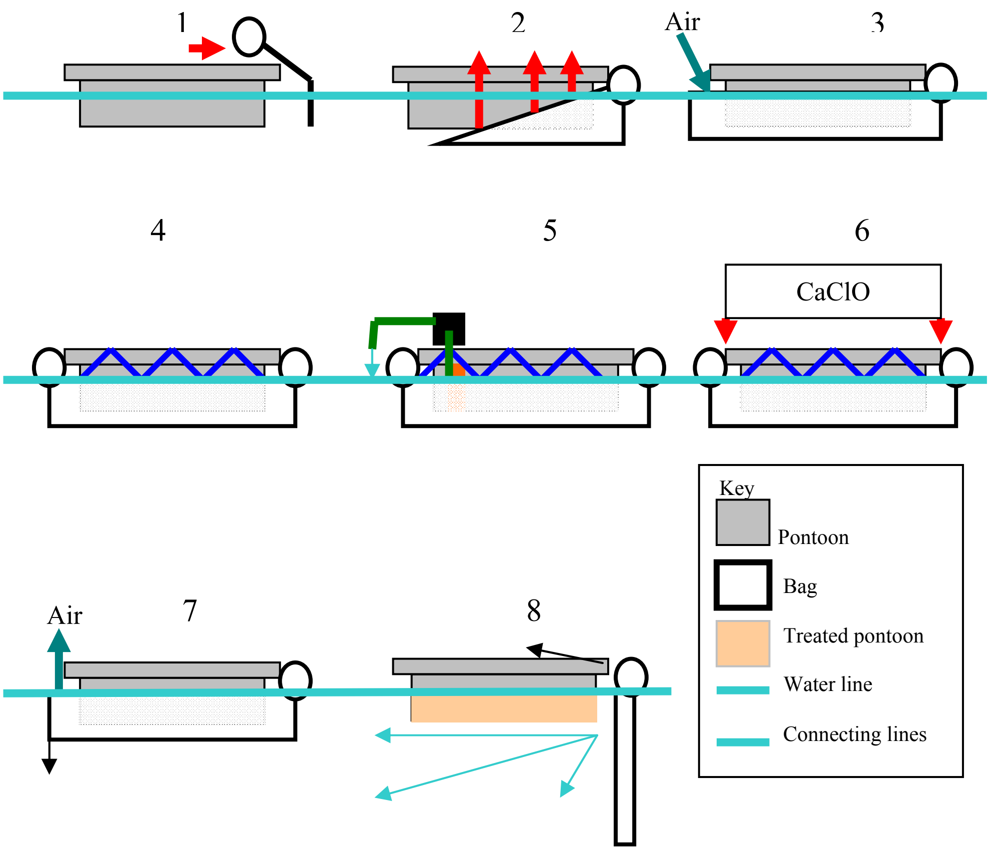



**Figure 9** Fitting procedures for inflatable collar walkway bags.


**Figure 10** Photographic guide to (**Figure 9**) A, stage 1; B, stage 2; C, stage 3; D, stage 5; E, stage 6

### *3.2.4 Inflatable collar breakwater bags*

Bags designed to wrap the 20 m x 4 m floating breakwater sections were very similar to the walkway bags described above but scaled-up to fit. They were made of 500 gsm PVC coated polyester and used two 570 gsm PVC coated polyester inflatable tubes. To reduce manual handling and ease deployment each 20 m long breakwater section was covered by two large overlapping bags sealed at the overlap with ratchet straps. Even at half-breakwater pontoon size they were heavy, requiring at least two divers and four surface personnel to handle and cannot be deployed against even a slight tidal flow, although with care they can be deployed to unfurl down-current. The bags were ‘tailored’ to fit the boat-bow-shaped ends of each breakwater section and had a plain ‘stern’ end to overlap with the adjacent bag (**Figure 11**). Each bag had seven high strength ratchet straps integrated into the design of the bag, running in sleeves welded transversely to each bag at regular intervals. The straps performed two main functions: to securely attach the bag to the pontoon and to compartmentalise the bags to reduce internal water flow.

It is important to note that the procedure required 2-3 days of calm weather as even slight wave action can destroy the fabric of the bag or overtop the bag sides and dilute the calcium hypochlorite.

There were two design variations of breakwater bag. The first type (used on the North-South breakwater) was used in conjunction with a separate wedge-shaped section (referred to as a ‘wedge’) to cover the complex surfaces where the mooring chains originated at the junctions between breakwater sections (eastern breakwater (**Figure 11**). **Wedge sections have to be fitted before the main breakwater bags on Pontoon E.** These were then adapted to the less complex pontoons on the East-West breakwater where the chains originated away from the junction and the wedge sections were not needed.

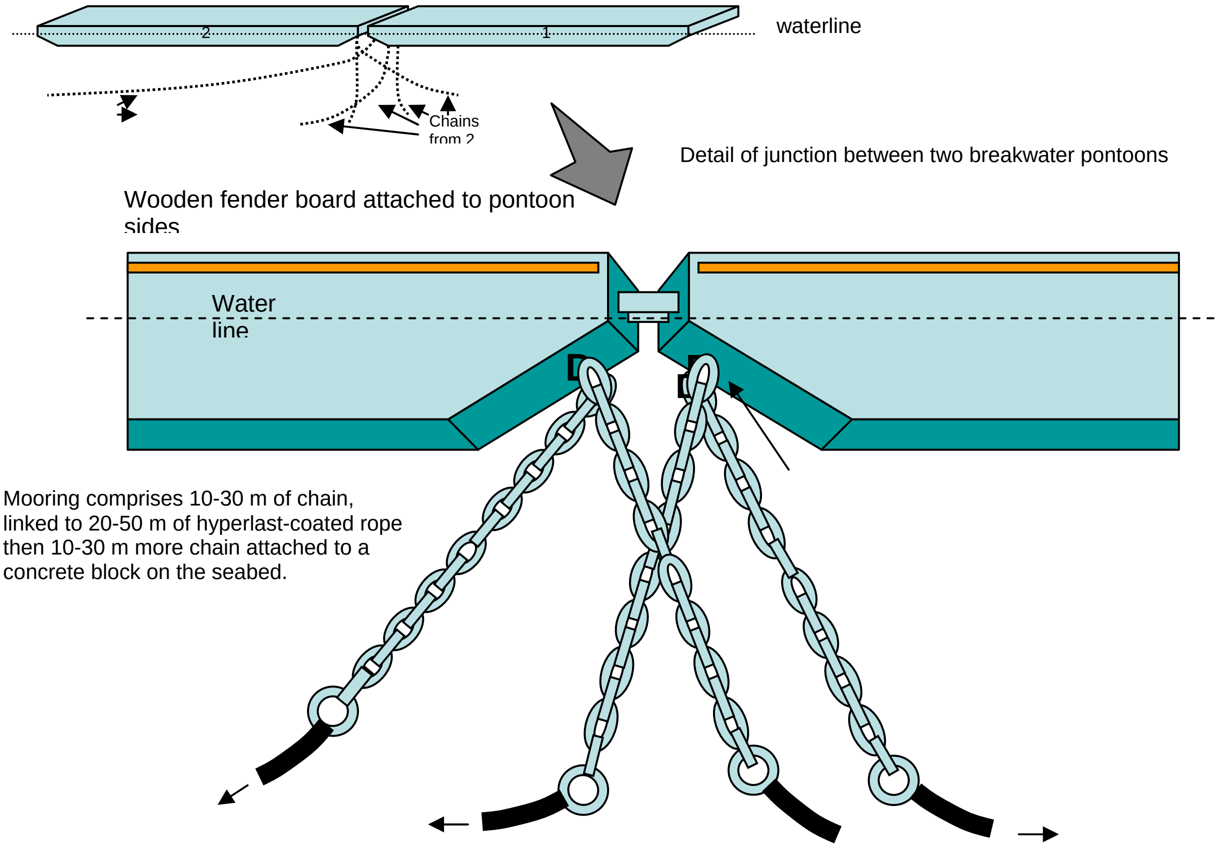



**Figure 11** Diagram of the N-S breakwater chain configuration.

## Breakwater bag deployment procedure

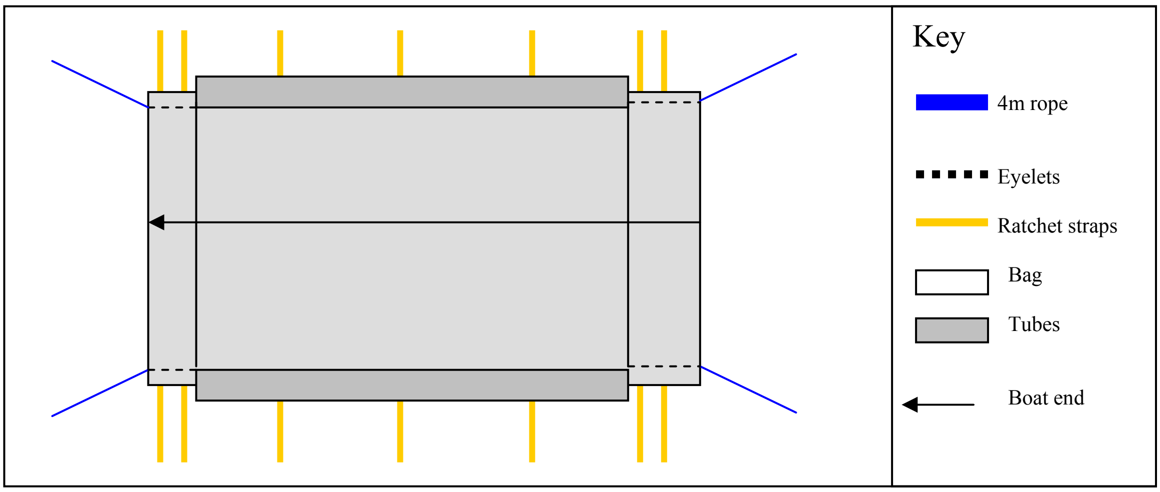



**Figure 12** North-South breakwater bag set-up diagram.

Pre-deployment (**Figure 12**): Label the fabric with indelible marker 'bow', 'stern', 'inside' and 'outside' to aid orientation once the bag has been launched. **Note - A wedge sections should be fitted to each end of the pontoons first before fitting any of the main North-South breakwater sections** (see **3.2.5** below ). Attach guide ropes (approx 6 m long lengths)

For additional fitting instructions refer to (**Figure 14).**

1. Inflate the trailing edge tubes and then lower the deflated leading edge of the bag into the water. It will be necessary to push the bag underwater as trapped air will tend to keep the bag afloat at first.
2. The diver pair should then take ropes from the leading edge and swim them under the pontoon to the opposite side, ensuring that they route the bag and ropes appropriately around the chains or any other obstruction, and then pass the ropes up to the surface support personnel.
3. The support team slowly hauls the bag into position with the deflated leading edge tube at or near the surface.
4. Locate the tube inflator valves and inflate the leading edge tube. This will lift any remaining sections out of the water.
5. Make final adjustments to the position of the bag to ensure that the 'bow' shaped end fits in place at the 'wedge' end of the pontoon.
6. Connect ratchet straps (**Figure 13**) (shackled on one side of the pontoon and tensioned using a ratchet cam on the other) from the fender boards of the pontoon, under the bag and up to the fender board on the opposite side of the pontoon. Tie up any loose ends of rope and check the bag has sufficient freeboard so that small waves will not over-top the bag.

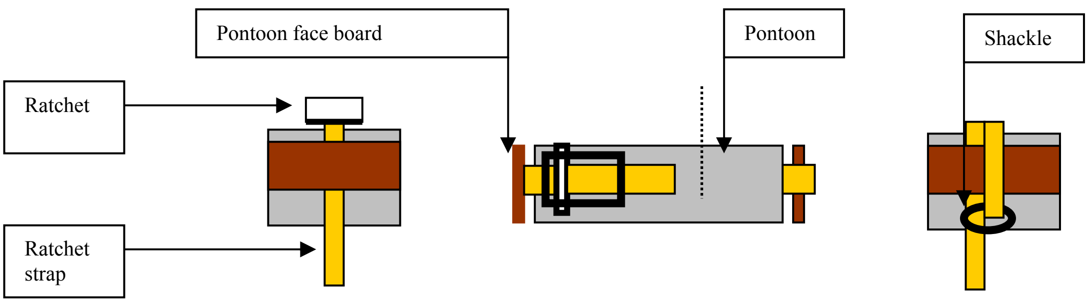
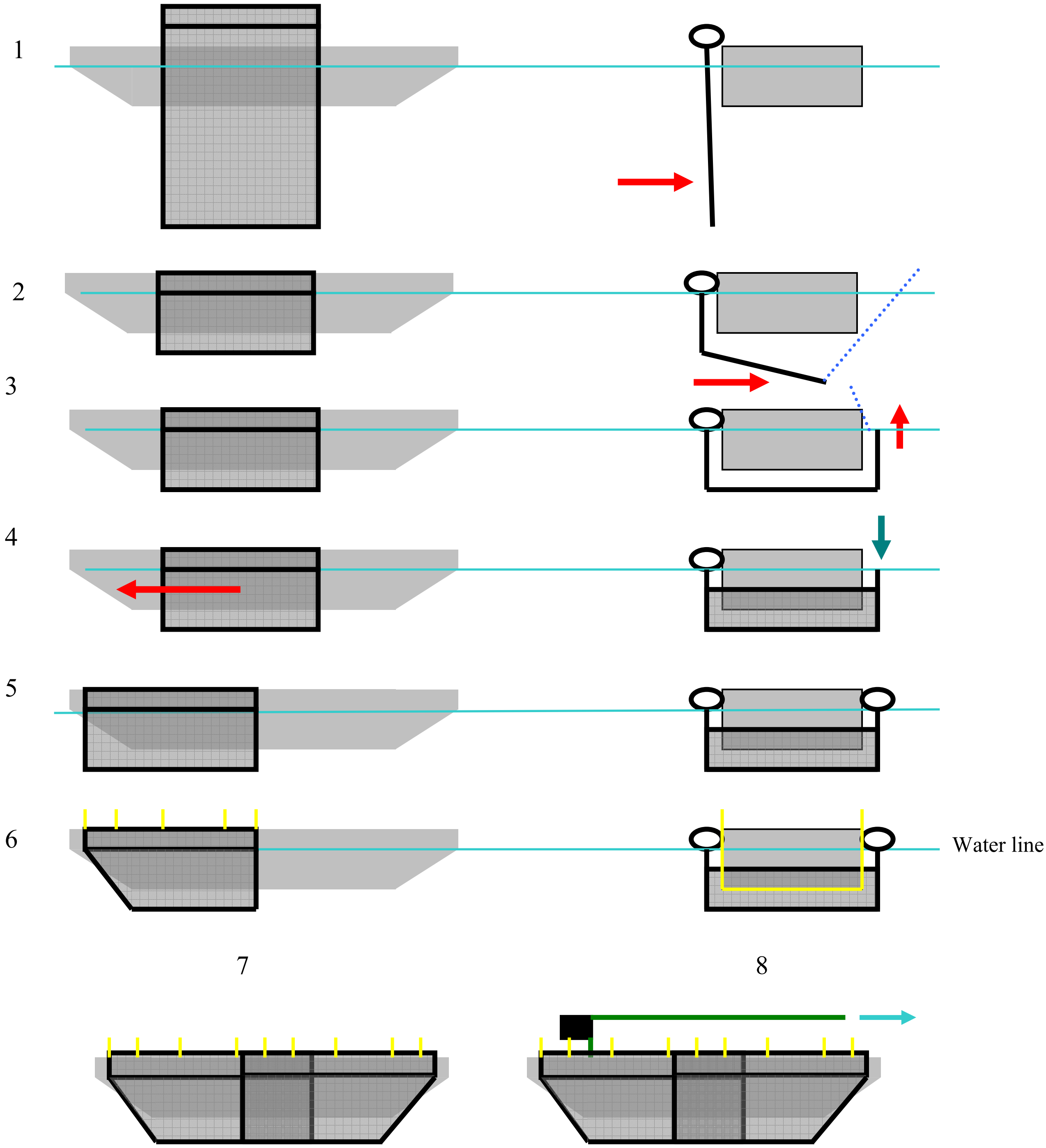



**Figure 13** Ratchet strap configuration for breakwater bags.

Repeat steps 1 to 6 to cover the other half of the pontoon with the second section of bag.

7. Ensure at least a 2 m overlap of fabric at the join between the two halves and that the submerged portions overlap smoothly.
8. Pump the excess water out of each pontoon half using a centrifugal pump and strainer. It may be necessary to pump out the sub-compartments between each strap separately. Each compartment takes approximately 10-20 minutes to drain; this process can be speeded up by using multiple pumps.
9. Dose the pontoon with the appropriate amount of calcium hypochlorite, (approx. 2 kg per compartment). Make sure to spread the dosing evenly over both sides of the pontoon to avoid "hot spot" areas of concentrated calcium hypochlorite.
10. Leave the bag for between 2-3days. Once the treatment is completed undo all fittings and deflate the tubes on one side. Allow the bag to sink and the contents to disperse. It is then possible to remove or relocate the bag in the water by hauling it from the pontoon deck.


1
2
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Water line
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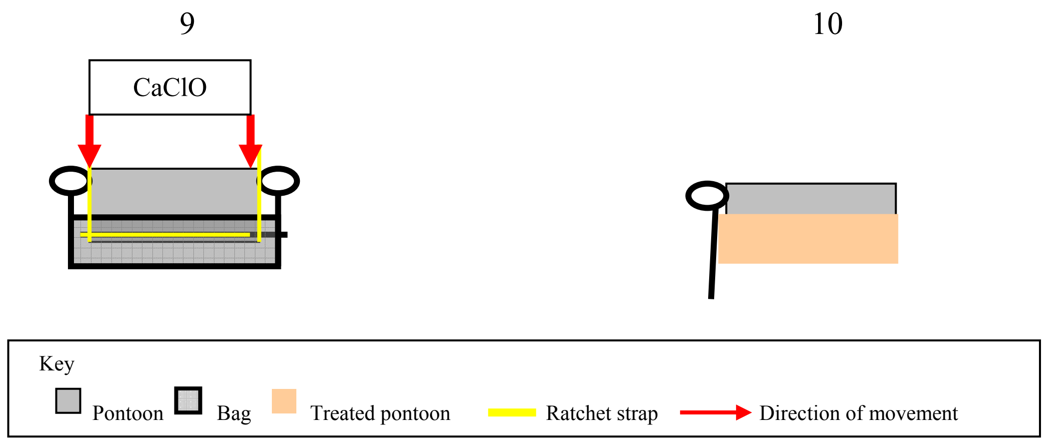



**Figure 14** Fitting procedure for North-South breakwater bags.

### *3.2.5 Breakwater junction 'wedge' sections on North-South breakwater pontoon.*

<u>Deployment procedure</u>

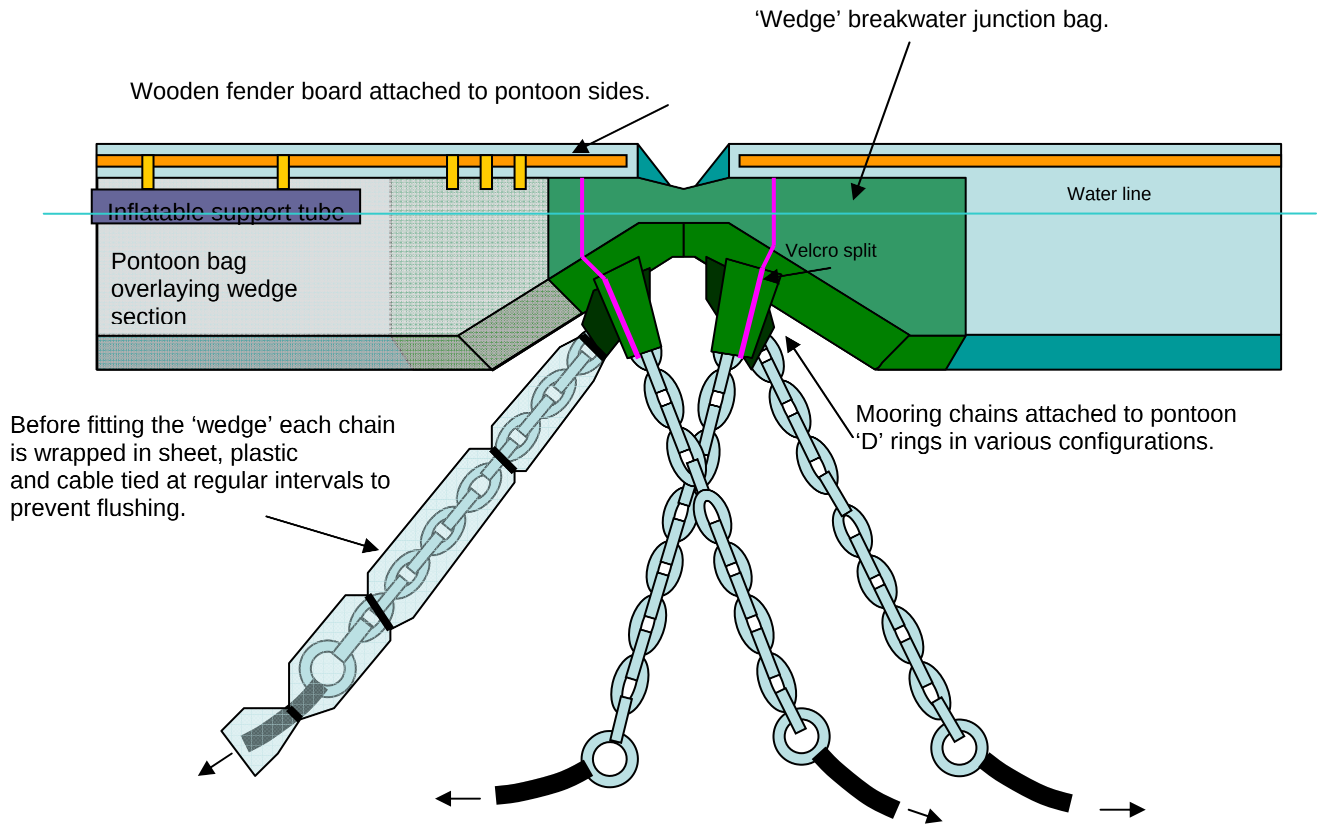



**Figure 15** Wedge section fitted to North-South breakwater E

For additional fitting instructions refer to **Figure 15** and **Figure 16**.

1. Firstly determine how many and where the chains originate at each junction between the breakwater pontoons. This can be carried out by snorkeling or diving.

2. Once the wedge has been correctly orientated, attach supporting lines to the central 'spine' and open all the Velcro flaps. The wedge can now be lowered into the water.
3. Pass the centre lines to a diver pair who then swim them into place and pass them back up through the gap between the two pontoons to the surface team standing over the junction.
4. Once in place the surface team hauls on the ropes to lift the wedge into position, the bar should rise into the gap between the two pontoons.
5. The bag will then have to be folded around the chains, if present, and the Velcro fastenings closed.
6. Connect the rest of the wedge sides to the fender boards on the pontoon.

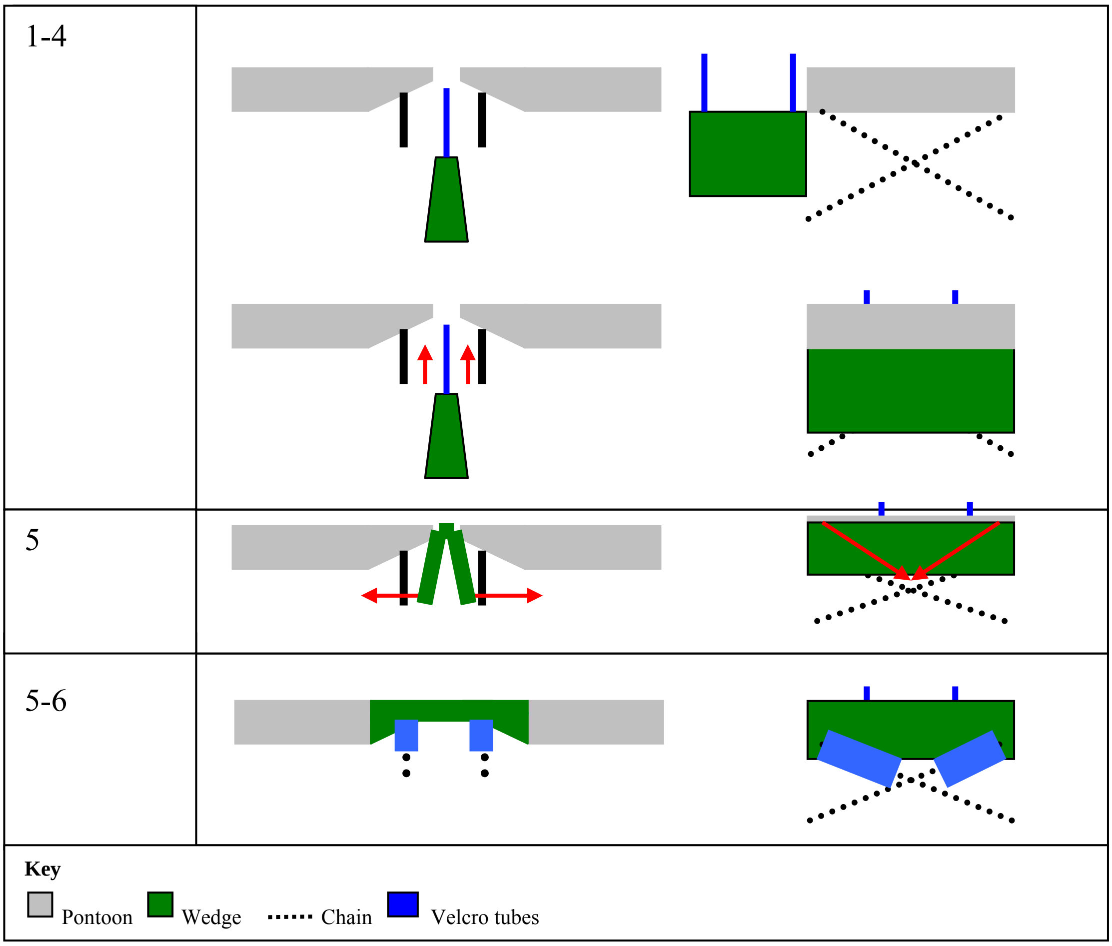



**Figure 16** Fitting procedure for North-South breakwater wedge.

*3.2.6 East-west breakwater*

<u>Fitting procedure for East-West breakwater</u>

For fitting instructions refer **Figure 17**, **Figure 18** and **Figure 19**

If this is the first deployment of the breakwater bag, it will be necessary to attach ropes and fittings (**Figure 17**) and mark bow and stern ends. As with the other breakwater E the procedure requires a 2-3 day calm weather window or wave action will damage the bag and dilute the calcium hypochlorite.

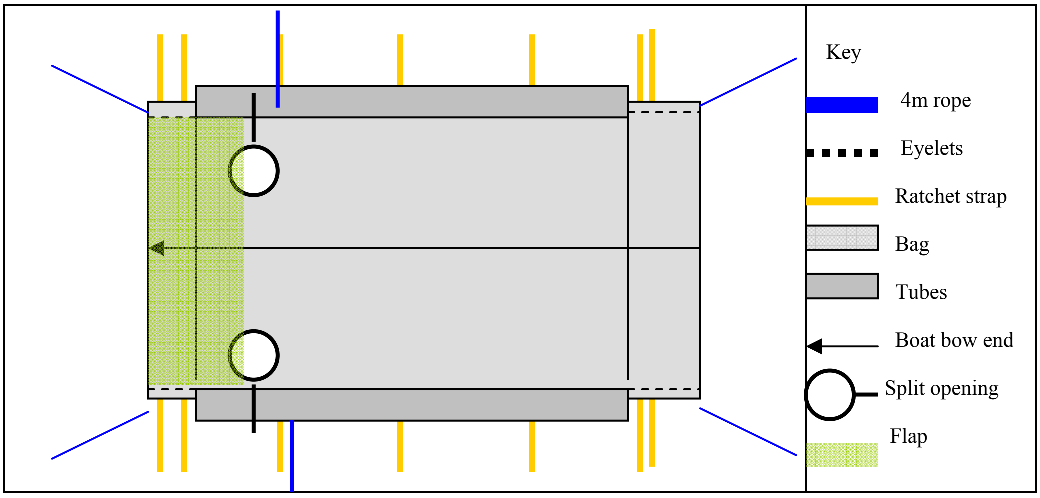



**Figure 17** East-West breakwater bag set-up.

The first part of the procedure is exactly the same as fitting an North-South breakwater pontoon (follow steps 1-6 in section 3.2.4.1 **Figure 14)**, making sure that all the Velcro fastenings are undone before deployment and the bag is properly aligned with respect to the chains.

1. Once the bag is in position the divers unfurl the bag around the chains making sure not to tangle or twist the bag. The operation of moving the bag against the water resistance can be physically demanding.
2. The leading edge of the bag can now be taken to the surface and…
3. … the Velcro joins sealed all the way up to the surface.
4. The surface team then fits the remaining inflatable tubes.

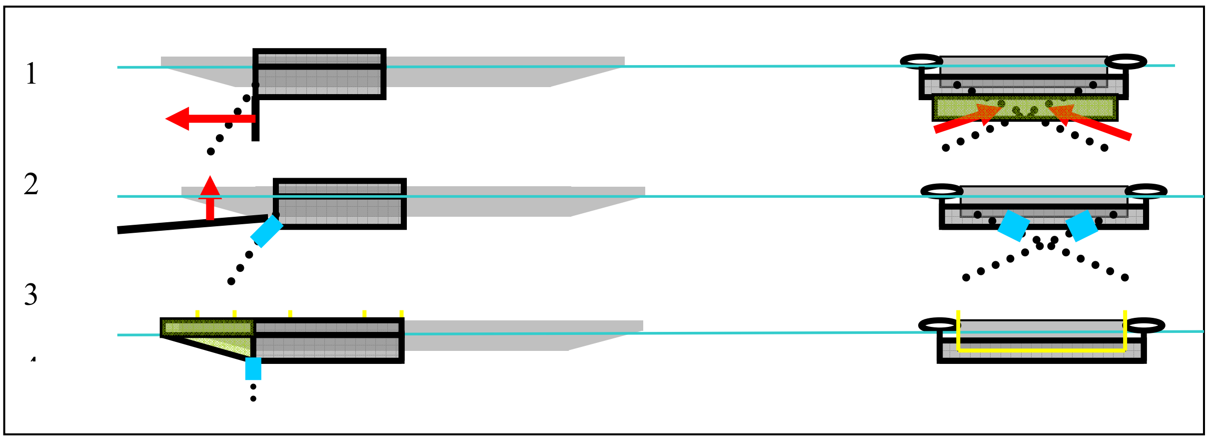



**Figure 18** Fitting procedures for E-W breakwater.

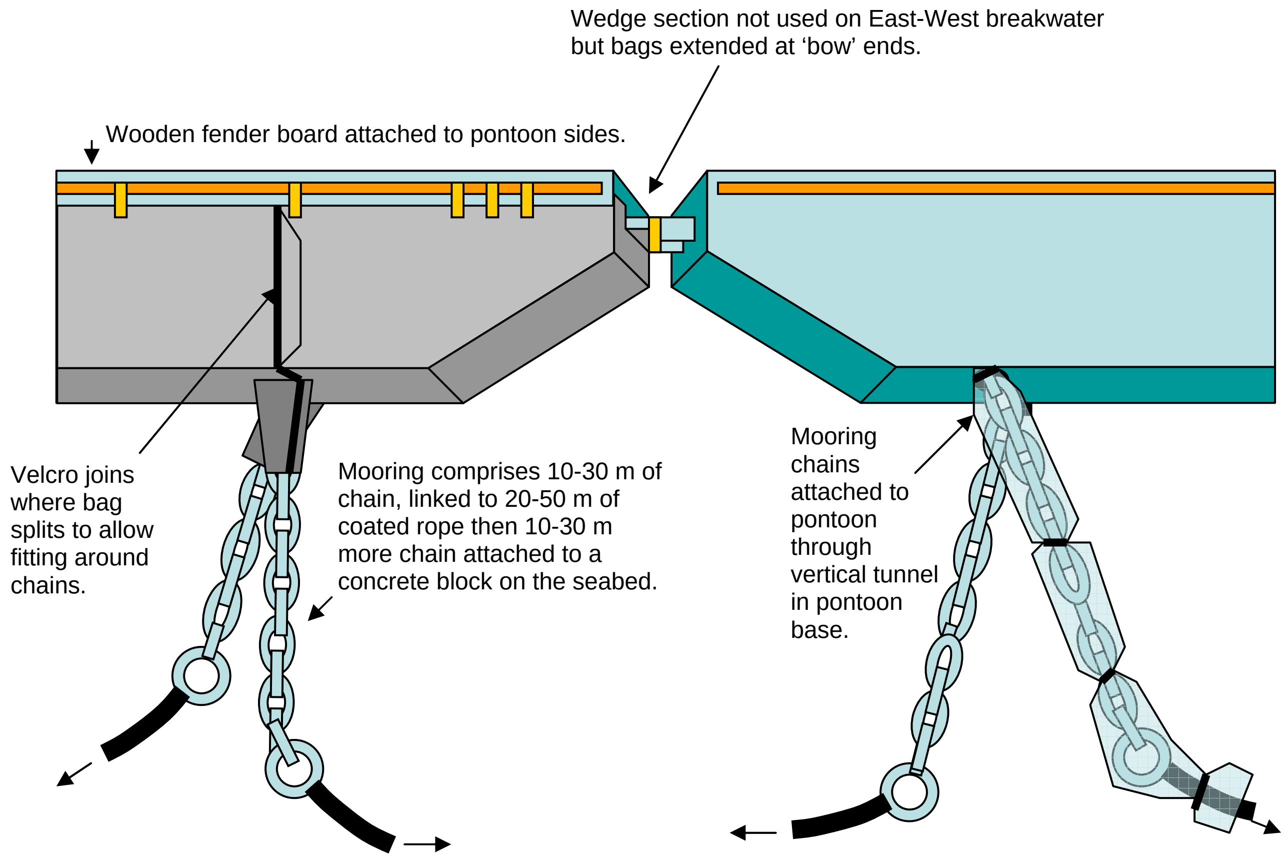



**Figure 19** bags and chain wrapping on the East-West breakwater

#### *3.2.7 Wrapping chains and moorings*

The methodology for wrapping chains and moorings is an adaptation of the techniques used in New Zealand for the treatment of wharf piles (Coutts 2007). Refinements were developed during the eradication programme to improve effectiveness and reduce the time taken to fit.

In total 110 moorings and 30 swinging mooring were treated using plastic wrapping to smother *D. vexillum*. Depending on chain type a variety of plastic grades and configurations were used. A light-weight 400 gsm clear polythene sheet on a roll tended to be used for pontoon chains and a heavier 500 gsm clear polythene tube for swinging moorings, although the two plastics were used interchangeably. The wrappings were left on for the duration of the eradication (referred to by Kleeman (2009) and others as 'set-and-forget'), although appeared to work after a week or so as evidenced by the contents turning black.

#### *3.2.8 Marina mooring chain wrapping*

<u>Preparation</u>

Ten metre lengths of sheet plastic were cut from the supplied roll and re-rolled onto plastic tubing (usually drain pipe slightly longer than the width of the plastic) with the loose end taped to prevent unrolling before deployment. A rope lanyard with a karabiner was fed through the tube to make a sling for the divers to carry.

Large (>500 mm long x 9 mm wide) cable ties were used to secure the sheeting around the chains. 'Dispensing racks' of 50-100 cable ties were prepared to allow the divers, wearing thick gloves, to pull them off the rack as required. A rack is prepared by simply cutting the ratchet head off one tie then feeding all the others on to that tie, leaving sufficient length at the 'tail' end to tape a loop into a karabiner to clip to the diver's harness.

Wrapping chains underwater

A dive pair need to work as a team; one diver dispensing and holding the plastic in place a couple metres at a time around the chain, while the second diver rolls and secures the sheeting onto the chain with cable ties to create a near-watertight seal. Cable ties need to be placed every 40-50 cm to prevent the plastic from unraveling and water from flushing along the wrappings (**Figure 20**). For the long chains on all the breakwater pontoons one or two additional rolls of plastic were used to reach the desired length.

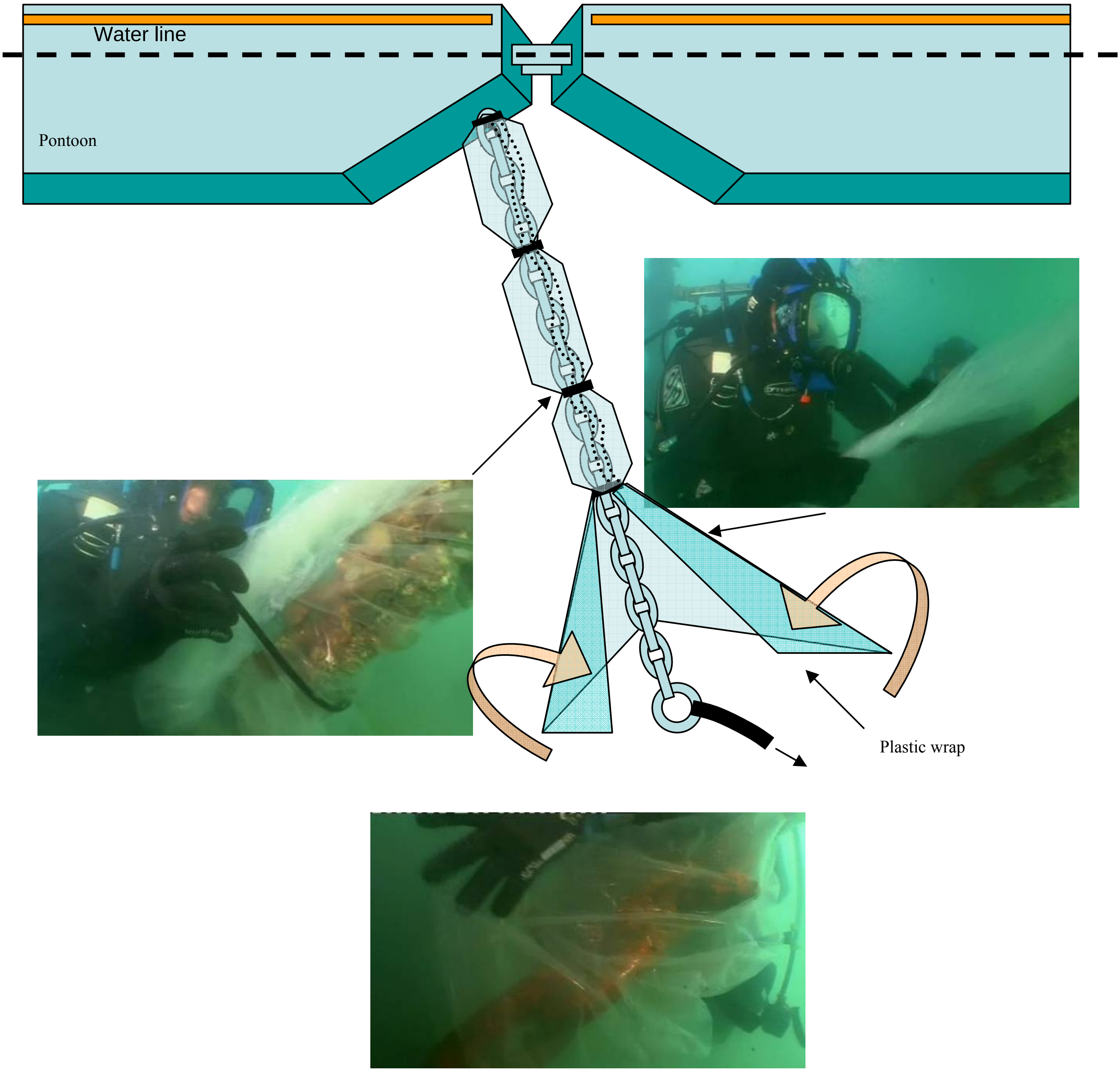



**Figure 20** Chain wrapping method **Removing wrappings from chains**

The plastic wrapping was only removed once the entire marina had been treated.

The removal process is simply the reverse of deployment. The divers start by cutting all the cable ties from the chain (making sure to collect the debris) - they then unroll the plastic. At this point the underwater visibility tended to decrease to zero. Surface personnel can help retrieve and tightly bundle the plastic to reduce the space required for disposal.

### *3.2.9 Wrapping swinging moorings*

Tubular 500 gsm clear plastic on a roll was ideal for covering swing mooring chains providing the diameter of the tube was large enough to fit over the mooring buoy at the surface. Large amounts of water can get trapped in the tube, and will rupture the plastic as the chain shortens on a falling tide, and therefore excess water must be expelled before the cable ties are secured. The method described in **3.2.8**, using sheet plastic, can be used if the buoy diameter is too large.

Preparation

The plastic tubing was deployed off the deck of CCW's dive support vessel *Pedryn* rather than being carried into the water on rolls by the divers. The flattened plastic tube, straight off the roll, was very difficult to peel open once submerged so pre-cut sections (usually 10m lengths) were opened up and passed to the divers once they were in the water.

Wrapping swinging moorings underwater

The process can be described in 5 steps (**Figure 21**):

1. The pair of divers work together to fit the tube of plastic over the mooring buoy, once the plastic is in place cable tie the top end, above the buoy, closed.
2. Once the top is secure work the plastic down the chain over kelp and other obstructions. At the bottom gather the plastic and secure it with a cable tie so that water can still move freely out of the tube by gathering and tying only one corner of the plastic.
3. Working from the top down, squash the bag by hand to remove the excess water and attach cable ties every 75 cm to 1 m.
4. Once all the excess water has been removed the lower-most cable tie can be replaced.
5. Leave for 2-3 weeks by which time the content should have turned anoxic.

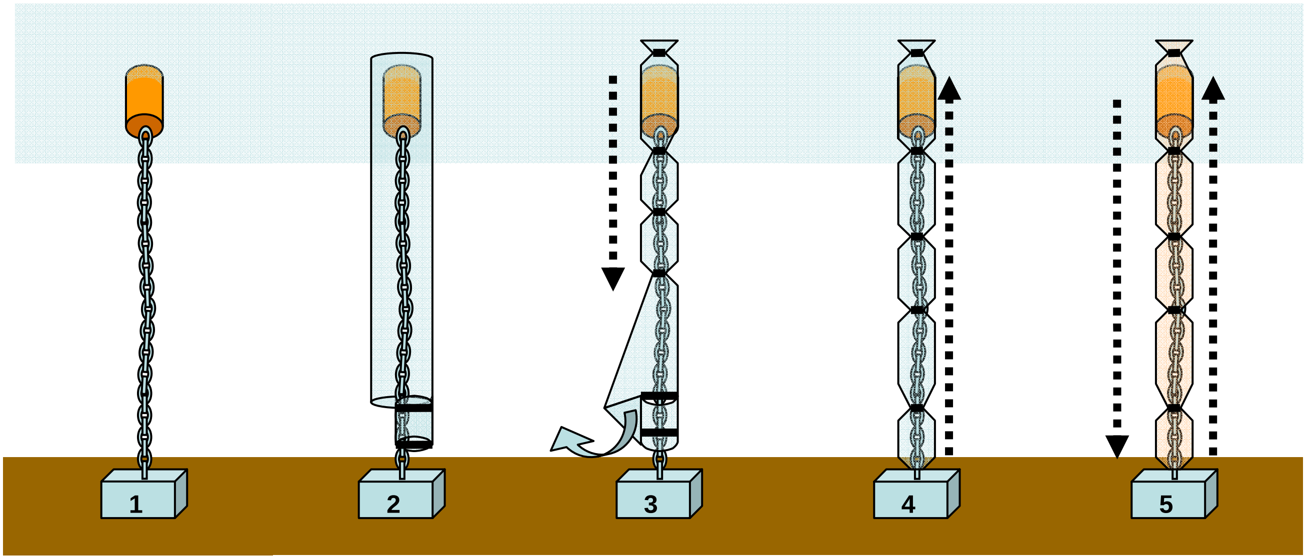



**Figure 21** Swinging mooring treatment using plastic tubing.

Removing tubular wrappings from swinging moorings

As described in section **3.2.8** removing the tubular plastic was the reverse process of deployment, although the process could be speeded up by slitting the bag from the bottom up at the same time as cutting the cable ties using diver's scissors. The surface support crew assisted by tightly rolling the contaminated plastic on the boat's deck.

#### *3.2.10 Alternative methods for treating pontoons and wrapping large buoys and moorings*

In several cases, it was necessary to use adapt the treatments described above to cope with odd sized or shaped pontoon floats, inaccessible areas where bags could not be deployed and very large mooring buoys.

*In-situ* jet washing

Jet washing was used to clean a small section (approximately 16 $m^2$) of breakwater pontoon where a mooring chain prevented bags from being fitted (**Figure 22**). The treatment was precautionary-only as there was no evidence of colonization by *D. vexillum* on this particular section of pontoon. A domestic Kärcher jet washer in conjunction with a two-inch centrifugal pump was operated by two divers working on the underside of the North-South breakwater – this one-off event taking approximately two hours.

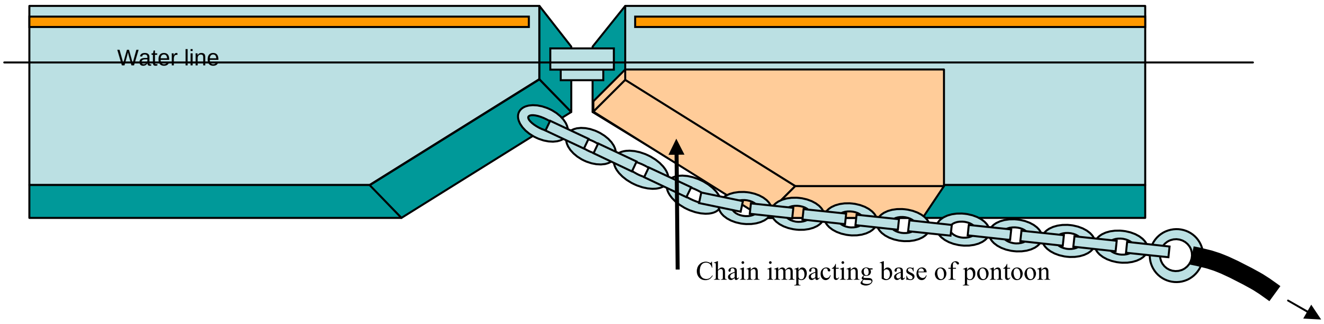



**Figure 22** Jet washed area on East-West breakwater.

Working as a pair, one diver jet washed while the other diver sucked up the dislodged debris using the pump intake hose. The pump's outlet filtered water through a 500µm sieve to catch any debris.

'Space fillers'

The complex topography associated with the undersides of many larger and older sections of the marina (RNLI and fuelling berths) necessitated the use of inert, water-filled 'sausages' to fill dead space (**Figure 23**, **Figure 24** and **Figure 25**). Tubes of 500 gsm clear polythene were cut to size then filled with water *in situ* to displace approximately 2.5 $m^3$ of 'free' water.

Without these, the freely circulating water would remain in the treatment bags, even after pumping, which would therefore require dosing with considerably more granular calcium hypochlorite than standard for the size of bag to reach a sufficiently high concentration to kill the sea squirts.

**Figure 23** Spacer deployment method. The rolled up bag attached to the water pump outlet on the surface

These were constructed from a length of polythene tubing just over double the length of the space to be filled. Each section was folded inside-out to form a double thickness tube just longer than the width of the pontoon. One end was sealed by folding over the material and cable-tying it in place, and then the bag was tightly rolled to exclude as much air as possible (**Figure 23**). The outflow pipe of the water pump was then inserted into the open end of the bag and temporarily secured in place with cable ties or cord.

**Figure 24** Diver deployment of space filler.

The dive team then positioned the tube in its place under the pontoon and the surface crew pumped water into the tube. Diver to surface communication equipment was essential for this operation to ensure the tubes were not over inflated with water.

Once the pump was stopped and its outflow pipe removed, the end of the bag was quickly sealed closed by tightening the cable ties. Any air bubbles in the tube added a small amount of buoyancy which kept it pressed gently in place against the underside of the pontoon (**Figure 24**). Lines were attached to both ends of the tube and secured in place on the pontoon superstructure. Once all the tubes were in place a walkway-sized bag was fitted over the whole pontoon.

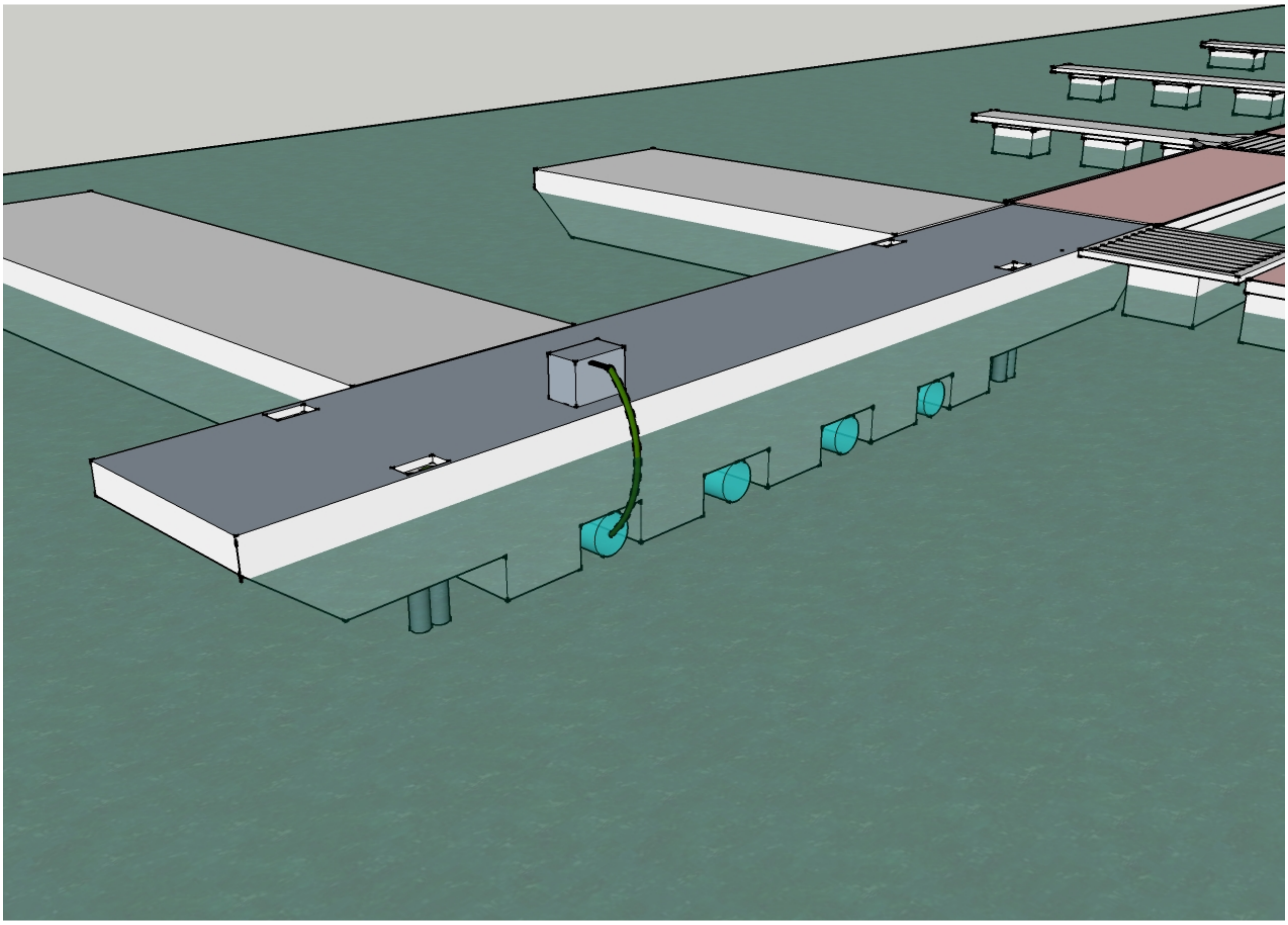


**Figure 25** Positioning of space fillers.

Treating large mooring buoys and chains

A combination of 500 gsm plastic and 'internode' bags were used to wrap the largest mooring buoys situated just north of the marina (**Figure 26**). Other medium-large buoys were treated using finger pontoon bags adapted to accommodate chains and mooring eyes. Calcium hypochlorite was used to accelerate the treatment in some cases.

A  B 

**Figure 26** Wrapping large mooring buoys. A, Largest of the mooring buoys treated; B, Medium-sized buoy wrapped using two adapted finger pontoon bags.

Cleaning larger vessels using sprayed bleach

A moderately large (~15 m) but only partially renovated motor cruiser *Gulf Streamer*, situated on a mooring just outside the NW corner of the marina, was found to be harbouring multiple *D. vexillum* colonies. The vessel was too large to bring ashore in the marina and due to an ownership dispute the boat had not been cleaned or antifouled for several years and was carrying a heavy fouling burden. In an attempt to kill the *D. vexillum* colonies the boat was brought along side the jetty to the south of the marina at high water and allowed to dry on the falling tide. A garden weedkiller sprayer, filled with undiluted bleach, was used to spray all the below-waterline surfaces. The household bleach had an almost instantaneous effect on the fouling biota, with both solitary and colonial sea squirts rapidly contracting and then losing their ability to respond to touch within minutes. The vessel was re-floated on the next incoming tide and positioned back on the mooring. Subsequent examination of the vessel's hull several weeks later showed that virtually all the biota had been killed – the remains of sea squirts and other animals now overgrown by fine filamentous algae.

## 3.3 Survey and monitoring techniques

Survey and monitoring techniques designed around two levels of resolution were established to detect:

1. Presence / absence and approximate distribution of *Didemnum vexillum* within a marina or on other structures.
2. Presence / absence and change in colony size over time at specific sites within the marina.

### *3.3.1 Rapid assessments for presence / absence within a marina.*

This technique was used regularly in Holyhead Marina as part of the QA process to check that effective eradication treatments had been carried out. It was also used on the survey of marinas throughout the rest of Wales to look for other occurrences of *D. vexillum*. The time of year is important when conducting presence / absence surveys as *D. vexillum* colonies are likely to be at their largest in mid to late summer, but much smaller and not easily detected during the winter.

The first stage was to walk, where possible, around the entire marina and related structures looking at the shallow submerged pontoon sides. Ideally this should be carried out at low water so that the sub-tidal portion of pilings, if present, may also be investigated. Any dangling ropes, heavily-fouled floating objects and fouling biota should be removed from the water for inspection. This will provide information on what might be living on the underside of the pontoons or in slightly deeper water where the salinity might be more favourable for growth. See **Figure 27** and **28**.

**Figure 27** Rapid assessment – inspection of ropes and **Figure 28** fenders and old tyres.

The community structure of native fouling biota gave important clues to the suitability of the marina habitat for *D. vexillum.* A lack of native sea squirts such as *Ciona intestinalis* and *Ascidiella aspersa* but abundance of mussels *Mytilus edulis* indicated that the salinity regime was too low. If presence of *D. vexillum* had been confirmed by this stage then information on abundance and distribution could be enhanced by conducting a snorkeling survey.

A snorkel survey should be carried out if the composition of the native biota suggests that the marina is suitable for *D. vexillum* colonization even if it has not been found from the surface. If required the entire area will need inspecting - this is best conducted in either a figure-of-eight or a saw-tooth pattern to ensure that all the surfaces of each structure are observed (**Figure 30**). Obstructions, such as kelp, should be parted to gain the best view of small colonies that may be in the 'understory'. Well-established colonies will be easy to spot (**Figure 29**), but smaller juvenile colonies will only be found through close inspection.

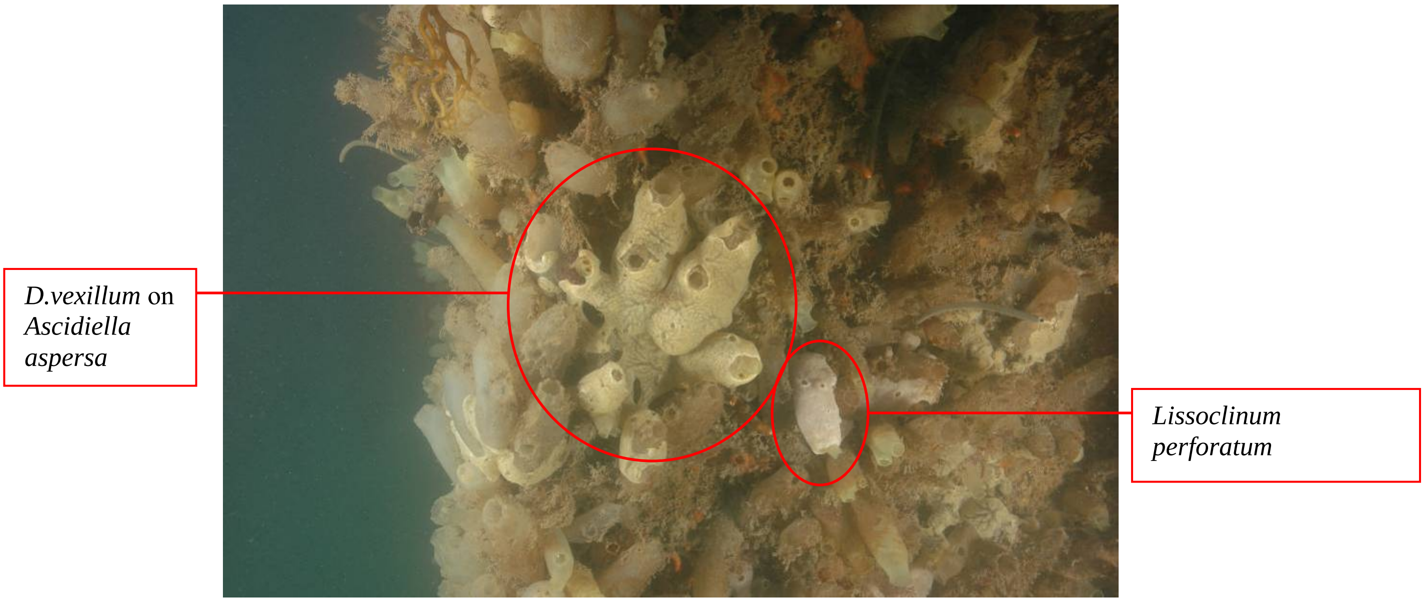



**Figure 29** *Didemnum vexillum* overgrowing native ascidians in Holyhead marina.

Colonies were commonly found amongst native solitary ascidians in Holyhead; at other sites they may grow on different substrata. Small colonies look like 'didemnid snow' (small white speckles of post-settlement colonies of *Didemnum maculosum*) and are very difficult to differentiate from other species.

Samples of suspect colonies should be gathered and, if practicable, kept alive in fresh seawater for microscopic examination and dissection.

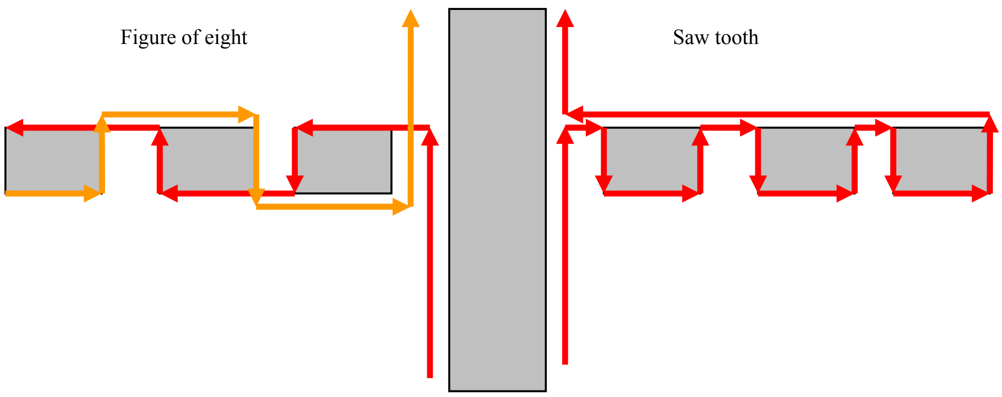



**Figure 30** Rapid assessment snorkel methods.

### *3.3.2 Diving surveys for detecting presence / absence*

Each survey location required site-specific planning and included a variety of structures and locations around Holyhead Harbour including:

1. Subtidal parts of the Holyhead breakwater wall.
2. Hard substrata – natural and rip-rap around the marina.
3. Undersides of large vessels and jack-up barge (**Figure 32**)
4. Commercial ferry terminal and fish dock structures.(**Figure 31**)
5. Jetty pilings (**Figure 33**).
6. Large mooring buoys.

Some of the diving survey had to be restricted to representative sub-samples of the larger of these structures which were otherwise far too extensive to examine in fine detail. The rate of sampling was dictated largely by the number of person-days available and was concentrated on areas which appeared most likely to harbour *D. vexillum* by supporting large numbers of native solitary and colonial sea squirts.

The divers developed patterns of searching appropriate to each type of structure. They concentrated on working below the low water mark and avoided searching over fine mud, structures devoid of other marine life and freshly cleaned surfaces. Structures such as the Tinto aluminium jetty and ferry terminals, which have many vertical stanchions and pilings reaching in to deep water, were best surveyed by spiraling up or down each structure but navigating horizontally between them underwater at around 4-10 m depth without surfacing between each one. This was primarily to avoid saw-tooth diving profiles which can be hazardous, by inducing decompression illness, to the divers (**Figure 33**).

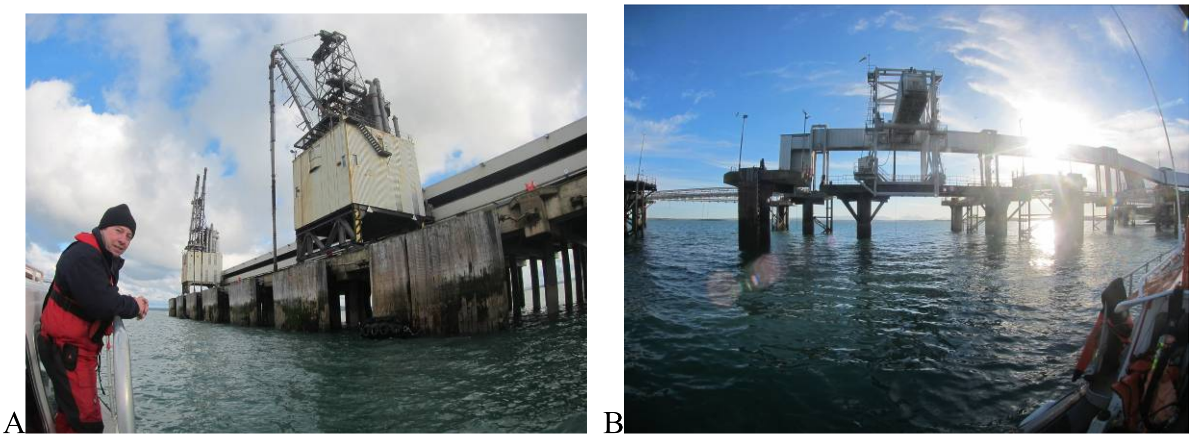



**Figure 31** A. Anglesey aluminium 'Tinto' jetty B. Terminal 3/5

**Figure 32** Diving the jack-up crane barge Excalibur.

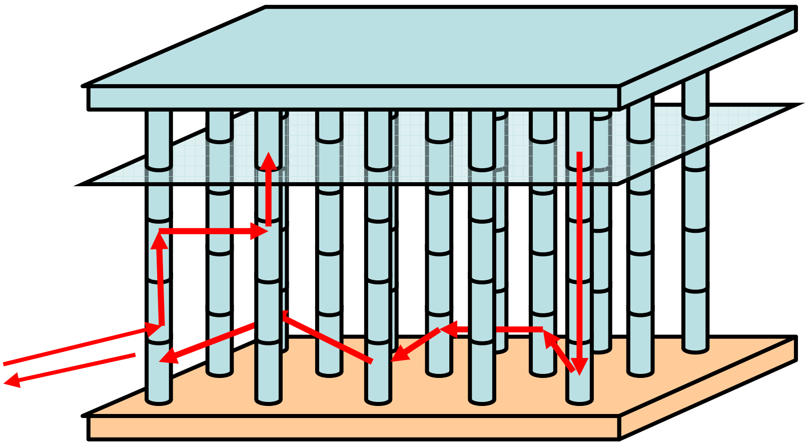


**Figure 33** Pilings and the survey pattern for investigating the Tinto jetty.

As part of the quality assurance and quality control measures with regard to specimen identification all samples were photographed *in situ* before they were collected and then photographed again in numbered sample bags once removed from the substratum (**Figure 34**). This assists significantly in training observers to identify species by their field characteristics. Samples were processed by first relaxing in menthol crystals dissolved in seawater then preserved in 98% ethanol.

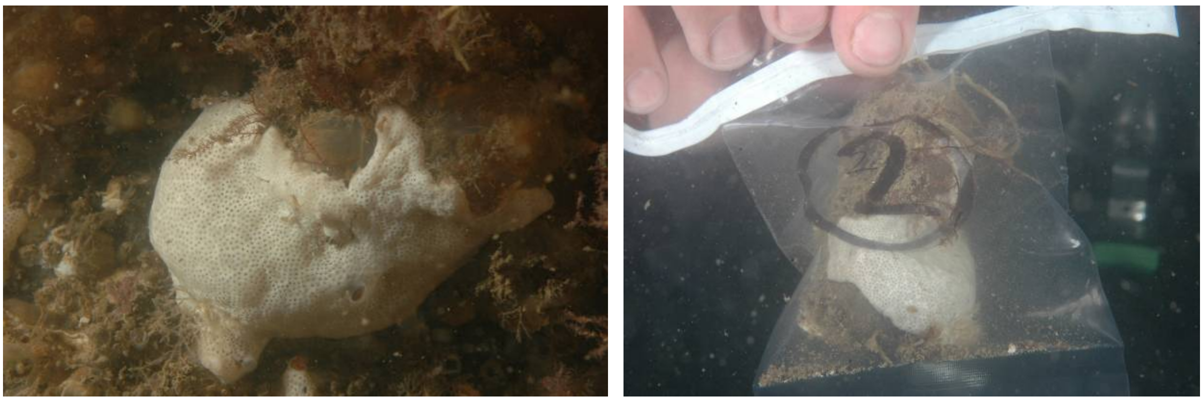


**Figure 34** Sampling methods using photography to aid *in situ* identification and QA/QC measures

### *3.3.3 Monitoring using fixed-location video quadrats in the marina*

Fixed location video quadrat sites have been established in the marina to replace existing photo quadrats sites set up by the School of Ocean Sciences, Menai Bridge. The original quadrats were set up before the start of the eradication project to monitor *D. vexillum* growth rates and colony size. The original method made use of CCW's Nikon D70 digital single lens reflex camera in a Seacam underwater housing set in a fixed frame. However, the images produced by this method were not sufficiently clear to count small colonies – mainly because of poor water clarity and kelp obscuring the substratum – so the stills camera was replaced with a high-definition hand-held video camera used on close-focus setting.

Video quadrat method

Video sampling points were established at 18 sites around the marina. A map (**Figure 35**) plus instructions (**Appendix II**) of exact locations and quadrat orientation was produced to ensure accurate repositioning at a later date. Two 0.5 m x 0.5 m quadrats with sub-frames at 10 cm intervals were used at each location. The quadrats were joined side-by-side with cable ties and were held in place against the sides of either the small finger pontoons or the walkways as shown on the map - the top left hand corner of the quadrat was orientated to the recorded location on the pontoon sides.

Before positioning the video quadrats, the kelp (mainly *Saccharina latissima*), was trimmed back to just above the holdfast. As a comparison both cut and uncut quadrats have been recorded, clearly showing that the kelp makes consistent counting of the sea squirt colonies almost impossible. The quadrats were positioned on the pontoon face with the top right-hand corner on the site marker. The quadrat was positioned so that the top of the quadrat is just under the water line. In such shallow water, the recorder only required snorkeling gear and was able to deploy the video camera in a zig-zag pattern down the quadrat as illustrated in (**Figure 36**).

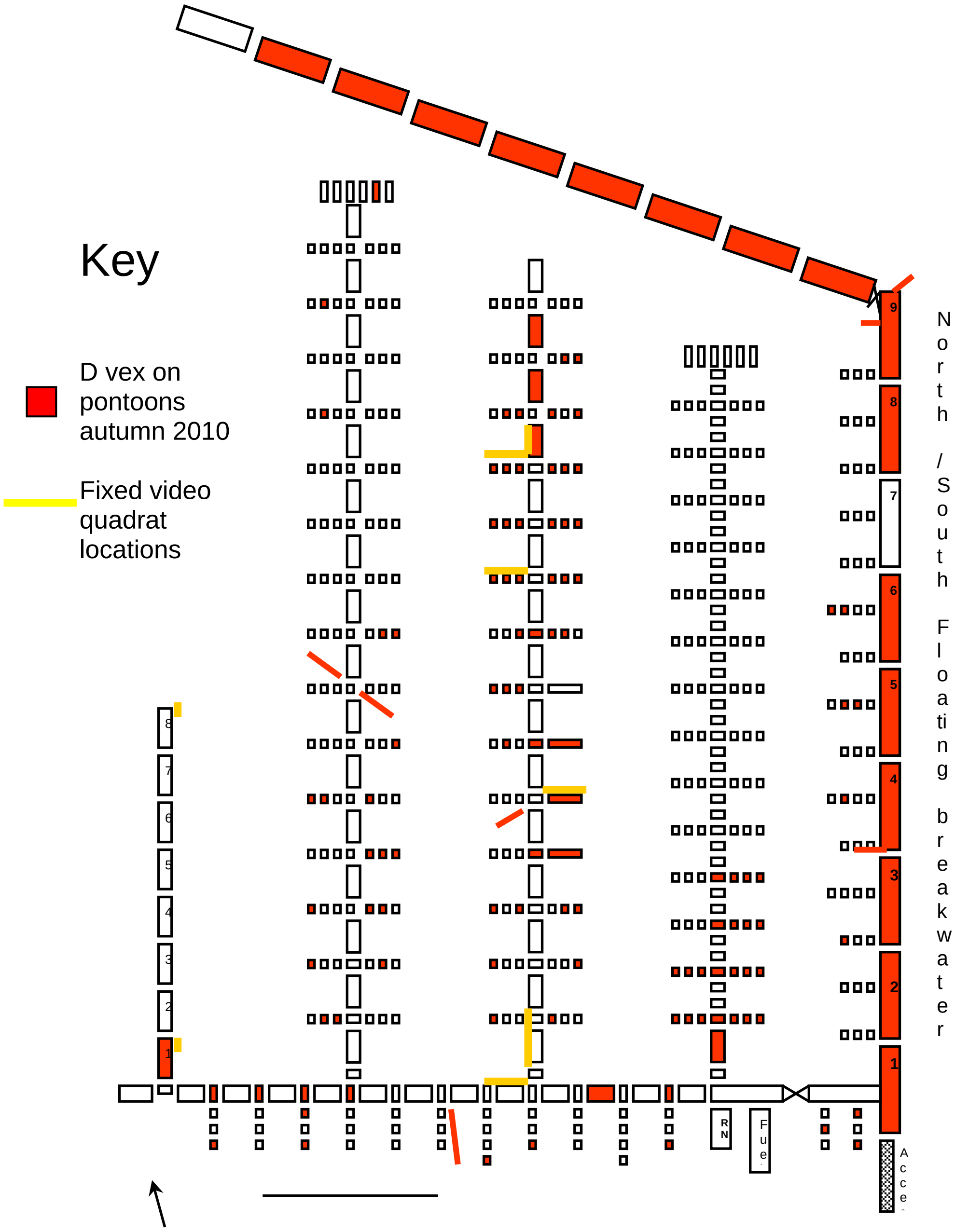



**Figure 35** Marina layout with locations of fixed point video quadrats

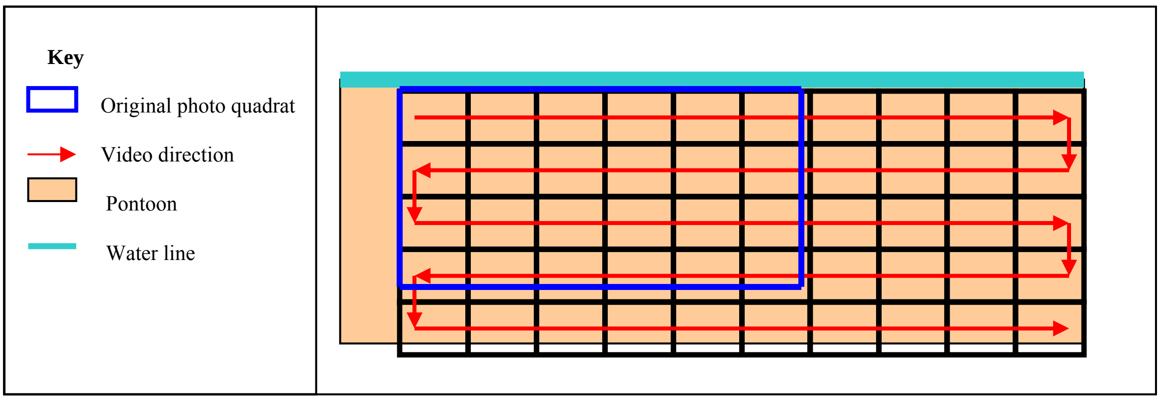



**Figure 36** Video quadrat methods diagram.

Best results are achieved by moving the video slowly and steadily from quadrat sub-cell to sub-cell and pausing on each cell for approximately four or five seconds (see also Figure 37).

**Figure 37** Video quadrat method.

# 4 ERADICATION LOGISTICS

## 4.1 Licensing issues

The Marine Management Organisation (previously DEFRA, and now in Wales, part of the Welsh Assembly Government) has a statutory duty to control the deposit of articles or materials in the sea; the primary objectives being to protect the marine ecosystem and human health, and minimising interference and nuisance to others.

This duty is exercised under powers conferred by the Food and Environment Protection Act 1985 Part II (FEPA), which require that a licence be obtained from the licensing authority to deposit any articles or substances in the sea or under the seabed.

See http://marinemanagement.org.uk/works/licensing/process_application.htm for details.

Exemptions to the act include scientific instruments, but as the none of our materials and methods used during the eradication attempt were regarded by DEFRA and the MFA as part of a scientific study nor part of the existing marina structure (for which a FEPA license had already been granted) we were asked to apply. This process can take up to ten weeks under standard application procedures, however special dispensation was made for this application and processing time was considerably shorter than normal.

A license was issued for user-defined quantities of bags, wraps and chemicals used in the project (see **Appendix III** for a copy of the application) with a requirement for periodic renewal.

Unfortunately the then Aberystwyth-based fisheries office did not receive the communication we had from the London DEFRA office to proceed with the project which resulted in delays of several weeks while the paperwork had to be put in order. Even more complications and delays occurred while the legal status of the shock treatment chemical, calcium hypochlorite, being used as a biocide, was debated within the Health and Safety Executive. After a month they concluded that they had no jurisdiction on how it was used in the first place and we were allowed to continue.

#### *4.1.1 Port authority and work permits*

Holyhead Marina, although privately owned, is under the jurisdiction of Holyhead Harbour controlled by Stena Line. Any work in the judicial remit of Stena Line requires a daily work permit – particularly when divers may be working in the busy commercial shipping areas. Separate permits were required for diving and surface work which had to be obtained from Holyhead port control office, issued by the daily duty officer, based within the harbour complex – access to which required a security pass specific to each individual person and each vehicle. Our diving risk assessment (**Appendix IV**) and list of personnel and their relevant qualifications had to be presented at Port Control prior to them issuing a work permit. Our arrangements were streamlined so that we only needed to visit Port Control once per week to pick up the relevant permits but called in by VHF each day at the start and finish of each diving operation.

#### *4.1.2 Limiting factors: weather and equipment*

Bad weather was our major limiting factor during the eradication attempt and must be considered carefully for any future work that, by necessity, has to occur during the winter while the sea squirt is not producing large numbers of larvae. Most marine biological survey work tends to occur during the summer months. In contrast we had to contend with:

- Low working temperatures – down to minus two degrees water temperature near the surface and minus ten air temperature plus added wind-chill. Our standard thermal protective equipment (dry suits) had to be supplemented with additional under layers and eventually electrically powered heated under-vests. The high abrasion environment working amongst the pontoons required the use of overalls being worn on top of dry suits and the need for constant suit repairs and maintenance. Even with additional thermal insulation the divers could not extend their in-water time to much beyond 90 minutes per dive. Most dives were conducted using full face masks which, apart from the advantages of built-in diver-to-diver and diver-to-surface communications, were significantly warmer than standard half-masks.
- Winter gales - particularly during the post-Christmas 2009 period when the 'worst north-easterly gales in 15 years' hit the marina. Many of our eradication bags were either lost or shredded by the violent wave action in the marina. Further days were lost due to elevated sea states making working amongst moving pontoons and boats too dangerous or the bags ineffective. We had one 'incident' when a small tornado blew through the marina, damaging yachts' rigging and blowing trolleys full of our equipment off the pontoons into the water.
- Sea ice formed within the marina, making work slow particularly when trying to move eradication bags through the water between adjacent pontoons.
- Poor underwater visibility caused by strong winds combined with spring tides. This made diving work difficult when it was not possible to see from more than a few centimetres when fitting the larger eradication bags around the chains on the breakwater sections.

### *4.1.3 The limitations of team size and material availability*

The table below summarises the maximum amount of work that could be achieved each day expressed in terms of numbers of bags fitted to the different pontoon types. In most cases the number of personnel working each day was between five and eight - all figures are based on this team size. For most of the small and medium sized bags, the numbers that were fitted per day were limited by the availability of ready-made bags rather than team size. Conversely two sets of the large breakwater bags required a full day to fit by the same team size. For any future eradication attempts in the marina these values can assist in gauging the rate at which bags can be fitted and the result of increasing team size and/or numbers of bags available of different size.

**Table 3** Table of bag types and deployment rates.

| Bag Type | To fit…: | Maximum number of units removed and re-fitted per day by 5 – 8 people team | Potential estimated maximum number of units re-fitted per day with same team size |
|---|---|---|---|
| None-inflatable | Small pontoon finger floats | 41 (limited by quantity) | 50 |
| | Walkway 'internodes' and most of walkway D | 15 (limited by quantity) | 25 |
| Inflatable | Finger – experimental inflatable bag | 1 (limited by quantity) | n/a – only used experimentally |
| | Walkway B, C, F | 4-6 (limited by quantity and personnel ) | 6 |
| | Walkway A | 6 (limited by quantity and personnel ) | 4-6 |
| | Walkway – west end F | 1 (limited by quantity) | n/a – only 1 pontoon of this type |
| | Walkway F - east end | 1 (limited by quantity) | n/a - only 1 pontoon of this type |
| | Breakwater E | 2 (limited by quantity and personnel ) | 2 |
| | Breakwater East-West | 2 (limited by quantity and personnel ) | 2 |
| | Fuel jetty | 1 (limited by quantity) | n/a - only 1 pontoon of this type |
| | RNLI jetty | 1 (limited by quantity) | n/a - only 1 pontoon of this type |
| Wedge | Covering chains and gap between breakwater pontoons on E | 3 (limited by quantity and personnel ) | 3-4 |

## 4.2 Chemical 'shock' Vs biological treatments

The term 'biological treatment' is used here to describe the stagnation reaction that kills the fauna by creating anoxic conditions in the bags or wraps. This method can be used all year round on treatments that are not time-limited and is ideal for 'set-and-forget' plastic wrapping of pontoon mooring and swinging moorings chains that are fully submerged and only slightly effected by wave action.

The stagnation method is effective in bags when the water temperature is over 11 degrees (Kleeman 2009) and will happen at lower temperatures but takes weeks rather than days. Water ingress through stitched seams, small punctures and overtopping by small waves tended to result in a higher rate of failed treatments in cool conditions.

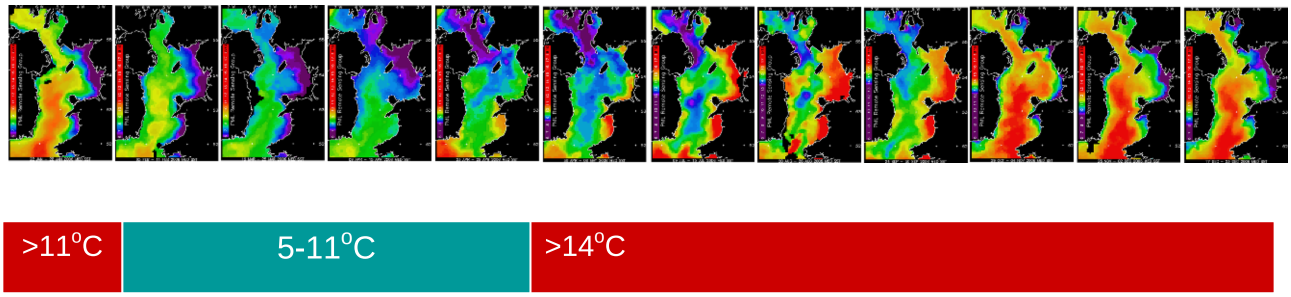



**Figure 38** Irish Sea monthly averaged Temperatures (Kleeman, 2009).

At lower temperatures and when the bags were at risk from over-topping or wave-driven mixing, chemical 'shock' treatment was a preferred option for effective eradication. Chemical treatments were calculated on an effective chemical dose of chlorine at 200 parts per million - derived from work by Denny (2008). Doses were calculated by estimating the amount of seawater remaining in the bags after being pumped out. The figures, particularly for the larger bags which were far more difficult to pump 'dry', were rounded up to allow for a potentially much higher dilution factor (**Table 4)**.

**Table 4** Calcium hypochlorite doses.

| Bag Type | Purpose | Dose Kg @ 200ppm (based on theoretical calculated water volume) | Dose used Kg |
|---|---|---|---|
| None-inflatable | Finger – small pontoon floats | 0.1 | 0.1 |
| | Internode – medium size floats | 0.6 | 0.6 |
| | Walkway D / internode / ends | 1.0 | 1.0 |
| | Finger – Type 2 | 0.6 | 0.6 |
| Inflatable | Walkway B, C, F | 1.9 | 2 |
| | Walkway A | 7.4 | 10 |
| | Walkway – west end F | 5.4 | 20 |
| | Walkway F - east end | 13.3 | 20 |
| | Breakwater | 15.8 | 20 |
| | Breakwater- Internal wedge | 15.8 | 20 |
| | Fuel jetty | 12.9 | 20 |
| | RNLI jetty | 8.8 | 10 |
| | Finger – solid | 1.2 | 2 |
| Wedge | Breakwater | N/A – not dosed separately from breakwater | N/A |

# 5 RESULTS

## 5.1 Rapid Assessment

### *5.1.1 Across-Wales survey*

The across Wales rapid assessment of marinas identified Holyhead Marina as the only location in Wales with *D. vexillum*. Other sites – Pwllheli, Neyland and Milford Haven marinas were identified as highly suitability for colonization, providing ideal substrata and conditions for settlement in at least part of each marina (**Figure 39**).

The majority of marinas around Wales were all or part brackish due to riverine inputs; the lowest salinity conditions found in Aberystwyth marina. Aberystwyth had little or no fouling from marine species on any substrata inside the marina, with fully marine intertidal species such as *Fucus serratus* reaching only as far up river as the harbour entrance.

Other marinas around Wales incorporate a tidal sill or lock gates, restricting the flow of water for up to 12 hours a day. Many tended to have fresh water inputs from rivers or storm drains, resulting in reduced salinity particularly after heavy rain. Such situations are unlikely to be suitable for colonization by *D. vexillum* (**Table 5**).


**Figure 39 Marina surveyed in Wales.**

**Table 5** Welsh marinas and their suitability to host *D. vexillum*

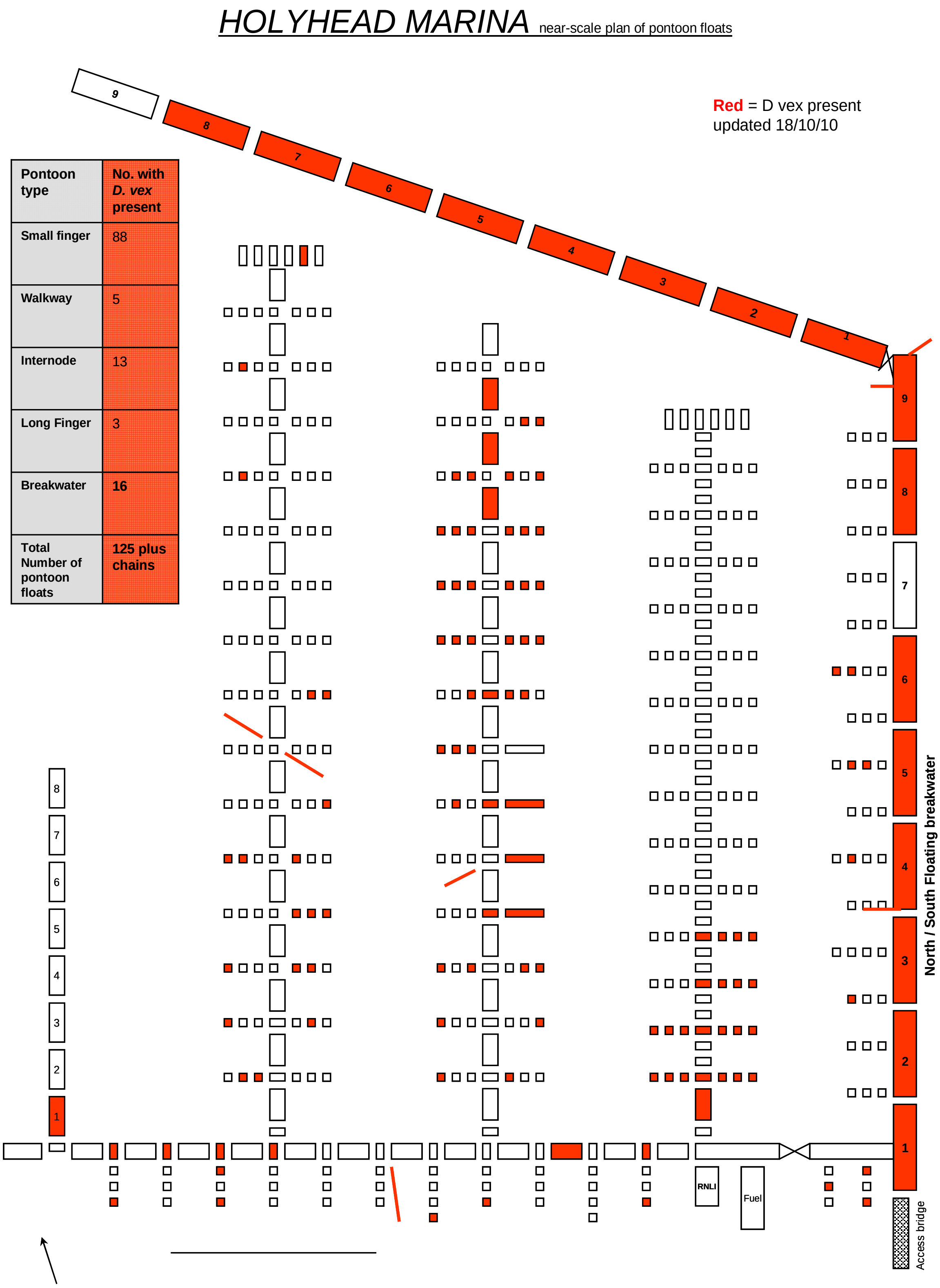
| Location | Latitude decimal minutes (ºN) | Longitude decimal minutes (ºW) | Date of survey | Marine species | Comments | *Didemnum vexillum* Present or Absent |
|---|---|---|---|---|---|---|
| Holyhead Marina | 53º 19.173 | 04º 38.488 | 10.12.08<br>25.05.10<br>ongoing | Moderate-high species abundance and richness of filter feeding invertebrates and algae. Fringing kelp. | Ideal – clean circulating seawater with minor sporadic freshwater input | Present |
| Deganwy marina | 53º 17.480 | 03º 49.673 | 11.12.08 | *Mytilus edulis* and filamentous algae, no ascidian species recorded. | Behind sill and with some freshwater input. | Absent |
| Conwy marina | 53º 17.458 | 03º 50.286 | 11.12.08<br>18.05.10 | *Mytilus edulis* and filamentous algae, no ascidian species recorded. | Behind sill and with some freshwater input. | Absent |
| Rhyl river mouth | | | 18.05.10 | Few barnacles and mussels only | River mouth – no ascidians present. | Absent |
| Port Penrhyn | 53º 14.182 | 04º 06.706 | 18.12.08 | Mainly bare substrate and no ascidian species recorded. | Intertidal substrata only. | Absent |
| Port Dinorwic | 53º 11.178 | 04º 12.582 | 12.12.08<br>18.05.10 | *Mytilus edulis*, filamentous algae, *Ascidella aspersa, Botryllus schlosser and, Botrylloides leach.* | Pontoon dock dries at LWS but a few ascidians found on hard substrata. | Absent |
| Victoria Dock - Caernarfon | 53º 08.562 | 04º 16.669 | 30.01.09<br>18.05.10 | Mytilus edulis, filamentous algae, occasional ascidian species (*Ascidella aspers and, Botryllus schlosseri*). | Behind sill and with minor freshwater input but ascidians present. | Absent |
| Pwllheli marina | 52º 53.191 | 04º 24.389 | 16.12.08<br>19.05.10 | *Mytilus edulis*, filamentous algae, *Ascidella aspersa, Ciona intestinalis, Botryllus schlosseri and Botrylloides leachi.* | Parts of marina at the seaward end have ascidians colonising the deeper parts of pilings. | Absent |
| Aberystwyth marina | 52º 24.595 | 04º 05.202 | 09.02.09<br>19.05.10 | Filamentous algae, no evidence no ascidian species recorded. | Virtually nothing growing on stuctures. Toxic metal riverine input antifouls the marina (anecdotal information). | Absent |
| Milford Haven marina | 51º 42.699 | 05º 02.270 | 11.02.09<br>12.02.09<br>28.07.10 | *Mytilus edulis*, filamentous algae, *Ascidella aspersa, Ciona intestinalis, Botryllus schlosseri, Botrylloides leachi and Styela clava.* | Behind sill/gates but salinity appears high. Other non-natives present including lots of ascidians. | Absent |
| Neyland marina | 51º 42.632 | 04º 56.544 | 12.02.09<br>28.07.10 | *Mytilus edulis*, filamentous algae, *Ascidella aspersa, Ciona intestinalis, Botryllus schlosseri, Borylloides leachi, Styela clava and Botrylloides violaceus.* | Open marina with freshwater input. Ascidians inc non-natives common especially near the mouth. | Absent |
| Swansea marina and Swansea yacht club marina | 51º 36.984 | 03º 56.048 | 10.02.09 | Barnacles and tubeworm casts, filamentous algae, no ascidian species recorded. | Behind gates / sill and very little colonisation. | Absent |

**Figure 40** A, Tidal sill at Conway marina; B, Fouling biota at Victoria Dock, Caernarfon; C, Fouling biota at Neyland marina; D, Fouling biota at Conwy marina

### *5.1.2 Holyhead rapid survey results*

After completing first full wave of eradication, a rapid assessment survey of Holyhead Marina was conducted to assess the effectiveness of the eradication process. On the 25$^{th}$ May 2010 *D. vexillum* was not found in Holyhead Marina. At this time all the pontoons and chains were at various stages of 'recovery' from their recent treatment and were either still obviously bleached, completely un-colonised, had a fine fuzzy coating of filamentous green and brown algae, or were supporting newly settled native species.

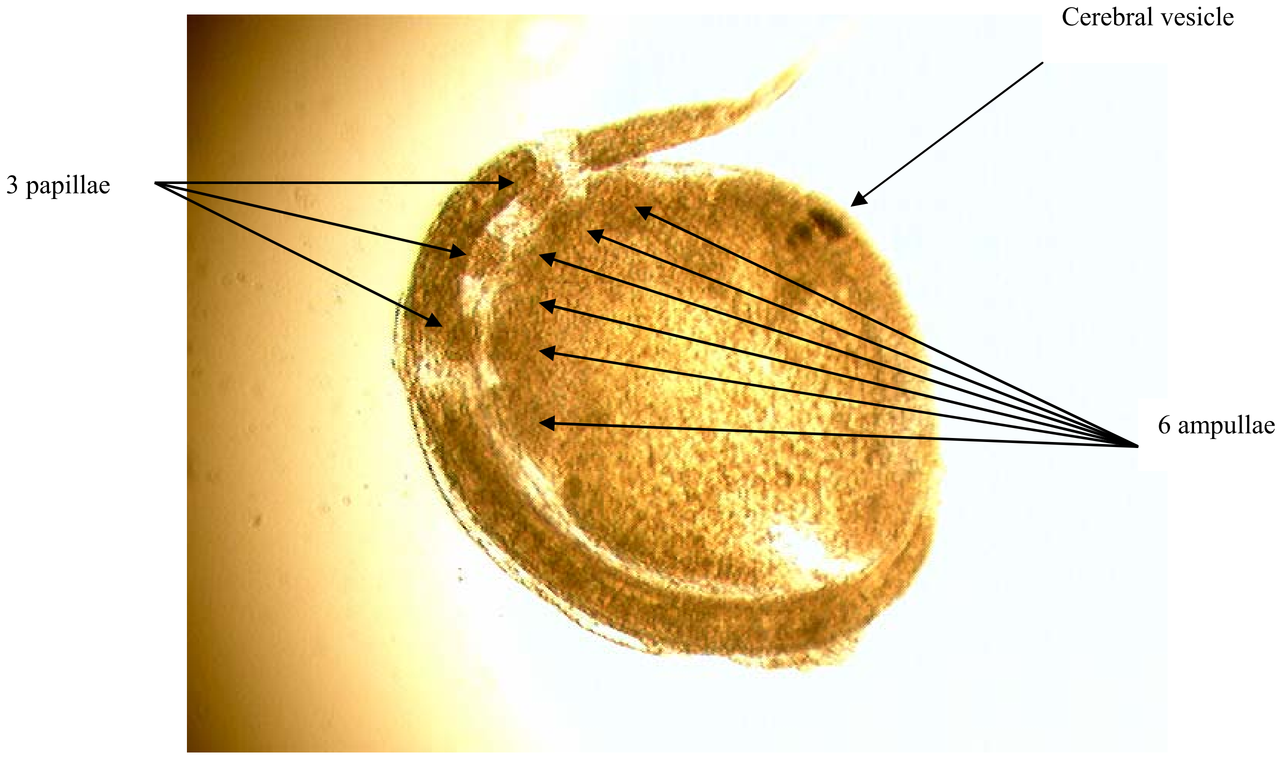
A later rapid assessment of Holyhead Marina made on the 30$^{th}$ September 2010 showed 19% of the inner portion of the marina structures had been re-colonised by *D. vexillum*. A second snorkel survey of the floating breakwater structures conducted on the 18$^{th}$ October 2010 revealed that 16 of the 18 breakwater pontoons had become re-colonised (**Figure 41**). Due to the relatively large size of the breakwater floats, this equated to 50% of the total submerged surface area of the marina had become re-colonised post eradication.



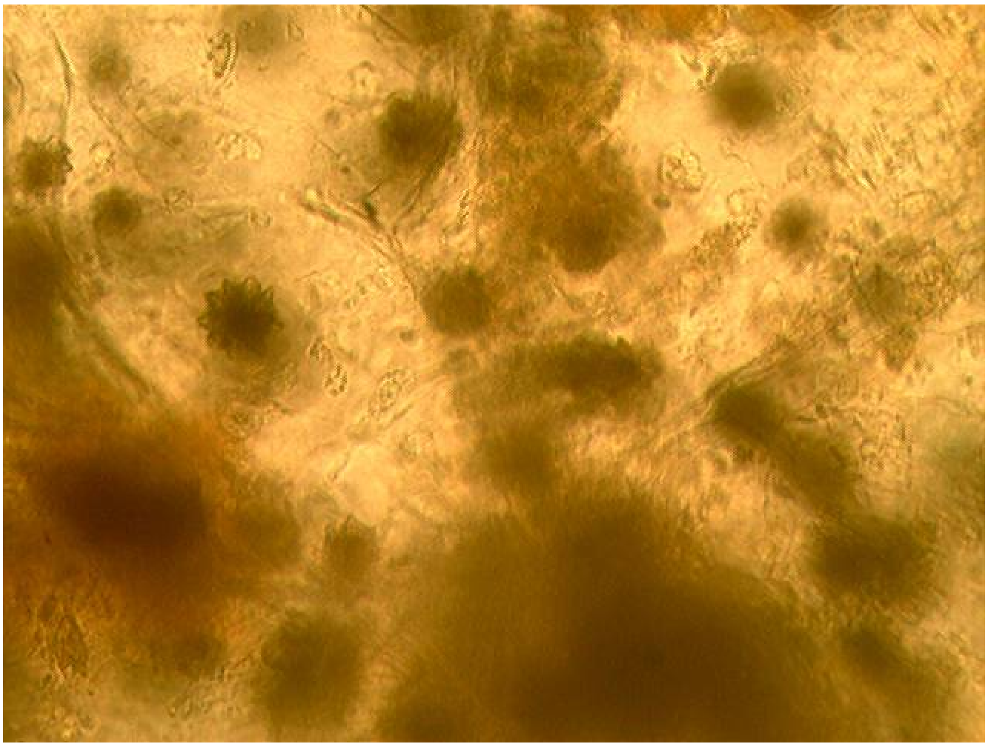

| Pontoon type | No. with *D. vex* present |
|---|---|
| Small finger | 88 |
| Walkway | 5 |
| Internode | 13 |
| Long Finger | 3 |
| Breakwater | 16 |
| Total Number of pontoon floats | 125 plus chains |

**Figure 41** Extent of *D. vexillum* re-colonisation on 18/10/10**.**

Samples taken on the 30th September 2010 from Holyhead Marina were confirmed as *D. vexillum* using several key taxonomic features outlined by (Kott 2002; Lambert 2009) (**Figure 42**).




A



B

**Figure 42** A, unhatched *Didemnum vexillum* brooded larva from Holyhead Marina with key features highlighted
B: *Didemnum vexillum* spicules from holyhead marina

### *5.1.3 Video quadrat monitoring results*

Our up-graded HD video survey method produced considerably more consistent results than the earlier digital stills photographs, with *D. vexillum* colonies being detected as small as 2-3 mm which were possibly missed earlier. The variation between cell frequency and percentage cover was due to a wide variety of colony sizes found in the different quadrats. Most of the videoed colonies were clustered on finger pontoons (quadrats 7-9) with relatively few colonies found on the walkway pontoons (**Table 6 and 7**).

**Table 6** Quadrat cell frequency and percentage data for 08/10/2010.

| Quadrat | Cell Frequency | Percentage Cover |
|---|---|---|
| **Q1** | 0 | 0 |
| **Q2** | 0 | 0 |
| **Q3** | 0 | 0 |
| **Q4** | 0 | 0 |
| **Q5** | 0 | 0 |
| **Q6** | 0 | 0 |
| **Q7** | 28 | 11.8 |
| **Q8** | 7 | 2.8 |
| **Q9** | 7 | 0.7 |
| **Q10** | 18 | 6.2 |
| **Q11** | 0 | 0 |
| **Q12** | 0 | 0 |
| **Q13** | 0 | 0 |
| **Q14** | 0 | 0 |
| **Q15** | 0 | 0 |
| **Q16** | 0 | 0 |
| **Q17** | 9 | 3.2 |

**Table 7** Video quadrat cell frequency and percentage data for 08/02/2011.

| Quadrat | Cell Frequency | Percentage Cover |
|---|---|---|
| **Q1** | 0 | 0 |
| **Q2** | 0 | 0 |
| **Q3** | 0 | 0 |
| **Q4** | 0 | 0 |
| **Q5** | 0 | 0 |
| **Q6** | 0 | 0 |
| **Q7** | 19 | 6 |
| **Q8** | 8 | 3.2 |
| **Q9** | 0 | 0 |
| **Q10** | 13 | 4.6 |
| **Q11** | 0 | 0 |
| **Q12** | 0 | 0 |
| **Q13** | 0 | 0 |
| **Q14** | 0 | 0 |
| **Q15** | 0 | 0 |
| **Q16** | 0 | 0 |
| **Q17** | 7 | 2.1 |

Comparison of results from the 08/10/2010 and 08/02/2011 video surveys show an overall reduction in *D. vexillum* colony size and distribution. Colony resilience appears to be directly linked to pre-winter colony size, with smaller colonies such as those found in quadrat 9 totally disappearing over winter. However quadrat 8 shows a slight increase in both colonies size and distribution - this is probably due to the deeper distribution of colonies in the lower 20 cm of the quadrat which therefore avoid the more stressful near-surface drop in salinity and temperature. Average percentage cover dropped from 1.5% to .99% and average cell count dropped from 4.3 to 2.9. All colonies show signs of stress particularly those in shallower water. Samples were collected to ascertain the reproductive status of *D. vexillum*: no brooded larvae were found in the February 2011 samples.

## 5.2 Diving surveys of the wider harbour area

To confirm whether *D. vexillum* was restricted to the marina and had not escaped into the wider harbour area, representative areas of all major structures in the harbour had to be surveyed.

The first surveys (04th - 6th May 2010) located a 'suspicious-looking' pale beige-coloured didemnid ascidian on the pilings of the Tinto jetty and terminal four. Samples were taken for laboratory analysis and sent to John Bishop at the Marine Biological Association. However, no characteristic identification features could be found at that stage. Many of the samples taken did not contain brooding larvae and made conclusive identification impossible.

The other structures at terminals 2, 3, 5, the HSS berth, Fish Dock and the harbour wall were either evidently unsuitable (had no native sea squirts) or were not fouled by the unknown didemnid.

Later in July, when brooding larvae had been found in samples of *D. vexillum* in the marina, a second survey was conducted, with the aim of collecting new samples of the unknown didemnid. Samples were again taken to John Bishop at the MBA and were identified as two species - *Didemnum maculosum* and *Lissoclinum perforatum*.

In preparation for a second attempt at eradicating *D. vexillum* from the marina the diving survey of the harbour was repeated during the autumn (10th, 14th and 15th October 2010). This most recent survey covered more locations than previous surveys including more of the Tinto jetty pilings and the supports of the HSS docking ramp. No colonies were found in the wider marina area (**Figure 43** and **Appendix VI**).

**Table 8** Holyhead diving survey locations, outcomes and dates.

| Date surveyed | Locations | Sample taken for analysis | Photo/video | *D. vexillum* |
|---|---|---|---|---|
| 04th/5th/6th/05/2010 | Tinto Jetty, outer Breakwater, Terminal 4 (T4), T1, T2, Fish Dock and T 3/5 | Yes | Yes | No |
| 27/07/2010 | T4, Tinto jetty | Yes | Yes | No |
| 10th/14th/15th/10/2010 | Tinto Jetty, Outer Breakwater, Terminal 4 (T4), T1, Fish Dock and T 3/5 | No | No | No |

### *5.2.1 Summary of survey results.*

**Tinto jetty**- Extensively colonised by native colonial and solitary ascidians. Heavily silted at lower levels and regularly disturbed by ship movements on the jetty and T3/5.

**Holyhead breakwater**- The breakwater wall does not appear suitable for *D. vexillum* due in part to its intertidal nature. However, the eastern tip is colonised by native colonial and solitary ascidians and could potentially provide suitable substrata.

**The Fish dock**- The area is potentially suitable and is heavily colonised by native colonial and solitary ascidians. Some fishing gear, such as caches and keep nets kept submerged at the dockside, appear heavily fouled and could potentially act as vectors for importing/exporting *D. vexillum*.

**Terminals 1-5**- Most of the structures at the terminals are already heavily colonised by native colonial and solitary ascidians.

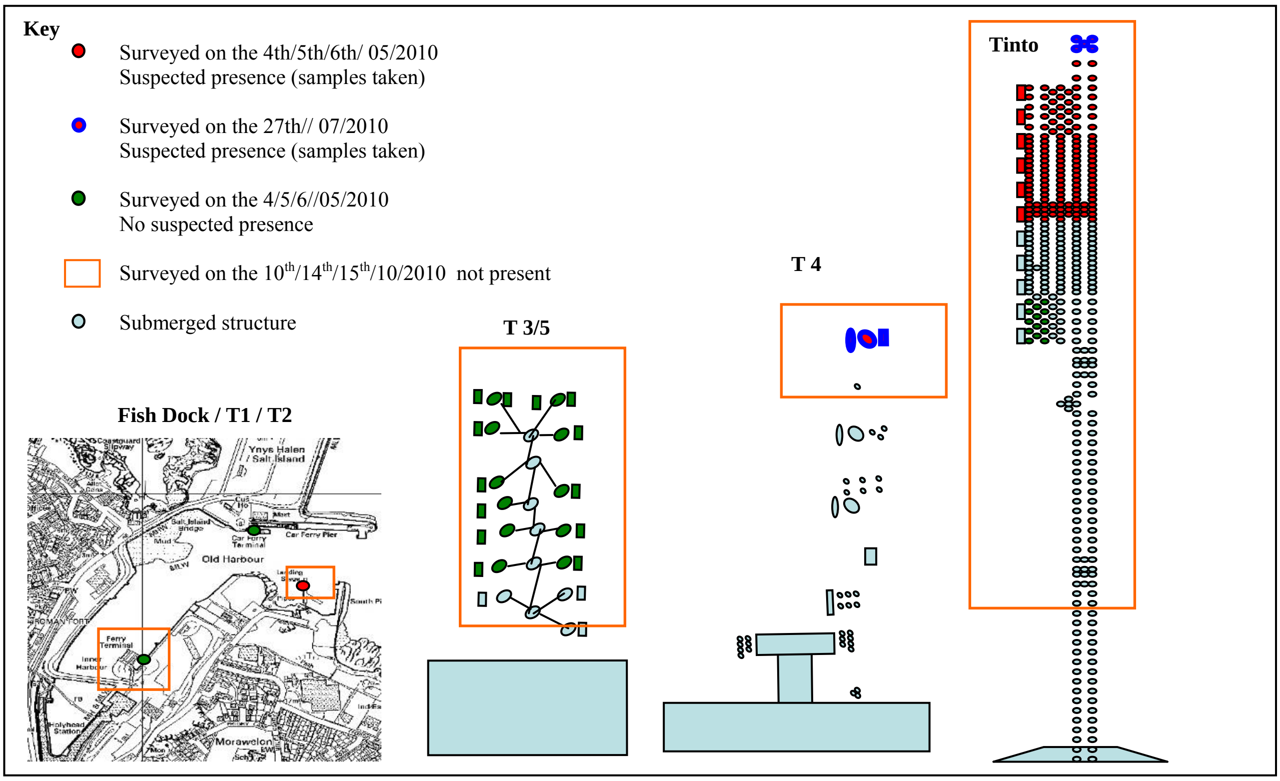



**Figure 43** Map of Holyhead diving survey locations, outcomes and dates.

## 5.3 Physical environment

### *5.3.1 Salinity*

Salinity data collected by (Jenkins *et al* 2010) in Practical Salinity Units (PSU) between the 05/08/2009 and 17/01/2010 clearly shows moderate seasonal variation (**Table 9**). Samples taken on 38 separate days displayed a range of 28, a maximum of 38 and a minimum of 15. Holyhead's average salinity (33) is comparable to that of the surrounding Irish Sea (33) and is likely to be maintained at this level much of the time by tidal circulation in the harbour and dropping or rising in response to extreme weather events only. Pulses of freshwater from the small river, just south-west of the marina, reduce salinity (e.g. 27/11/2009) and in the absence of wind a thin layer (approximately 10 cm) of brackish water will briefly remain on the surface within the confines of the marina with apparently little effect on *D. vexillum*.

**Table 9** Holyhead Marina salinity data in PSU between 05/08/2009 to 17/01/2010**.**

| Date | Reading | Date | Reading |
|---|---|---|---|
| 05/08/2009 | 35 | 25/11/2009 | 30 |
| 06/08/2009 | 38 | 26/11/2009 | 35 |
| 07/08/2009 | 32 | 27/11/2009 | 28 |
| 09/08/2009 | 34 | 28/11/2009 | 35 |
| 13/08/2009 | 34 | 03/12/2009 | 33 |
| 14/08/2009 | 35 | 04/12/2009 | 35 |
| 15/08/2009 | 33 | 05/12/2009 | 35 |
| 16/08/2009 | 33 | 06/12/2009 | 32 |
| 19/08/2009 | 30 | 09/12/2009 | 32 |
| 20/08/2009 | 30 | 10/12/2009 | 15 |
| 21/08/2009 | 33 | 11/12/2009 | 30 |
| 22/08/2009 | 35 | 12/12/2009 | 29 |
| 23/08/2009 | 35 | 17/12/2009 | 33 |
| 24/08/2009 | 34 | 23/12/2009 | 35 |
| 26/08/2009 | 33 | 07/01/2010 | 33 |
| 28/08/2009 | 32 | 08/01/2010 | 37 |
| 05/09/2009 | 33 | 09/01/2010 | 38 |
| 06/09/2009 | 34 | 17/01/2010 | 35 |
| 12/09/2009 | 34 | | |
| 13/09/2009 | 32 | | |

### *5.3.2 Temperature*

Near-surface temperature data was collected using a Vemco Minilog 12-TR 16k temperature logger. This was placed on pontoon C32 suspended on a weighted line 60 cm below sea surface, recording temperature every three hours from the 25$^{th}$ September 2009 to the 15$^{th}$ February 2011. The data shows a maximum temperature of 18˚C in late September 2010 and minimum temperature of 2˚C in December 2010. **Figure 44a and 44b** show minor diurnal temperature differences of around 1 ˚C superimposed on other patterns related to seasonal influences and the spring-neap tidal cycle.

2009-10 eradication year

In 2009 average temperatures did not drop below the theoretical 11˚C threshold for larval production (Kleeman 2009) until early December and persisted for 170 days (based on averaged temperature data). However, Coutts' (2010), our field observations and (Jenkins *et al* 2010) settlement panel data suggests that the cut off for larval production is lower at 8˚C. Using this assumption, the window of opportunity for conducting the eradication, when larval production is at its lowest, was only 109 days between the 11th December 2009 to the 07th April 2010 based on average temperatures (**Figure 44a**). Note, however, that temperatures only consistently stay beneath 8˚C between late December and mid March. Using temperature maximums this window is even smaller at only 101 suitable days between the 29th December 2009 and the 25th March 2010.

2010-11 comparison

In comparison the 2010-11 winter started much earlier with sea temperatures dropping below the 8˚C threshold on the 26th November and remaining below this level until mid February, corresponding with the heavy and prolonged snow falls leading up to Christmas 2010. Despite 2010 having some sharp temperature drops in mid December and late January, the average daily temperature for the winter period has remained near to 8˚C since November (**Figure 44b**). In terms of a window of opportunity for an eradication attempt this is a much shorter period compared to the previous winter with only 81 days. This stresses the importance of regular monitoring to identify suitable times to conduct an eradication programme.

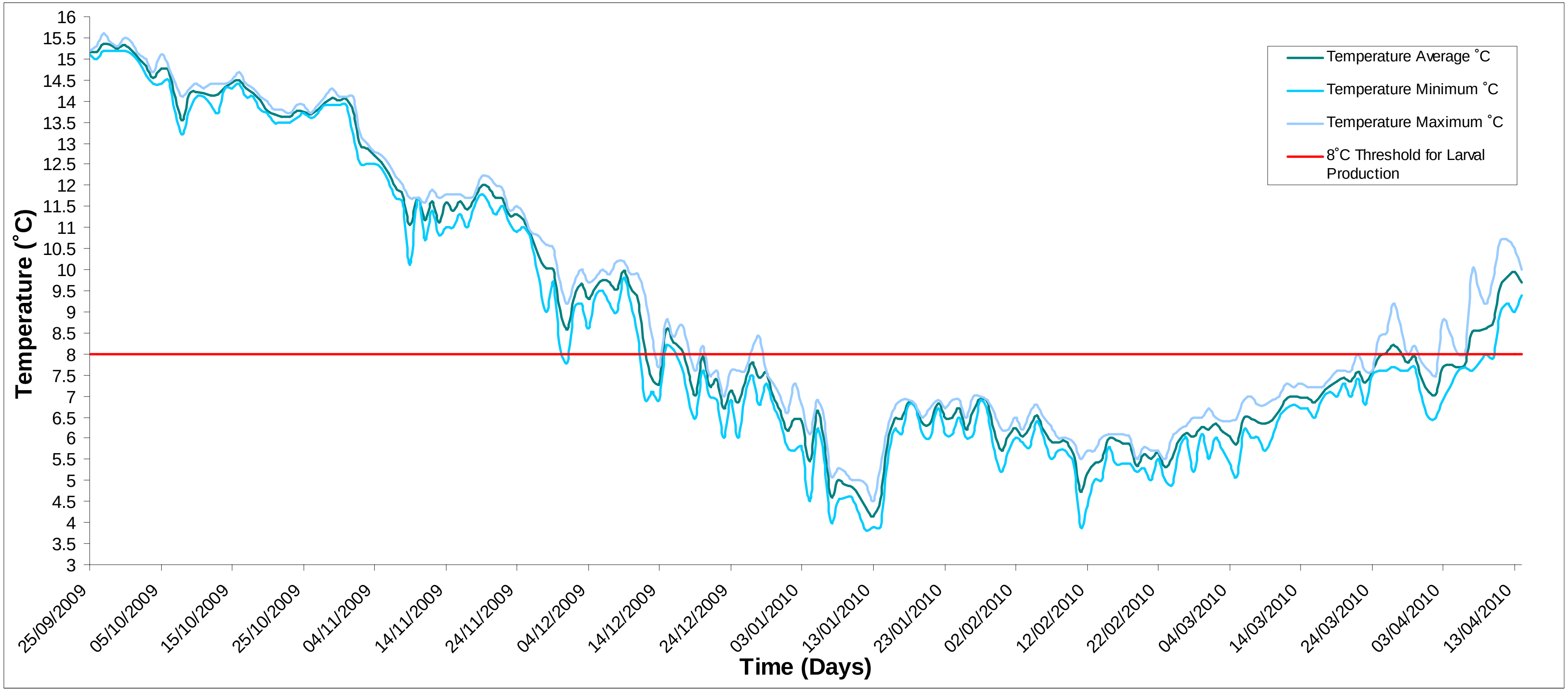



**Figure 44** A, Minimum, maximum and averaged temperature data from Holyhead Marina. (25/09/09 to 13/4/10).

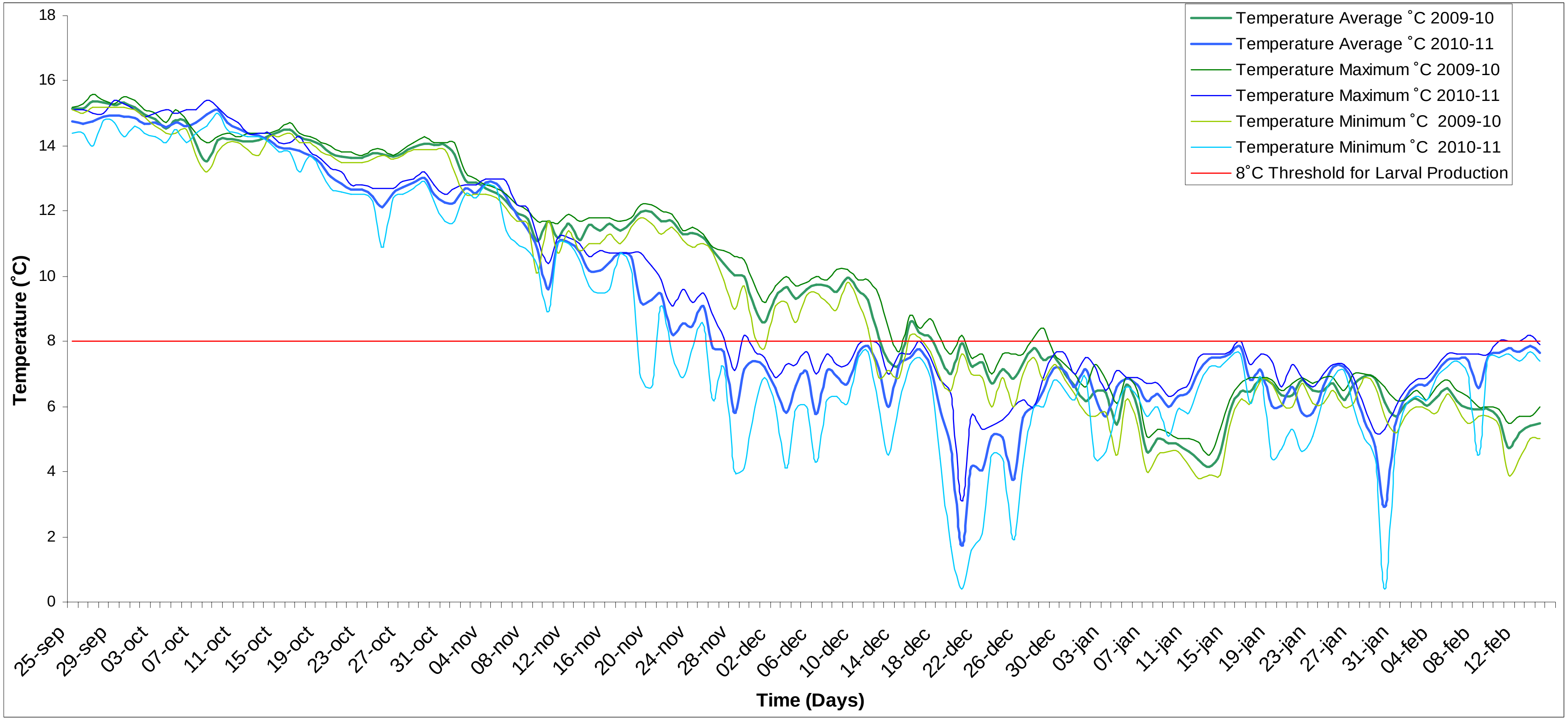



**Figure 44** B: Comparison of averaged temperature data from Holyhead Marina from 25/09/09 to 15/2/10 and 25/09/10 to 15/2/11

# 6 DISCUSSION

## 6.1 Ecological impacts

The highly recoverable nature of habitats in the marina and the lack of any endangered or protected species/sites, suggest the long-term effects to biodiversity from the eradication programme are less damaging than allowing *D. vexillum* to escape into the wider marine environment.

## 6.2 How successful was each type of treatment?

The initial results of the eradication were very promising. As with other similar eradication attempts around the World (Coutts 2007; Pannell and Coutts 2007; Kleeman 2009), we have found that isolating and smothering the biota using waterproof bags and wraps effectively kills most of the flora and fauna including *Didemnum vexillum* by inducing a stagnation reaction that results in anoxic conditions within the enclosed water layer. Introducing a chemical agent (in our case calcium hypochlorite) as a 'toxic shock' treatment speeds up the killing process so that structures can be treated within a few days. The most obvious effect of the treatment was bleaching of algae and this was used as a visual indicator to confirm that treatment had been carried out – in particular sugar kelp (*Saccharina latissima*) and all fine, filamentous reds and browns appeared white or disintegrated completely. Limpets (*Patella vulgata*) at or just above the waterline became moribund and easily dislodged, and all large solitary sea squirts such as *Ascidiella aspersa* and *Ciona intestinalis* had lost their turgidity and disintegrated on touch. The non-native sea squirt *Styela clava*, which has a very tough outer tunic, was also effectively eradicated. Where any sign of *D. vexillum* could be found post-treatment the structural integrity of the animal had broken down and bacterial/fungal growths quickly caused complete degradation.

One of the few animals to occasionally survive the treatment was the mussel *Mytilus edulis* - a small proportion of which remained alive when the rest of the biota had gone. Presumably they were able to tightly close their shells and in the low temperatures during the eradication programme their respiratory and energetic requirements were sufficiently low to survive remaining closed for several days.

Failure of the integrity of the bags and wraps was the most common cause of localised reduction in effectiveness of the treatment. This occurred either through damage to the bags and wraps - wave action and abrasion creating holes in the material or tearing seams apart – or ill-fitting bags overtopping or leaking at junctions and Velcro seals. Where any damage or reduced effectiveness was noted the bags were replaced and the treatment repeated. The most successful treatments were on the simple-shaped small finger pontoon floats where failure rate was low (less than 5%) and mainly related to the intensity of wind and wave action. Similarly the 'set-and-forget' plastic wrappings around the submerged mooring chains were normally very effective but failed at localised abrasion points where chains chaff against one another on the rise and fall of the tide.

Attaining a near watertight seal was more difficult where chain attachment points had to be accommodated into the larger breakwater and walkways bags. The bags with Velcro or ratchet strapped seals had to be examined carefully by the divers after fitting and polythene sheeting used to double-wrap areas where water would otherwise flow freely in or out of small gaps in the bags. To accommodate for likely flushing or signs of incomplete treatment (e.g. un-bleached kelp) these bags occasionally received a second dose of calcium hypochlorite. Wind and wave action also significantly influenced the effectiveness of treating these larger structures.

## 6.3 Why did the overall eradication programme fail to eliminate *Didemnum vexillum* completely?

There are various hypotheses as to why the marina structures were re-colonised by *D. vexillum* within a few months of completing the treatment. These are outlined below with an assessment of the most likely causes.

#### *6.3.1 The marina was re-colonised by a newly arrived colony*

Although it is possible that newly arrived individual colonies or larvae have arrived from another location via a visiting boat's hull, the rate of spread and the wide and fairly even distribution of the re-established colonies suggest that there was no point source for the re-inoculation. It is also possible that multiple vessels have arrived with *D. vexillum* on their hulls but to do so un-noticed is unlikely as incoming vessels were regularly inspected and none were seen to be carrying a significant fouling burden.

#### *6.3.2 The marina was re-colonised by larvae or fragments originating from an unknown source close to the marina.*

There is no shortage of alternative substratum for *D. vexillum* to colonise in Holyhead Harbour. Man-made harbour walls, shipping jetties, abandoned or neglected vessel hulls and rip-rap are all close by and all these support similar suites of species as the pontoons. However, repeated and detailed surveys of these areas have been made at various times of year – including during late summer when the *D. vexillum* colonies in the marina are large and very obvious – and nothing has been found. It would appear that the marina itself and a few adjacent mooring chains are the only location for this species. The reason for this apparent confinement is not clear, but it is possible that local conditions are not suitable for *D. vexillum* to thrive on hard substrata subject to normal tidal regimes - although it does elsewhere (see Bullard *et al.* 2007 and Kleeman 2009 for an overview on its habitat preferences) - or it simply has not had time since the original inoculation to reach these areas.

#### *6.3.3 The marina was re-colonised by fragmented colonies broken away from the pontoons during treatment.*

The fragile lobe-like growths that *D. vexillum* produces as it matures can effectively re-attach if they become dislodged (Bullard *et al*. 2007). Most of the *D. vexillum* colonies observed in the marina formed epifaunal plaques on native solitary sea squirts such as *Ascidiella aspersa*, *Ciona intestinalis* and the non-native *Styela clava,* as well as forming loosely attached crusts on pontoon and chain surfaces, and were vulnerable to being dislodged when the bags and wraps were being fitted. *D. vexillum*-coated native solitary sea squirts growing on redundant ropes, old tyres, fenders and lines hanging from the pontoon sides were even more prone to being dislodged.

Observations of solitary sea squirts experimentally dislodged or dropped into the water column showed that they would invariably sink to the seabed rather than float away near the surface. The seabed around virtually the entire marina is soft mud with a shallow anoxic layer and is unsuitable for long-term survival of native sea squirt species as well as *D. vexillum*. Algae such as *Saccharina latissima* with *D. vexillum* overgrowing it was also observed to sink towards the seabed in most cases. The marina being an 'island' of suitable hard substratum surrounded by soft mud has probably played an important role in the prevention of this species spreading by natural means.

#### *6.3.4 The marina was re-colonised by larvae from untreated sections moving to freshly treated sections.*

Experimental studies on the production and distribution of *D. vexillum* larvae (Kott 2002; Valentine *et al.* 2009) suggests that they are short lived in the plankton – only a few hours – and do not travel more than a few metres. The literature suggested that larvae are only produced when water temperatures are above a threshold of approximately 11 degrees (in Kleeman 2009). Various assumptions were made during the eradication programme based on this information - particularly that *D. vexillum* was not producing larvae during the winter period when the bulk of the work was being carried out although there is evidence that small numbers of larvae might be being released during the colder parts of the year (Coutts 2002b).

In retrospect it would seem that re-settlement of larvae is the most likely cause of re-colonisation around the marina. The evidence for this comes from monitoring the pontoons post-treatment in the late summer of 2010 when large numbers of well scattered, small and rapidly growing colonies were located. Although microscopic post-larval colonies were probably missed in late winter and early spring 2010 the pattern of re-colonisation by *D. vexillum* approximates to the progression of bagging and wrapping around the marina. It appears as though the freshly treated surfaces were ideal habitat for larvae to settle which then remained 'dormant' and virtually invisible amongst the re-colonising floral and faunal turfs until the water warmed later in the summer. The failure of the initial monitoring technique to resolve microscopic post-larval colonies in early spring 2010 could be attributed to poor water clarity and the original photographic techniques used which were later improved by using a close-up high definition video camera and cutting the kelp canopy back to provide an uninterrupted view of the underlying substratum.

# 7 RECOMMENDATIONS FOR FUTURE ERADICATION ATTEMPTS

This section discusses the possibility of making improvements to our original programme, described in the methods and results sections, to take into account the variable risk of larval or fragment transfer at virtually all times of year. This mainly involves improvements in the quality assurance and intensity of the techniques but also needs to consider re-designing some of the wraps and equipment. These measure may involve unavoidable increases in cost and labour-intensity, although savings may be made through economies of scale when sourcing bags and wrapping material.

### *7.1.1 Sea temperature must be low*

Only carry out the eradication when seawater temperatures are at their lowest and larval production has ceased or is at a minimum for the winter.

It is unlikely that *D. vexillum* produces many larvae when water temperatures drop below 8 or 9 degrees (Valentine *et al.* 2009; Jenkins *et al.* 2010). From temperature logger data recorded in Holyhead Marina during the 2009-2010 winter this occurs approximately between January and the end of March – although this could vary inter-annually. Our temperature logger data for the 2009-2010 winter gives a window of approximately 100 days at less than 8 degrees, although this being an atypically cold winter, future attempts might have an even shorter window of opportunity when risk of larval transfer is at a minimum.

### *7.1.2 Monitoring*

Continue regular monitoring for larval settlement and reproductive potential.

Settlement plates should be used to monitor for freshly settled larvae or juvenile colonies and these should be inspected regularly (Jenkins *et al.* 2010 study adopted a two week cycle) and positioned to 'catch' larvae originating from anywhere in the marina. Beware, though, that settlement plates will not catch all larvae and can lead to underestimates of their abundance (Martin *et al.* 2010).

Treated surfaces should be monitored carefully on a regular basis in sufficient detail to spot newly settled colonies, although it may be necessary to sub-sample representative sections of the marina and its different structures.

Examining samples of mature colonies for larvae within their brood chambers would help determine the reproductive potential at the time of year, although the source of mature colonies will diminish as the eradication process progresses.

### *7.1.3 Treated surfaces must not be left uncovered adjacent to untreated surfaces.*

The initial eradication attempt involved deploying a limited number of bags and wraps across the marina in multiple waves during the seven months of field work. This should be avoided by reducing the number of waves to a minimum within the three month temperature window. Ideally all structures should be covered at once, but this would probably be impracticable through factors of cost, lack of time to produce a full set of bags and wraps, insufficient numbers of suitably qualified personnel and lack of suitable weather windows. If more than one wave of deployment is intended then a 'fire-break' of covered pontoons and chains must be maintained to separate the treated and uncovered surfaces from the untreated 'infected' structures. The width of this fire-break should be at least the width of one of the main walkways which means, for example, that to treat the marina in two waves would require considerably more than 50% of the pontoon structures covering at once – including covering more than half of the large floating breakwater sections as well as most of the chains.

Larval dispersal might have been accidentally augmented through the process of pumping out the bags immediately after fitting. Although we have no evidence as to whether this occurred or not it would be prudent to improve filtration on the pumps' seawater outflow so that as the excess water is pumped out of the bags prior to dosing with calcium hypochlorite any free larvae are not jetted back into the marina. For this to be effective a fine mesh filter array is required. Coutts (2002) developed an underwater vacuum cleaner for scraping *D. vexillum* off pontoons and hulls, and although the method did not remove 100% of the sea squirt their work on the filtration of the effluent produced by this method suggested that they could prevent any larvae, which have a trunk diameter of 300 µm, escaping back into the environment. They tested an array of filters that included a coarse sacking layer then 200, 100, 50, 25, 10, 5 and 1 µm meshes and predicted that the 50 µm mesh in the third stage of filtration would be adequate.

### *7.1.4 Air drying and experimental trials on the efficacy of antifouling paints.*

Treating the marina structures using divers and bagging and wrapping techniques *in-situ* is both labour intensive and expensive but largely unavoidable due to the design and layout of the marina. Air-drying, involving complete removal of the smaller components of the marina, taking them well above the shore line and allowing them to dry for a few days (and perhaps jet washing to speed up the process) could be more cost-effective than *in-situ* treatments but will not work for the larger floating breakwater sections which are too heavy and large to remove using on-site facilities at Holyhead marina. The finger pontoons at each berth could be treated this way but will need careful, slow-speed transportation to beach them at high water and at the same time avoid fragmentation of *D. vexillum* colonies.

Once dried and cleaned these smaller units would make ideal test panels for antifouling paints which could help improve biosecurity by reducing the risk of re-colonisation (see section 6.4.4 on improving biosecuirity and marina design).

As a general note to anyone attempting an eradication programme at other marinas we suggest that the first line of 'attack' would be to investigate removing sections for air drying as this would reduce the need for expensive *in-situ* treatment. The ideal time for removing sections of marina is during the winter when many boat owners will be have their boats on hard standing preparing for the next year's use. See also section below on marina design and biosecurity.

*7.1.5 Improving bag and wrap design.*

The suite of eradication bags described in the materials and methods sections varied in price per $m^2$ coverage. The cheaper, simpler designs appeared to just as effective as the more complex, heavy duty bags with floatation chambers although none of the materials could cope with the wave action induced by north-easterly gales. For any subsequent wave of eradication we would recommend opting for simpler, cheaper designs without floatation chambers but with greater freeboard (i.e. sufficient material to draw the bags up the level of the walkways), and plenty of attachment points for hooking the bag to the pontoon superstructure. More emphasis should be placed on ensuring that the bags seal better at the junctions between the chains and the pontoon floats and that these are easier to put in place underwater by divers.

Most of the bags used during the first eradication attempt were intended for multiple deployments. The large breakwater and walkway bags were deployed at least five times and had to be regularly customised to fit the different shaped pontoon structures and chain configurations. The smaller, cheaper finger pontoon bags were often too badly worn or holed to cope with re-deployment after around four or five uses. If the next stage of the eradication were to be completed in only two or three waves the durability, weight and need for continual reshaping could be reduced and thereby reduce cost per unit. Bags should also be designed for a one-off fit on the more complex pontoons where multiple chains, added buoyancy blocks and marina service ducting can be incorporated into the designs – for example at the RNLI and fuelling berths.

## 7.2 Biosecurity

Protecting the investment put into the eradication of a marine species by preventing re-colonisation requires long-term commitment in terms of improving and maintaining biosecurity and the consequent requirements of sustaining budget and staffing. The topic of pathway management for *Didemnum vexillum* is dealt with in detail in a report prepared by the *Didemnum vexillum* GB working group (Recommendations for Reducing the Rate of Spread and Potential Re-Invasion of *Didemnum vexillum*). Some of the site-specific issues are dealt with below.

*7.2.1 Legislation to prevent the spread of non-native species.*

Article 22 of the Habitats Directive regulates the deliberate introduction of non-native species into the wild so as to protect natural habitats and native flora and fauna. However, in this case *D. vexillum* is arguably a non native species that is already present as a result of non-deliberate action by parties unknown, therefore this should be seen a management response need to an environmental threat rather than a regulatory issue. The Habitats Directive cannot therefore be used to prevent, for example, yachts with *D. vexillum* on their hulls leaving the marina.

There is no specific requirement in the Habitats Directive (other than Article 22) to take action to protect the environment or biodiversity generally. But if the spread of *Didemnum* could conceivably pose a threat to any N2K site, the obligations in Article 6(2) could justify treating the need for eradication as a 'biological emergency' and accelerate various bureaucratic process such as contract letting: "Member states shall take appropriate steps to avoid....deterioration of natural habitats..." in SACs and SPAs. If feasible and proportionate steps were not taken to protect N2K sites – in this case take prompt and reasonable action to control / eradicate *D. vexillum* - and damage occurred as a result, WAG could conceivably be in breach of Article 6(2).

*7.2.2 Pathway management*

Vessels arriving from contaminated marinas with a high fouling burden, whether *D. vexillum* is confirmed amongst the attached biota or not, should be removed from the water as soon as possible for cleaning in a closed-loop or hard standing washdown facility and antifouled. A 'clean hull policy' can be legitimately enforced by the host marina staff who can also assist in this respect by keeping records of ports of origin for all visiting boats and reporting any boat suspected of harbouring *D. vexillum* to CCW for rapid inspection. While Holyhead Marina has a resident *D. vexillum* population, they must also consider the potential of exporting the problem to other marinas and the wider marine environment. Any vessel that has not moved for a prolonged period and is in obvious need of hull-cleaning and antifouling should be prevented from leaving the marina until this has been carried out. See quarantine section – below. Of the marinas and popular boating areas in Wales there are several listed by Somerwill (2011) as being at risk of colonisation by *D .vexillum*. These are the Menai Strait and Pwllheli marina in Gwynedd and Nayland and Milford Haven marinas in Pembrokeshire. Control measures to prevent the incursion of *D. vexillum* and the spread of non-native species generally should be instigated as a matter of urgency.

One location that has escaped consideration as being at risk so far is the naturally sheltered embayment at Porth Dinllaen on the north side of the Llyn Peninsula. During the summer this is a popular weekend stop-over anchorage for yachts normally based in Holyhed. The eelgrass *Zostera marina* beds in Porth Dinllaen are of high conservation importance and similar habitats have been recently been found to support *D. vexillum* in New England (Carman and Grunden 2010).

An Australian government initiative – The National System for the Prevention and Management of Marine Pest Incursions (see http://www.marinepests.gov.au/) – produce management guidelines for recreational vessels that put the above principles into action. These include boat owners having to carry appropriate paperwork as evidence of having fresh antifouling, records of journey details (log book) and the need for vessel inspections before being issued a clearance certificate to enter important conservation zones such as Darwin. They also manage vessel movements and hull cleaning in response to specific pest outbreaks or eradication attempts. If a vessel's hull, bilge or cooling water is found in need of treatment in the Northern Territories (NT) this is carried out at government-run cleaning stations at the expense of the NT.

### *7.2.3 Quarantine*

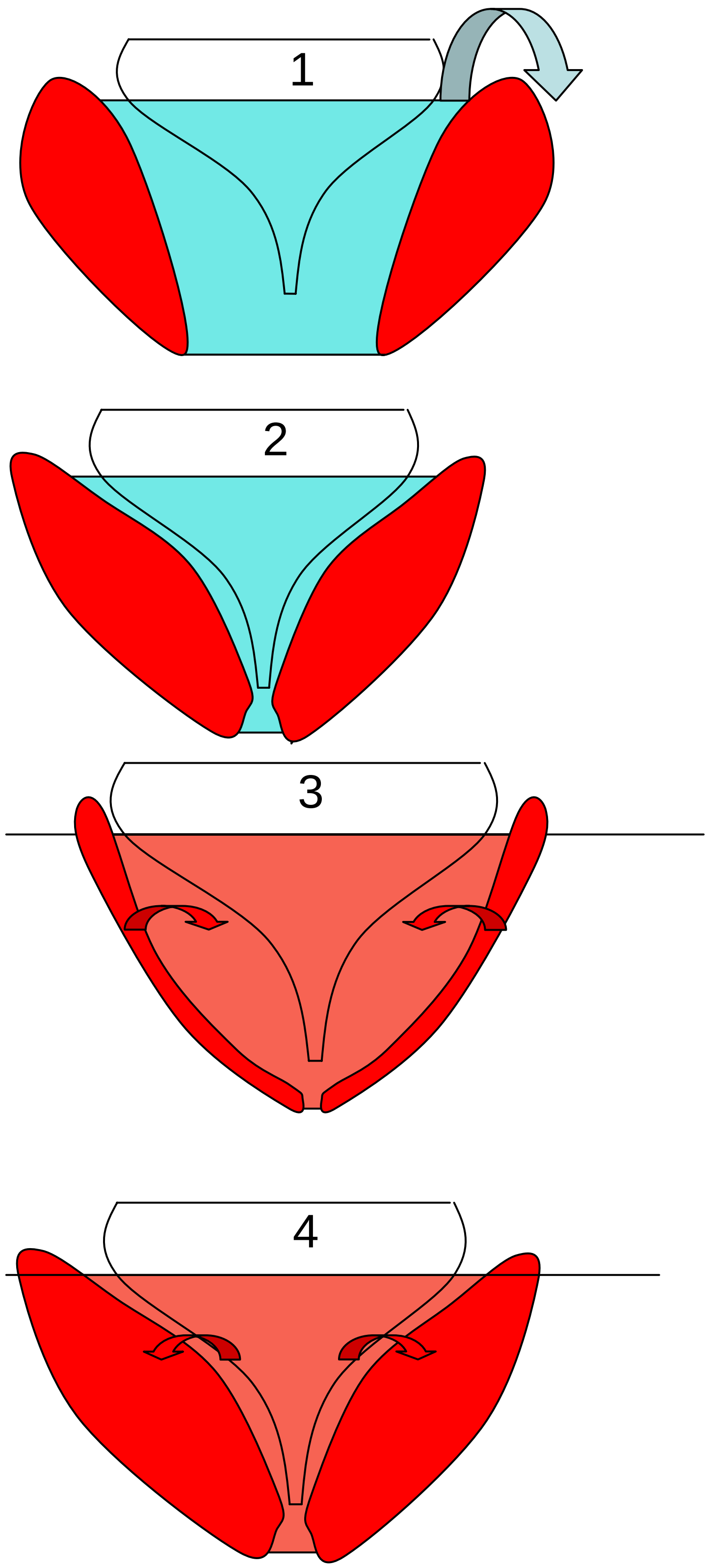
In an effort to reduce the risk of importing or exporting marine non-natives some form of quarantine system could be arranged in the marina. The aim of a quarantine berth would be to isolate and treat any vessel that may be carrying *D. vexillum* or any other invasive non-native on its hull and thereby prevent further spread either within the marina or exporting it elsewhere. Quarantine should not be seen as a cheap and convenient alternative to periodic hull cleaning and antifouling – more as an effective way of killing this species on the hulls of vessels considered at risk.

Ideally the quarantine process should be quick, reasonably priced (or at no cost to the boat owner) and easy to run; it should be able to deal with the majority of hull shapes and sizes expected in the marina and not seen as a deterrent to visiting the marina.

Plans for creating a quarantine berth in Holyhead Marina are under development. The following figures (**Figures 45-47**) represent two of the concepts under consideration at the moment.

Wet dock

The wet-dock quarantine berth comprises a double-skinned bag sufficiently large to accommodate any of the regular vessels. A shock-treatment chemical (acetic acid / bleach etc.) is contained in the bladder between the two skins of the bag. The advantage of this design is that the chemicals can be recycled between each treatment with only a small drop in concentration and a limited amount being released to the environment at each cycle. There would be a need for some form of hull inspection (e.g. small video camera on a pole) to compliment this method.




1. Vessel enters the quarantine dock and a flap-door closed behind it. Sea water is pumped out of the dock via a fine mesh filter to prevent larval transfer.

2. As the sea water is pumped out, the bag deforms to the shape of the hull.

3. The chemical treatment is pumped out of the bladders into contact with the vessel's hull. Duration of treatment depends on concentration of chemical, degree of fouling etc.

4. Once treatment is completed the chemical is pumped back into the bladders for the next treatment cycle. The dock is then opened and the vessel departs.

**Figure 45** Cross section view of the proposed 'wet' quarantine dock

One of the disadvantages of this system is the need for periodic disposal of depleted chemicals which might require specialist disposal rather than being released into the sea at the end of its useful life. A flexible wet-dock, even if built from heavy duty PVC coated fabric, might still be at risk from puncturing or damage in heavy wave action.

Dry dock

Various designs of 'personal' floating dock have been in production for a number of years (e.g. Hydro Hoist see http://www.boatlift.com/; Hydrodock International see http://www.hydrodock.com/index.html; Sunstream Float Lift see http://www.sunstreamboatlifts.com/floatlift.htm and image below) to keep mainly motor vessel's hulls dry and free of fouling organisms when not in use but conveniently ready to launch in minutes. There are many other advantages to the boat owner – including reducing the need for painting, far less corrosion to engine parts, ease of maintenance etc. Their operating principle involves having a cradle supported by floodable pontoons that are filled with air from a small compressor to raise the craft out of the water as required. Although perhaps beyond the financial scope of many boat owners such a device, or large scale 'commercial' version of this principle may be financially viable for a marina / agency to run as a service (see http://sealift2.com/Brochures/Sealift2-SD%20Shallow%20Draught%20Draft%20Dock%20-%20Data%20Sheet.pdf and **Figure 47** for the Sealift floating drydock system). The disadvantage of such a system is that if an invasive non-native is found on a hull it cannot be simply jet washed off into the surrounding water unless there is some form of curtain or catchment system included in the design, although high strength bleach sprayed on to the hull should be very effective at killing biota (Denny, 2008) without risk of fragmentation of the sea squirts or damage to the hull of the boat. There is also the risk of the floating dock itself harbouring non-natives, although this can be minimised by following basic house-keeping steps to keep it clean particularly if needing to translocate it to another marina.


**Figure 46** Photo: R. Holt. *Sunstream* boat lift supporting an rigid hulled inflatable at Deganwy marina, Conwy.

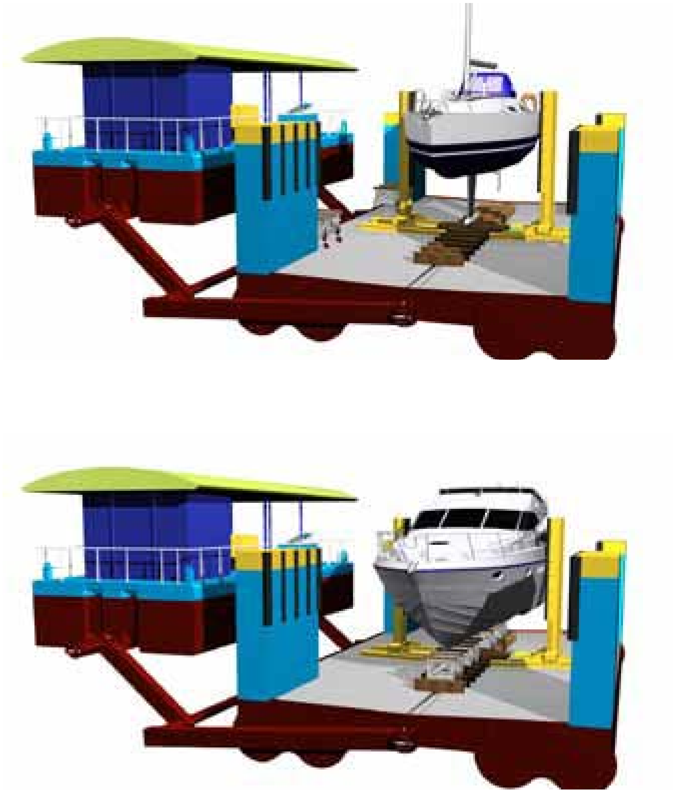


**Figure 47** *Sealift 2* – shallow draft floating dry dock that can accommodate keeled yachts and motor vessels. Images from http://sealift2.com

***7.2.4 Biosecurity and marina design***

Rather than expensively attempting to treat invasive non-native species once they have colonised an area, steps may be taken to build biosecurity measures in to a marina at the planning stages. This is specifically mentioned here as large-scale redevelopment plans have recently been unveiled to place a large marina adjacent to the existing marina in Holyhead Harbour (http://www.holyheadwaterfront.co.uk/).

Artificial substrata seem to favour colonisation by non-indigenous species (Tyrrell & Byers, 2006) and with a large increase in man-made structures in the water close to the existing marina it would seem logical to expect a high risk of transfer of *Didemnum vexillum* from one to the other.

The design of some marinas may even aggravate the problem. Floerl *et al.* (http://www.reef.crc.org.au/publications/explore/feat53.html) working on Australian marine non natives have found that breakwater walls create circular eddies that retain water for much longer than marinas without breakwaters. This can lead to an entrapment of the planktonic larvae of fouling species within enclosed marinas. Rates of recruitment by fouling organisms were found to be between two to nine times greater in these enclosed environments than in unenclosed marinas, and between three to 19 times greater than in adjacent coastal environments.

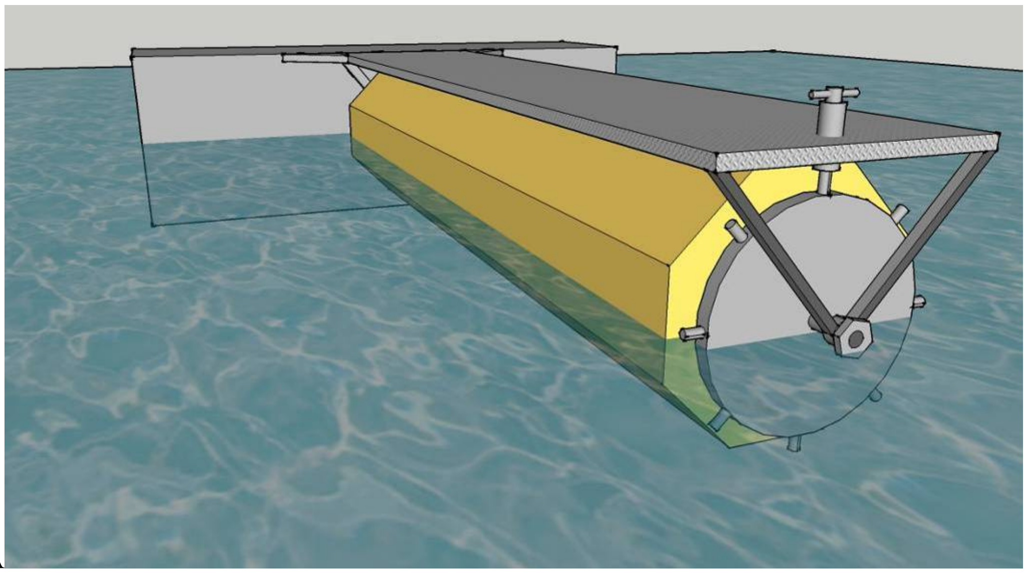
These results have major implications. For boat owners, they suggest that maintenance will be required more often in marinas with poor tidal flushing, because fouling is considerably greater in these environments. Second, because water circulation in enclosed marinas appears to trap marine larvae for a significant period of time, the spread of established introduced species from the marina may depend more on patterns of vessel movement than on larval dispersion by ocean currents. The outline plan for the new marina in Holyhead incorporates a large stone-built breakwater inside the existing harbour wall.

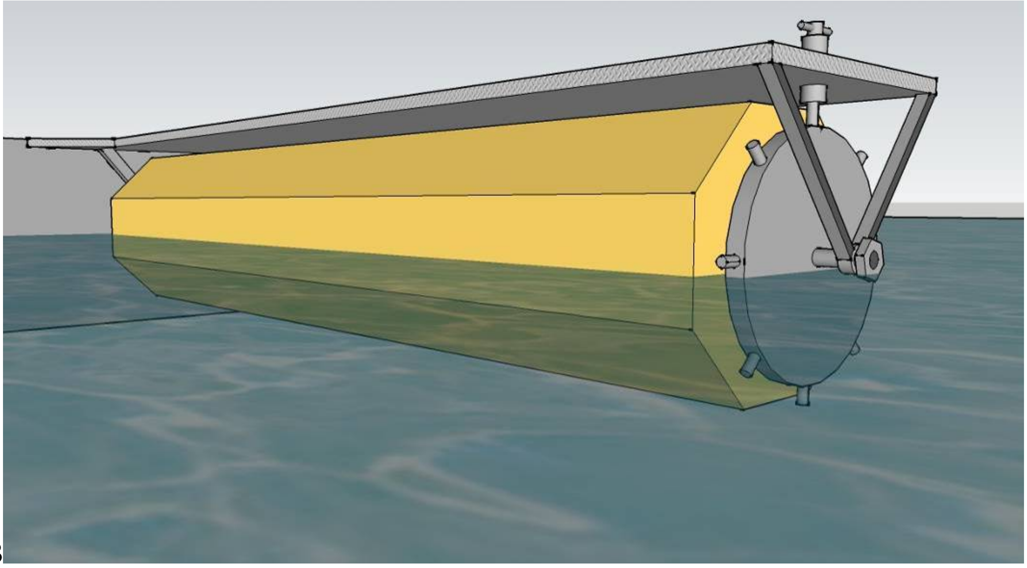
Of the marinas investigated for the presence of *D. vexillum* around the whole of Wales the least likely to become colonised appeared to be Aberystwyth. Not only is the water largely riverine and very low salinity but also, anecdotally, contains high level of heavy metals from mine workings in the catchment area. Although, reproducing the water quality conditions experienced in Aberystwyth might not be achievable (or allowable) it may be possible to direct a naturally occurring freshwater source into a semi-enclosed marina. Keeping the salinity down to 20 ppt or less has been shown to kill off *D. vexillum* after exposure of more than two weeks (Bullard and Whitlatch, 2009), but will not prevent other brackish-tolerant non-native species such as the invasive 'killer shrimp' amphipod *Dikerogammarus villosus* from establishing which thrives in salinities below 10 ppt and survives at 20 ppt (Bruijs *et al*. 2001).

#### *7.2.5 Air drying as a means of antifouling*

Harnessing freely available resources such as freshwater and air are perhaps the most sustainable means of dealing with invasive non-natives in the long term – particularly those that have established a permanent population in the marine environment but need to be contained and controlled locally. We have shown that air drying small sections of pontoon is a particularly effective means of killing *D. vexillum*. If a means of periodically air drying section of marina could be incorporated into the design at a large scale, even if this raised the build-cost, this could make considerable savings in the long term.

By using rotating pontoon floats (Figure 6.4) instead of fixed floats, the submerged portions can be brought above the waterline on a regular basis thereby keeping fouling to a minimum. The speed of rotation can be varied but most fully marine species cold not survive being out of the water for more than a few days, particularly in warm, dry weather. The following drawing shows how this concept may take shape and could possibly form the basis of an MSC/PhD style project. On the example shown in the drawing we would suggest a rotation rate of one 'notch' every two week would probably suffice to bring the submerged portion of the float above the waterline. Whether this idea could be effectively and economically scaled up so that larger walkways could be supported by multiple rolling pontoons is worth investigating, and perhaps make use of available materials such as using foam-filled drums as floatation pontoons. Designs could include automated mechanisms to rotate the pontoons, driven perhaps by harnessing low-amplitude wave action in the marina.

A




B

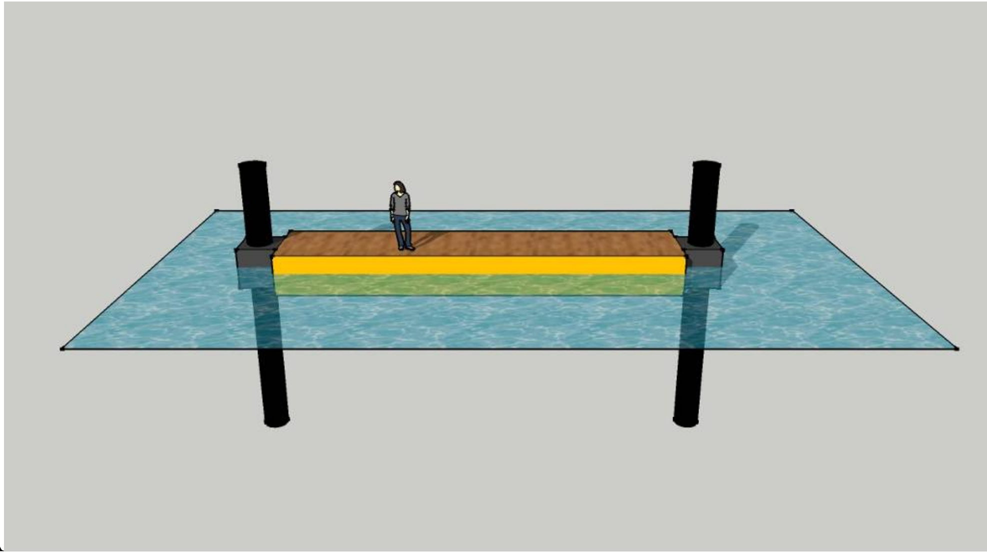
**Figure 48** A & B. Concept drawings of a rotating ‘finger’ pontoon. Note that the axle needs to be just above the waterline to prevent fouling at each end.

Air drying the larger structures in the marina *in situ* is more of an engineering challenge and, although not possible in the existing marina, could be built-in to a new design or achieved where the pontoons are held in position on pilings instead of chains. The following drawing (**Figure 49**) illustrates the principle whereby a high water of a spring tide is used to float a walkway pontoon to its highest position. It would then be locked in place and the tide allowed to drop beneath it leaving it suspended. The pontoon must be of sufficient strength to bear its own unsupported weight and the pilings also capable of load bearing.

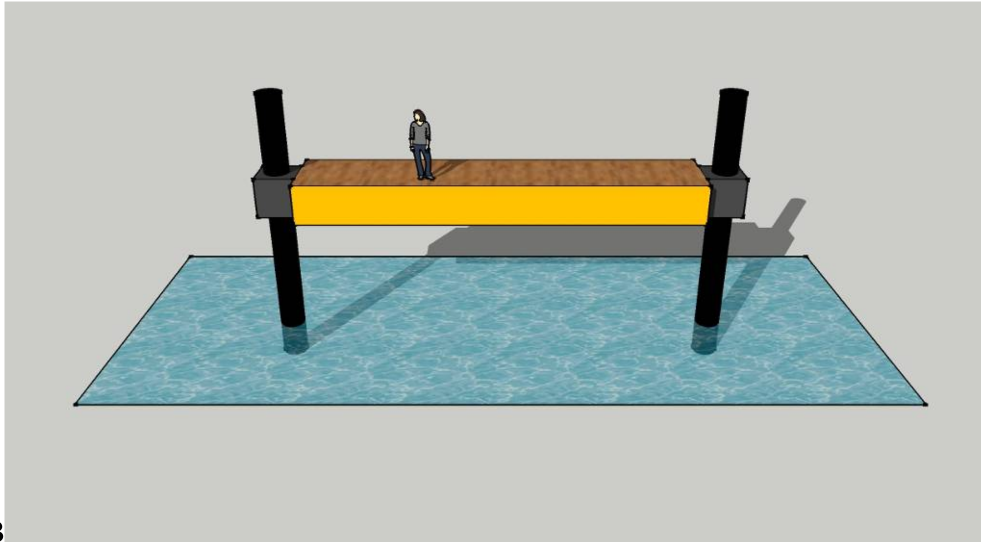
Additional ‘clean’ pontoons or small barges could then be placed underneath the suspended walkway on the next rising tide to keep it above sea level but avoid the need to keep it locked in place. The main limitation of this idea is that any sub-units would have to be removed from the main structure (e.g. finger pontoons at each berth). It also requires pilings to be in place between every section, whereas in reality the frequency of piling is often less and offset to one side of the walkway.

Once securely suspended above the waterline the pontoon could be simply left to air dry for a few days, or if required back in the water more urgently the undersides could be sprayed with a chemical agent such as concentrated bleach which has already been shown to be very effective in killing *D. vexillum* (Denny, 2008).

The pilings themselves are, of course, susceptible to fouling with non-native species and would have to be treated in a similar manner to other submerged structures. If designed into the marina at the construction phase plastic sleeves could be fitted and periodically removed or raised above the waterline without requiring divers.



A



B

**Figure 49** Conceptual drawing showing method of air drying pontoons suspended between pilings. A. Pontoon locked in position at high water. B. Pontoon suspended once the tide has dropped.

***7.2.6 Public awareness and disseminating information***

Raising awareness is fundamental to promoting biosecurity which requires public participation and support as well as funds. Our experiences in this respect during the eradication programme were of particular value and the main points are discussed here.

<u>Poster campaign.</u>

We promoted a 'wanted poster' campaign through the RYA's Green-Blue organisation. The poster (see **Appendix V**) was planned in conjunction with the GB *Didemnum* group - and various versions launched based on which region of the UK it was aimed at (e.g. we had bi-lingual versions and a local 'hotline' phone contact details etc). It was targeted primarily at recreational boat users to inform them of the problem and how to identify *D. vexillum*.

We were surprised by the lack of hotline calls – only one to inform us of a suspect-looking growth on the hull of a boat in Deganwy marina that turned out to be a native sponge species.

Video and YouTube.com

Having had some success with publicising CCW marine monitoring work on the You Tube internet site we downloaded several video clips to describe the organism and the eradication process – see http://www.youtube.com/watch?v=P3YAPLJGuZY .

Similar short video clips were used to report on progress to CCW management and proved to be a very useful tool in conveying large amounts of information very quickly via e-mail without needing to resort to lengthy written accounts. This was achieved using only a compact digital camera (Canon Ixus), on its video recording mode, and simple video editing software included as standard on most Windows-based pc's (Windows Movie Maker). The videos used as a reporting tool were very well received and are now being considered as a model for an alternative means of reporting within CCW.

Press release and media coverage

CCW promoted the start of the eradication programme with a press release which coincided with the You Tube broadcast. This immediately grabbed the interest of the local and then national press, radio and TV. The BBC One Show broadcast went into most detail on the eradication attempt and included underwater footage of interviews with a BBC presenter and the eradication in progress. See Figure 50.

This coverage generated most public interest in the project and from then on we rarely had to explain to visitors what we were doing in the marina. Unprompted, they more often asked how the eradication programme was going and were quite supportive of our efforts and were aware of the scale and nature of the problem we were tackling. The only negative feedback was in the form of 'good luck but you'll never do it'. Many people asked appropriate questions such as 'can I catch it on my boat?' and 'what will it do if it gets out? And a frequently asked question was 'Can you eat it?' In retrospect we would have prepared more hand-out literature and made even more of our Youtube facility while working in the field (e.g. have a small display stand on site) but time and the 'emergency' nature of the eradication action prevented this.

**Figure 50** Miranda Krestovnikoff presenting the BBC's One Show coverage of the eradication process in Holyhead.

# 8 POLICY RECOMMENDATIONS

Coutts and Forrest's (2007) paper 'Development and application of tools for incursion response: Lessons learned from the management of the fouling pest *Didemnum vexillum*' gives a very valuable summary of the steps towards a successful eradication programme and why so many have failed. Their 7 steps are used here as headings to summarise our own successes and shortcomings.

## 8.1 Baseline knowledge and an effective surveillance regime.

Prior to the discovery of *D. vexillum* in 2008 in Holyhead, this species seemed to cause little concern in the UK. Its discovery was almost 'accidental' rather than part of a GB-wide effort to monitor the arrival of or existing invasive non-native species. Since then there have been significant improvements towards national coordinated approach to non-natives (see https://secure.fera.defra.gov.uk/nonnativespecies/home/index.cfm) although no single agency has yet developed a specialisation or responsibility in this field. A regular monitoring programme of the marinas across Wales has been established by CCW, but must be continued into the future.

## 8.2 Clear lines of authority and rapid decision-making.

Treating the initial eradication attempt as a 'biological emergency' gave the project the same status as dealing with an oil spill with regard to the contractual and purchasing processes within CCW. This allowed rapid mobilisation of a field eradication team but this status was dropped after the first year of the project. Although, arguably, the long-term detrimental effects of non-native incursions are orders of magnitude worse than an oil spill in terms of financial and conservation/biodiversity losses, there is still no consistent approach GB-wide to deal with non-natives. We encountered many and various bureaucratic 'brick-walls' with regard to progressing the eradication programme – many of which were there simply because agencies and government departments have never had to deal with such matters with the urgency they require. Adopting a policy of 'shoot first and ask questions later' may be the only way to establish some level of control/containment when dealing with a new incursion which will buy time for further evaluation of the potential threat.

## 8.3 Commitment of sufficient resources to meet project goals.

Lack of clearly targeted funds in a climate of government spending cuts has been the main limitation of the eradication programme. *In situ* working using divers is very expensive – alternatives using land-based resources should be investigated further.

Funding biosecurity and eradication measures in the long-run is an issue that has to be tackled at a GB and preferably international level. Following the Australian and New Zealand models we should be considering stricter measures regarding pathway management which could include installing quarantine facilities at a network of sites throughout the UK. Funding so far has been on an ad-hoc basis to respond to a localized problem and has been sourced from a variety of government and commercial sources. There has been little regard to the future need to maintain a heightened level of vigilance or asking the 'polluter' to contribute in a consistent manner. To date Holyhead Marina have contributed considerable amounts of staff time and effort towards the eradication; CCW and WAG have sourced funding from various departments and the mussel growers, based in the Menai Strait, have offered a contribution towards the programme.

A more 'fair' approach would perhaps be to incorporate a biosecurity levy on leisure craft moorings and marina fees in addition to asking the industry and government to contribute to such measures. This would help fund a GB-wide approach to both monitoring and tackling non-natives which could, perhaps, be based in a government agency such as the Joint Nature Conservation Committee or the Environment Agency.

## 8.4 Proven treatment methods.

With suggested modifications and improvements to the first eradication attempt put in place the chances of a successful eradication in the marina are high. Long-term improvements to biosecurity, incorporating engineering and policy measures, have to be brought into effect to protect the investment put into the eradication.

## 8.5 Buy-in from stakeholders, and incentives for exacerbators to participate in management.

This requires high level cooperation to tackle biosecurity issues at a GB-wide scale. New marina developments must have built-in biosecurity at the planning stage but this will only be effective if all stakeholders take a share of the responsibility in tackling invasive non-native species. This will take a combination of public awareness raising, clean-hull policies and strict and monitored controls on the movement of commercial marine species.

## 8.6 Effective quarantine to prevent spread.

We have proposed solutions to control the spread of this species but these will only work if they are adopted widely. Perhaps the way forward is to fund certain aspects of quarantine and control in much the same way as agri-environment schemes have been run in the past and assist established marinas to set up quarantine facilities.

## 8.7 Effective project management and quality assurance procedures.

If one microscopic *D. vexillum* larva manages to escape and re-colonise a submerged structure then has the eradication process failed? Possibly... particularly if inadequate monitoring allows the newly developing colony to reach its reproductive stage. If many larvae or colony fragments escape the eradication process, or surfaces are inadequately treated and not monitored then the eradication is bound to fail.

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# Appendix I Data Archive

Data outputs associated with this project are archived as Project No. 214 and Media No. 580 on server–based storage at the Countryside Council for Wales.

The data archive contains:

[A] The final report in Microsoft Word and Adobe PDF formats.

[B] A set of images used within the report produced in JPEG format.

[C] A series of GIS layers on which the maps in the report are based.

[D] Associated video clips are also available under media 901, 906, 908, 982, 1145 & 1146

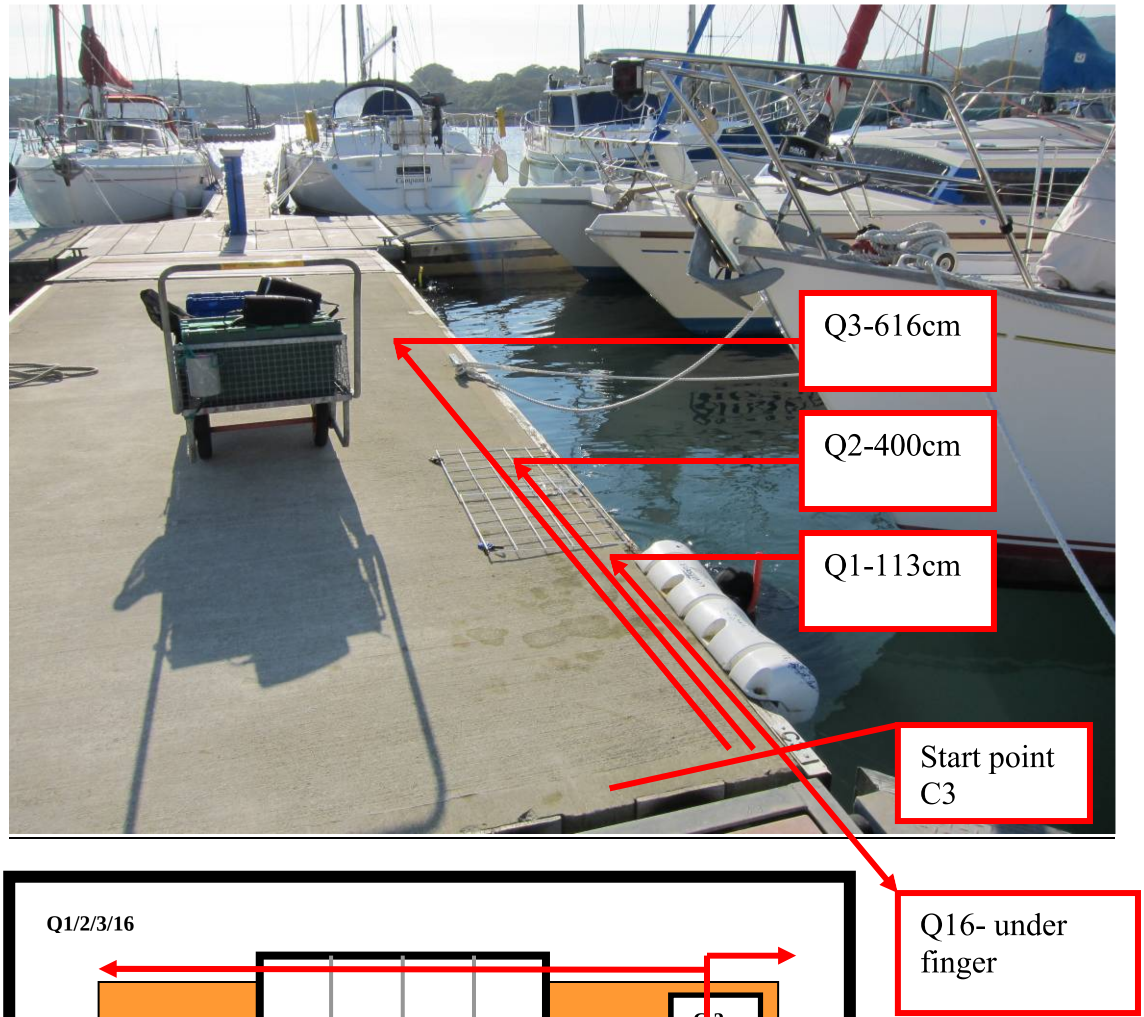
[E] Contextual temperature data is archived separately in project 224, media 590

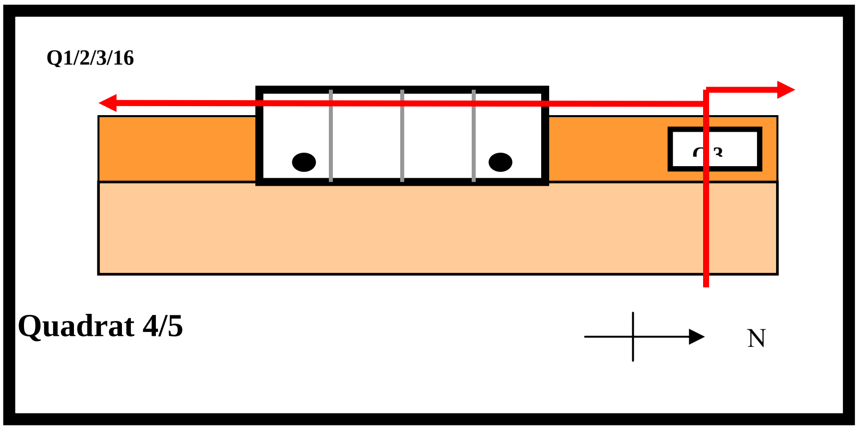
Metadata for this project is publicly accessible through Countryside Council for Wales' Library Catalogue http://www-library.ccw.gov.uk/olibcgi/w24.cgi by searching 'Dataset Titles'. The metadata is held as record no [xxxxx]

# Appendix II Fixed point quadrat locations in Holyhead Marina

**Quadrat 1/2/3/16**

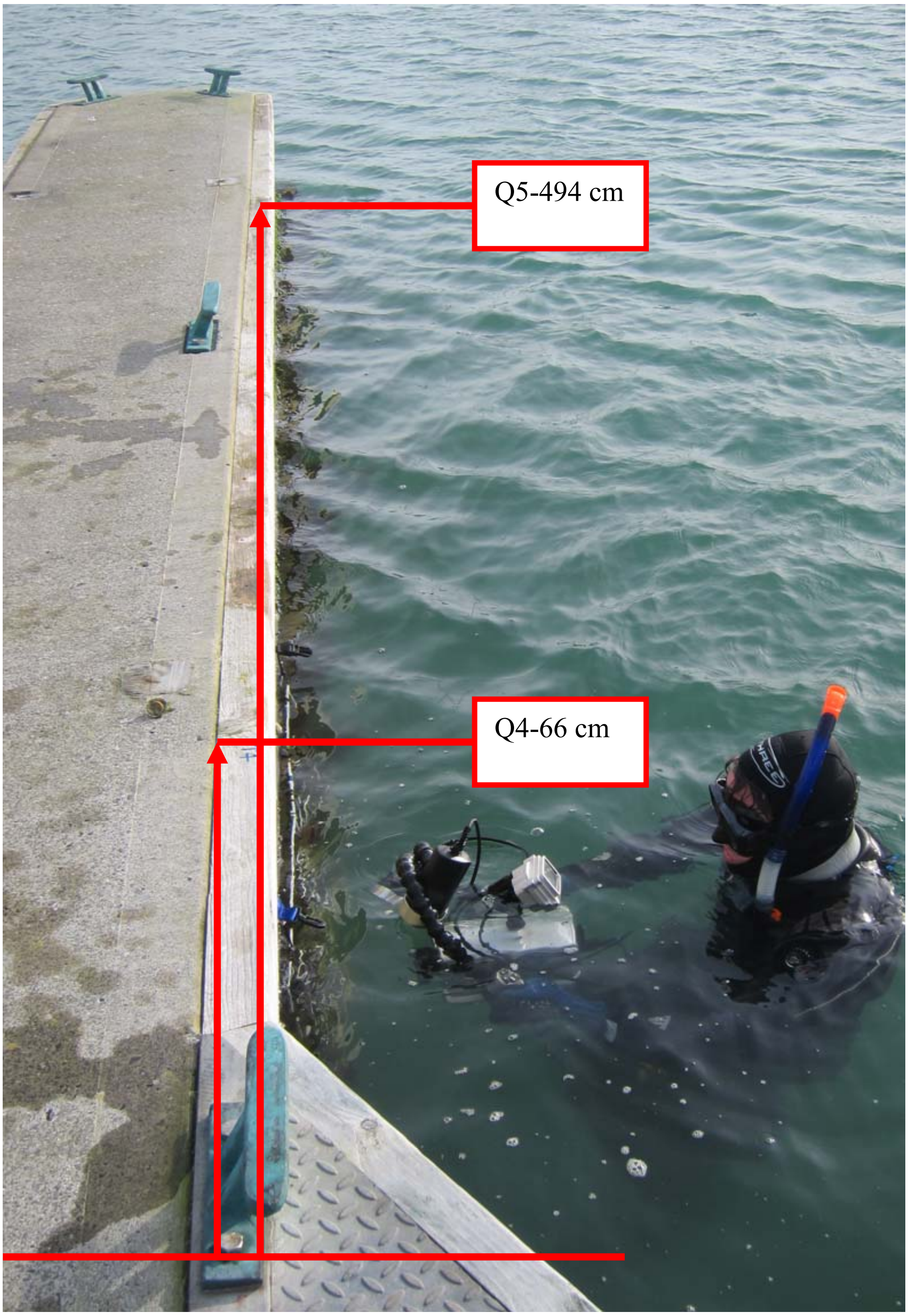
| Quadrat number | location | orientation | marker used | Distance from marker to top left of Quadrat |
|---|---|---|---|---|
| 1 | C3 Walkway | North / South | Using the central point on the C3 berth marker, measure 113 cm to the top left corner of the quadrat in a Southerly direction. | 113cm |
| 2 | C3 Walkway | North / South | Using the central point on the C3 berth marker, measure 400 cm to the top left corner of the quadrat in a Southerly direction. | 400cm |
| 3 | C3 Walkway | North / South | Using the central point on the C3 berth marker, measure 616 cm to the top left corner of the quadrat in a Southerly direction. | 616cm |
| 16 | C3 | East / West | Under the finger of c3-c5 on the first block, western face. | Under finger |

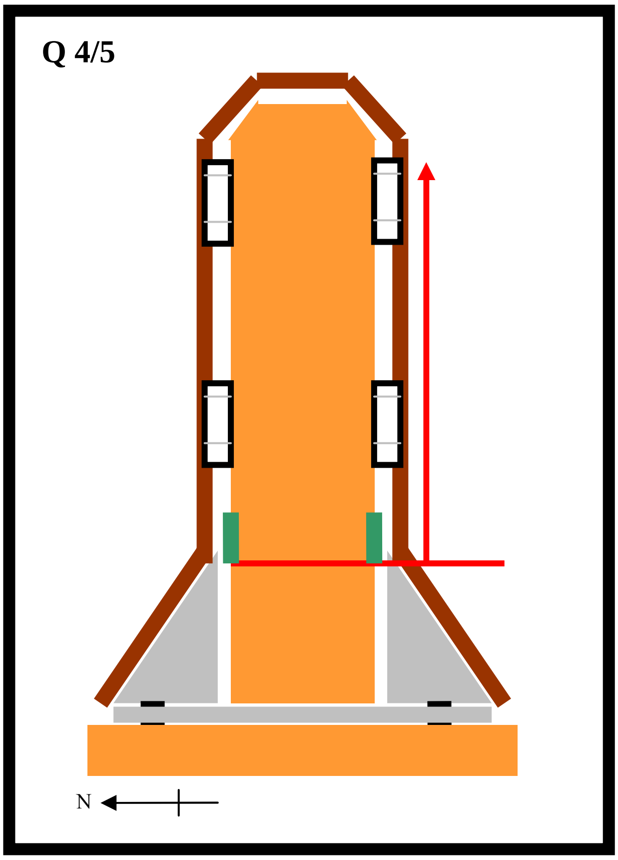



**Quadrat 4/5**

| Quadrat number | location | orientation | marker used | distance from marker to top left of Quadrat |
|---|---|---|---|---|
| 4 | C20 Long solid Finger | E/W | On the Southern face of the pontoon, measure 66 cm in an Easterly direction to the top left corner of the quadrat. Using the eastern tip of the metal triangle as the start location | 66cm |
| 5 | C20 Long solid Finger | E/W | On the Southern face of the pontoon, measure 494 cm to the top left corner of the quadrat. Using the Eastern tip of the metal triangle as the start location | 494cm |

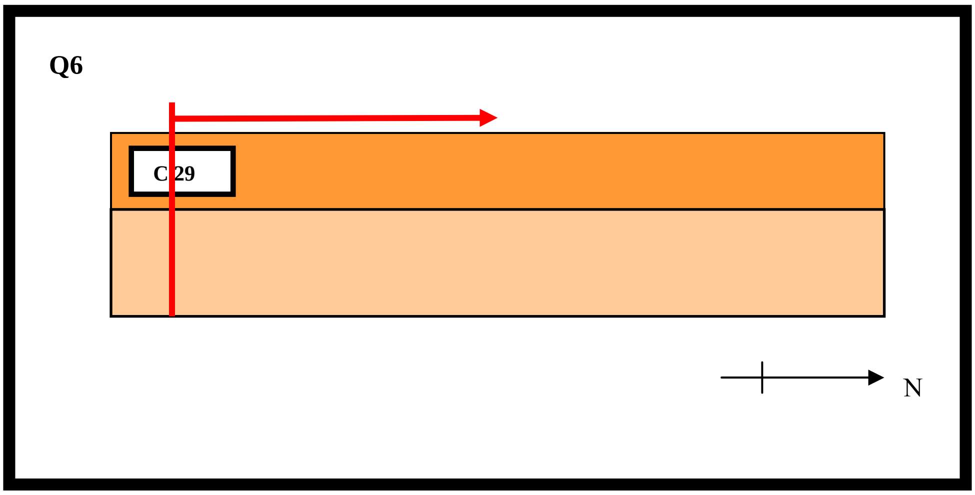


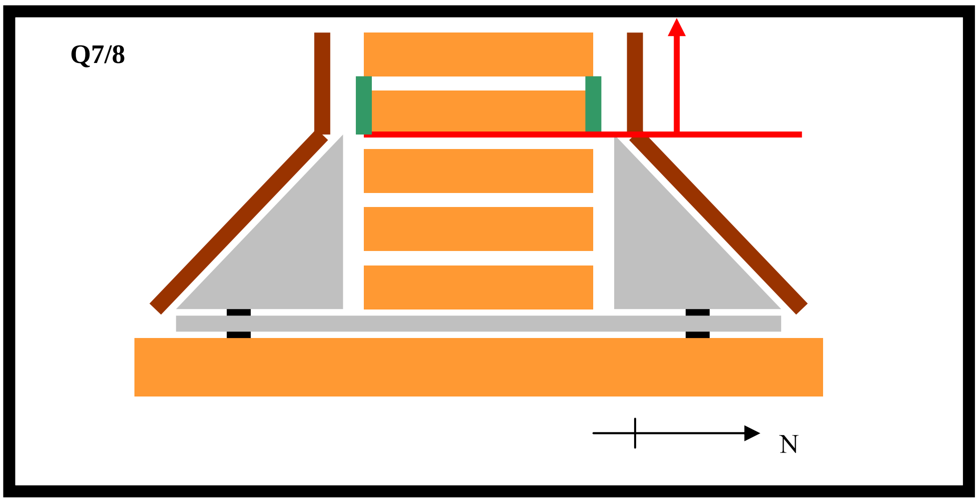



**Quadrat number 6/7/8**

| Quadrat number | location | orientation | marker used | distance from marker to top left of Quadrat |
|---|---|---|---|---|
| 6 | C29 walkway | N/S | Using the central point on the C29 berth marker, measure 109 cm in a westerly direction to the top left corner of the quadrat in a northerly direction. | 109cm |
| 7 | C29 Finger | E/W | On the Northern face of the pontoon, measure 127 cm to the top left corner of the quadrat. Using the western tip of the metal triangle as the start location | 127cm |
| 8 | C29 Finger | E/W | On the Northern face of the pontoon, measure 422 cm to the top left corner of the quadrat. Using the western tip of the metal triangle as the start location. Film the complete finger. | 422cm |

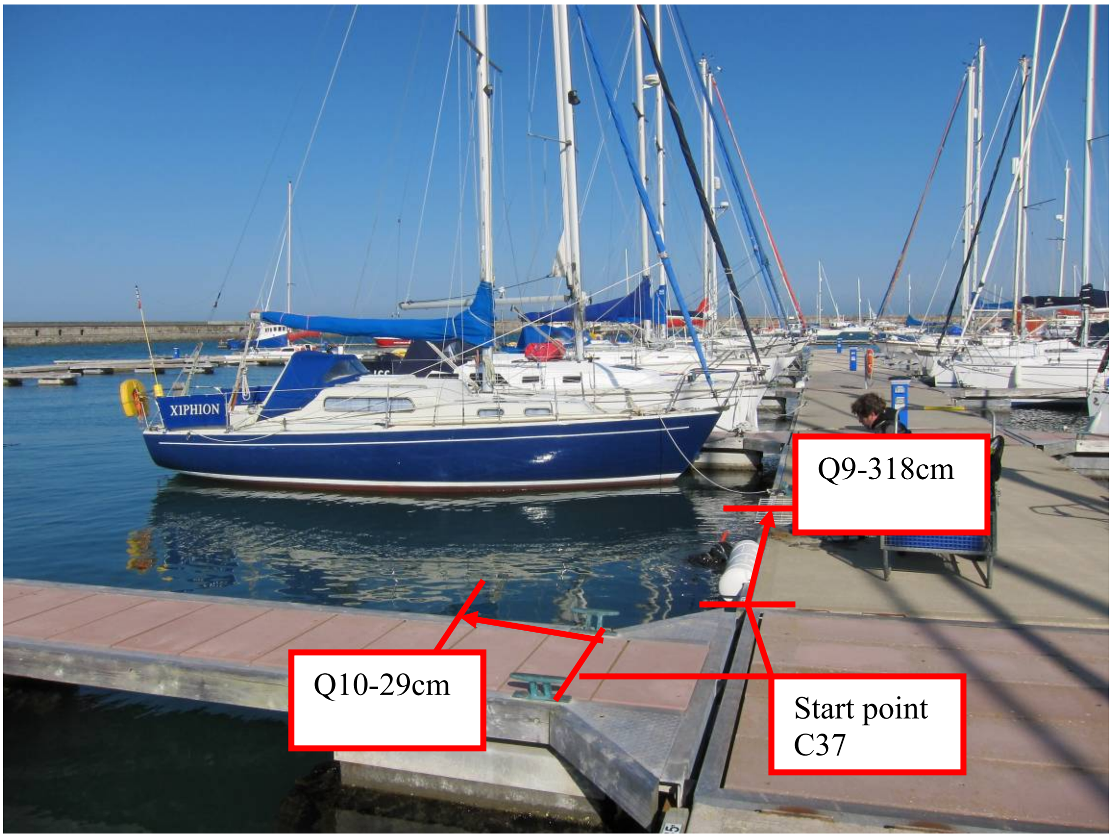

Q8-422cm
Start point
C29
Q7-127cm
Q6-109cm

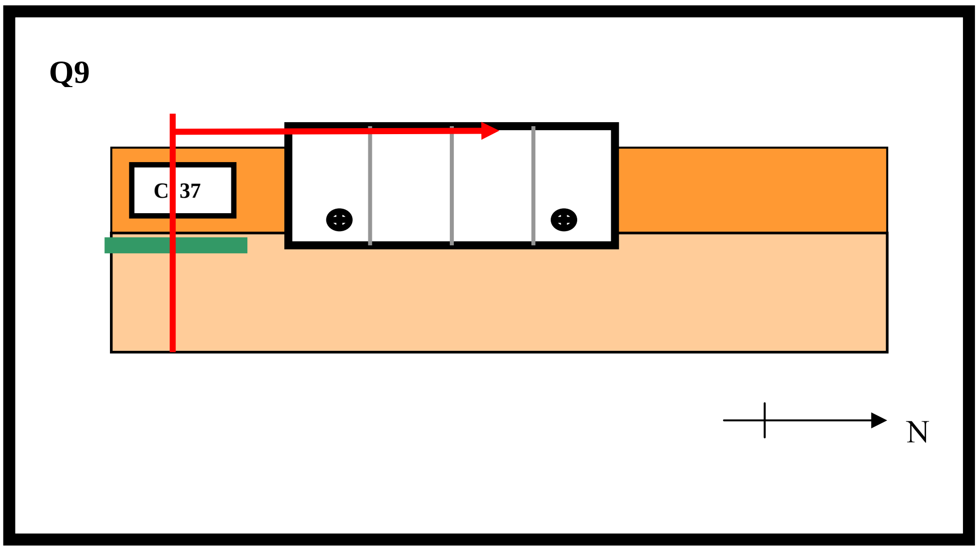

Q6
C29
N

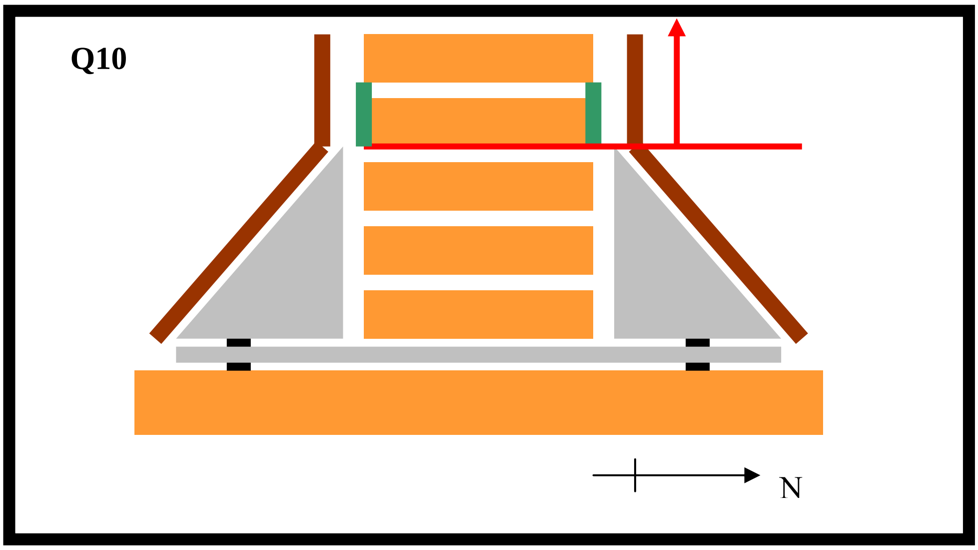

Q7/8
N

**Quadrat number 9/10**

| Quadrat number | location | orientation | marker used | distance from marker to top left of Quadrat |
|---|---|---|---|---|
| 9 | C37 walkway | N/S | Using the central point on the C37 berth marker, measure 318 cm to the top left corner of the quadrat in a northerly direction. | 318cm |
| 10 | C37 Finger | EW | On the northern face of the pontoon, measure 29 cm in a westerly direction to the top left corner of the quadrat. Using the western tip of the metal triangle as the start location | 29cm |

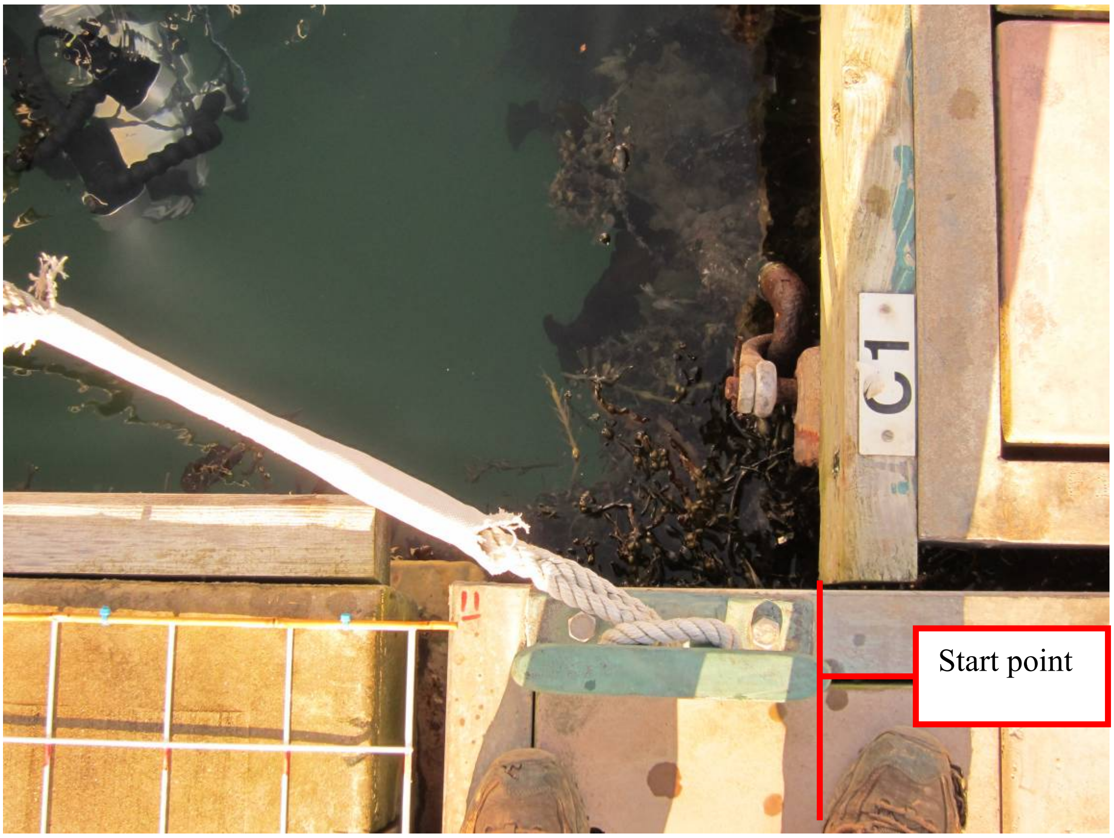


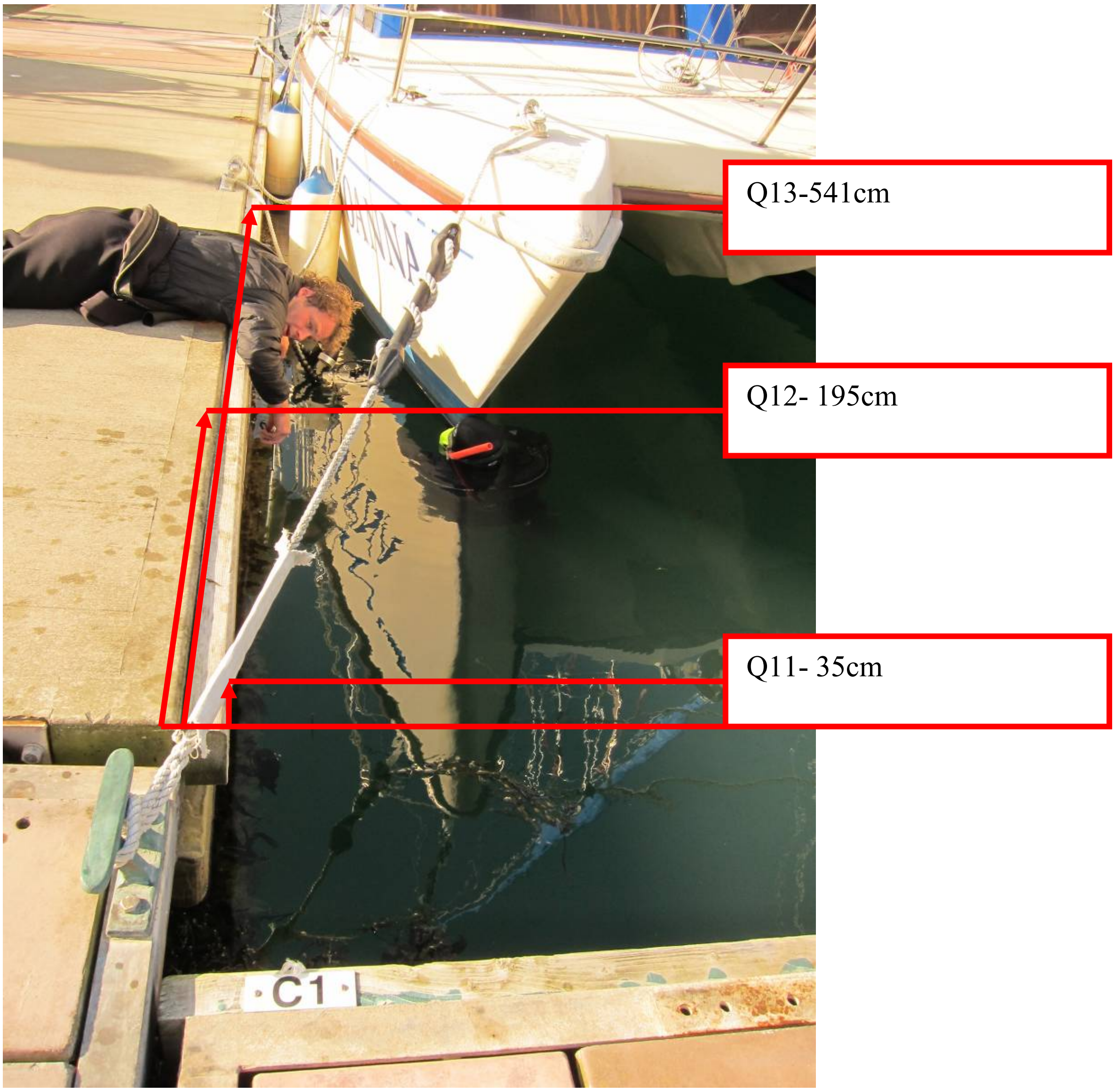






### Quadrat number 11/12/13

| Quadrat number | location | orientation | marker used | distance from marker to top left of Quadrat |
|---|---|---|---|---|
| 11 | Walkway ED XVIII | E/W | Using the C1 berth marker, measure 35 cm to the top left corner of the quadrat in a westerly direction. Along the northern face of the walkway ED XVIII | 35cm |
| 12 | Walkway ED XVIII | E/W | Using the C1 berth marker, measure 195 cm to the top left corner of the quadrat in a westerly direction. Along the northern face of the walkway ED XVIII | 195cm |
| 13 | Walkway ED XVIII | E/W I | Using the C1 berth marker, measure 541 cm to the top left corner of the quadrat in a westerly direction. Along the northern face of the walkway ED XVIII | 541cm |

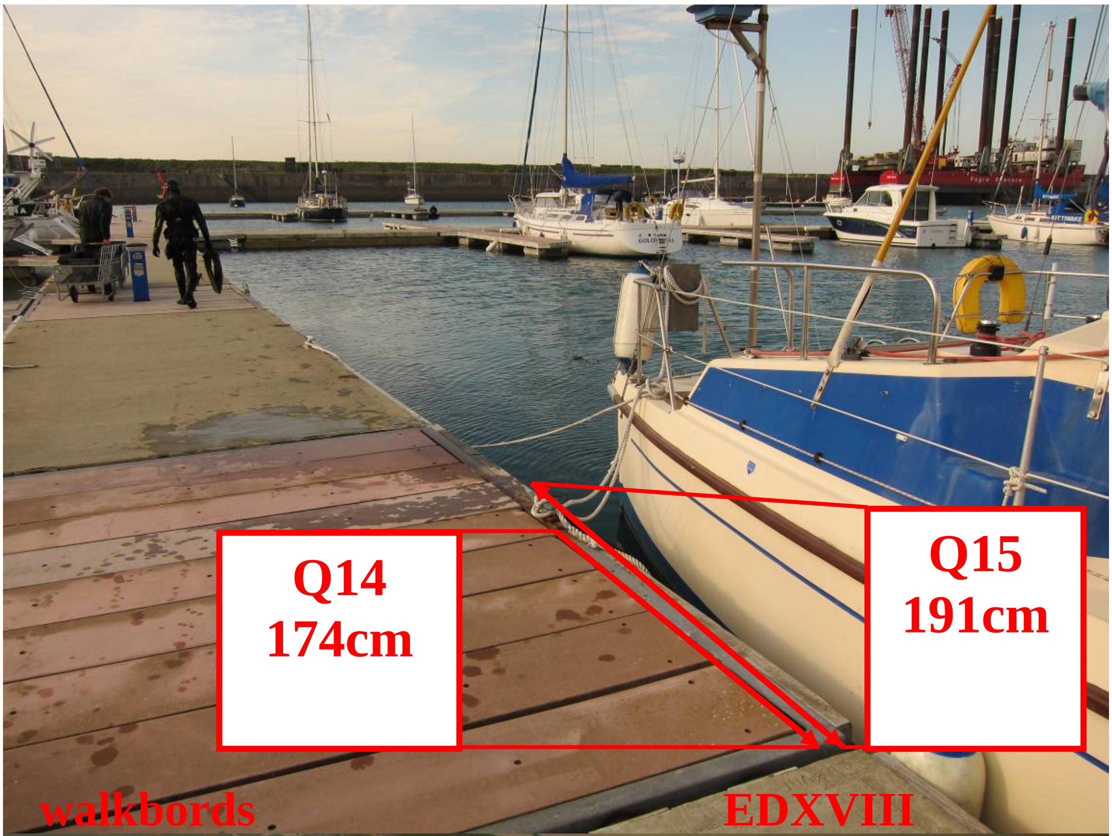

C1
Start point




**Quadrat number14/15**

| Quadrat number | location | orientation | marker used | distance from marker to top left of Quadrat |
|---|---|---|---|---|
| 14 | F15 Internode | E/W | On the North face of the F15 internode, measure in a westerly direction 174 cm. using the north west corner of ED XVIII as the start point. | 174cm |
| 15 | F15 Internode | E/W | On the North face of the F15 internode, measure in a westerly direction 191 cm. using the north west corner of ED XVIII as the start point. | 191cm |

**Notes** –
Record 14/15 as one quadrat.


Q14
174cm
Q15
191cm
walkbords
EDXVIII

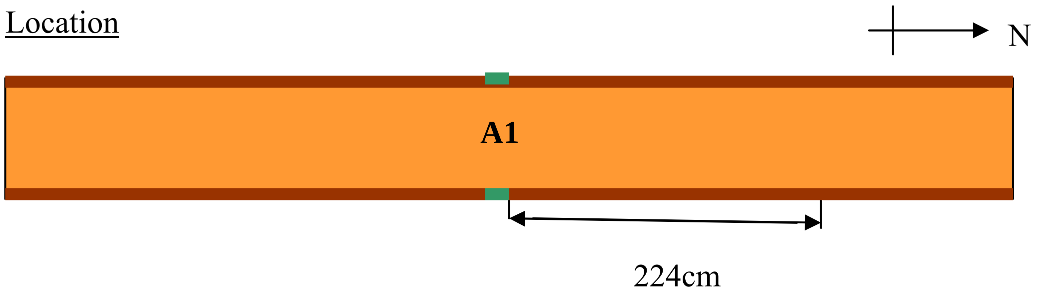

15
14

**Quadrat 17/18**

| Quadrat number | location | orientation | marker used | distance from marker to top left of Quadrat |
|---|---|---|---|---|
| 17 | A1 Walkway | N/S | Using the central eastern cleat. Measure 2.24m north, using the northern bolt on the cleat as the start point. | 224cm |
| 18 | A8 walkway | N/S | Using the central eastern cleat. Measure 2.24m north, using the northern bolt on the cleat as the start point. | 224cm |

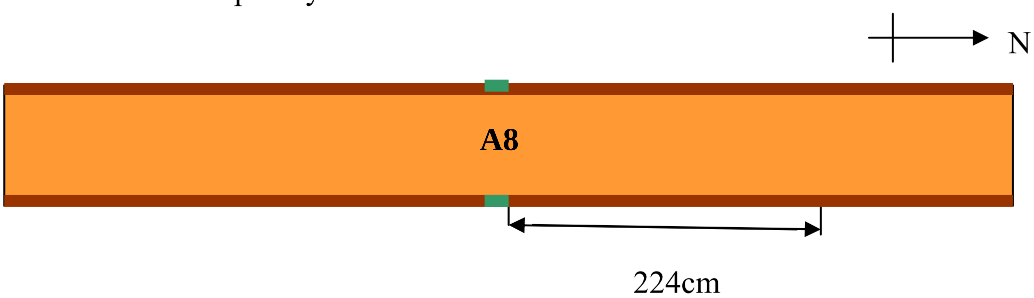
**Quadrat number 17**
Q17 was installed on the new A pontoon section as this area was previously uninfected. However there are now 2 large colonies of D.vexillum present. Q17 is also the only reinfected walkway.

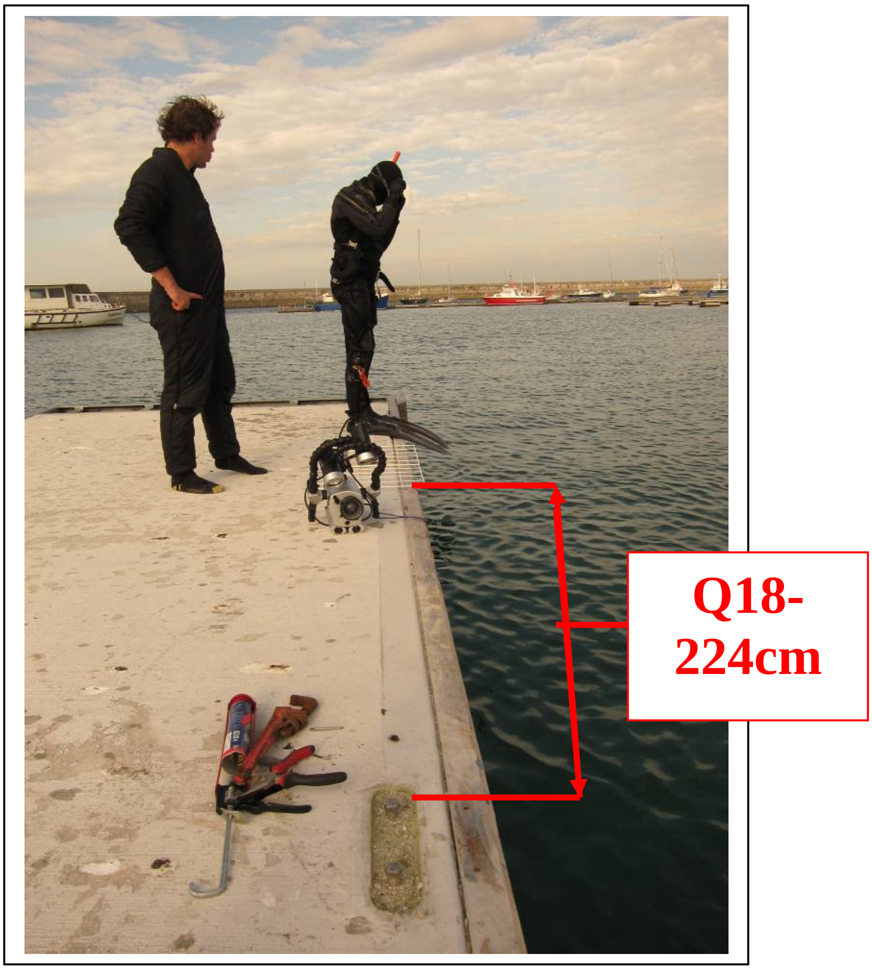
Location



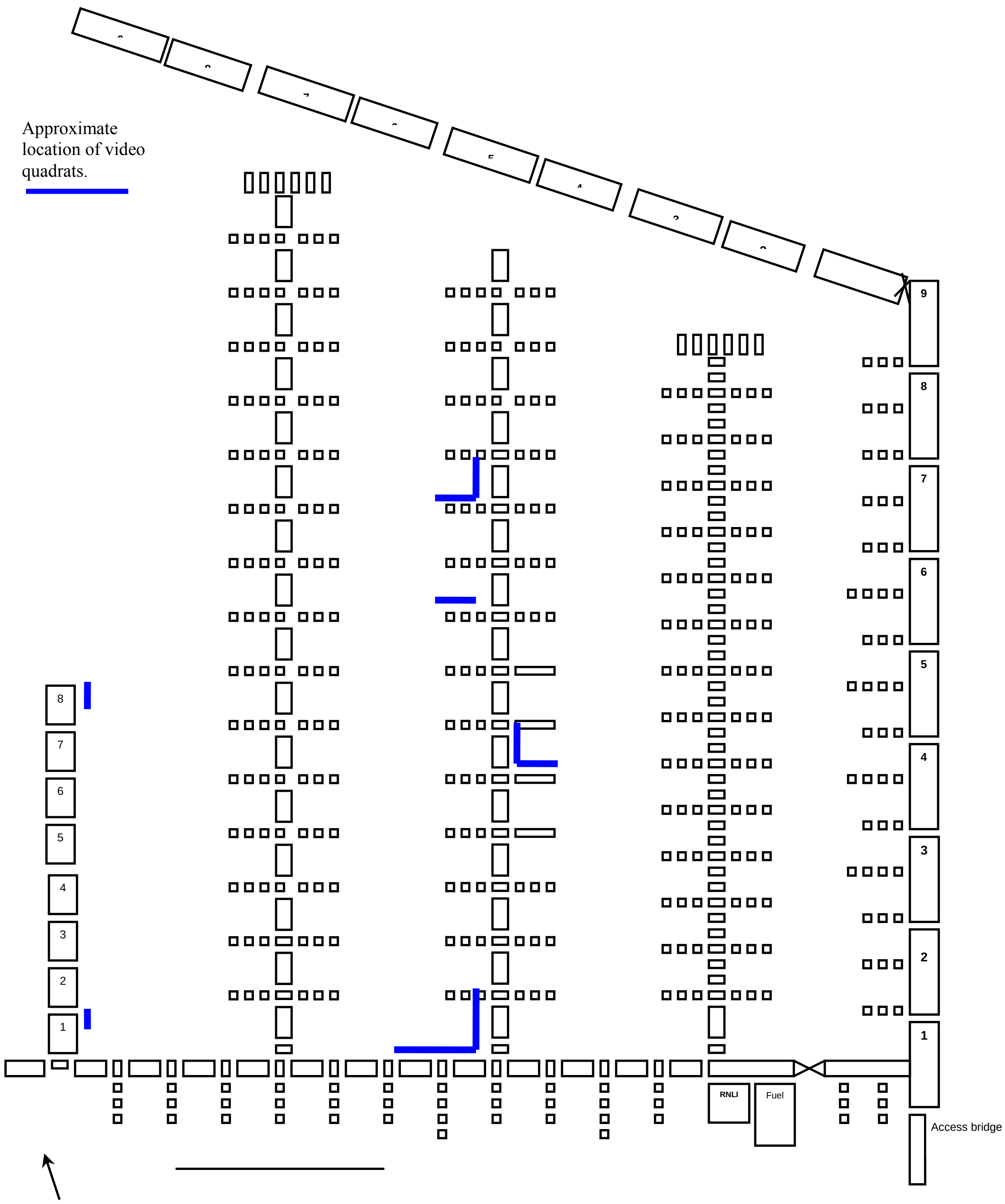

**Quadrat number 18**

Q17 was installed on the new “A pontoon” section as it was previously uninfected, pontoon A8 is also new and completely uncolonised.




Q18-
224cm

# *HOLYHEAD MARINA* near-scale plan of pontoon floats



## Appendix III Original FEPA application

*This application was later amended to include contractors' details, work-boat details and the amounts of calcium hypochlorite to be used.*

To:
FAO Michael Meekums
Marine Consents and Environment Unit
Room 309
Eastbury House
30-34 Albert Embankment
London
SE1 7TL

From:
Rohan Holt (*Didemnum vexillum* eradication project)
Countryside Council for Wales
Maes Y Ffynnon
Ffordd Penrhos
Bangor
Gwynedd LL57 2DW

**URGENT**: Adaptation of:

‘Application for a licence to deposit tracers and other materials in the sea’ - regarding **Eradication of non-native sea squirt *Didemnum vexillum* from Holyhead marina, N Wales.**

**Section 1-3. Details of current licence:**
No current licence or any previous licences held for this operation.

**Section 4. Applicant details**
Dr Rohan H F Holt
*Didemnum vexillum* eradication project manager
Countryside Council for Wales
Maes Y Ffynnon
Ffordd Penrhos
Bangor
Gwynedd LL57 2DW

Direct line 01248 387172 or mobile 07788910***
e-mail r.holt@ccw.gov.uk

**Section 5. Name and address of company**
See above CCW address.

**Section 6. Licence start date**

a. Start date: As soon as possible from date of application (or retrospectively to start on the 1st October 2009).
b. End date: Full scale eradication due to take place during winter and spring 2009 – 2010. Even if initial attempts at eradication are successful it is likely that repeat infections will

have to be treated using similar methods – therefore end date unknown, but DEFRA will be kept informed of progress throughout.

**Section 7**
**Materials and methods**
## 7. 1 Introduction

The invasive non-native sea squirt *Didemnum vexillum* was found in Holyhead Marinalast summer (2008). This was the first record of an established population of this species in the UK. The species is thought to originate in Japan and has become a pest in other countries such as the east coast of America and New Zealand (Lambert 2009). It has also been found on the east coast of Ireland (Malahide) and has just been confirmed from the Dart estuary in Devon (John Bishop pers. com.). It can grow very quickly, smothering native habitats and species and can also interfere with fishing, aquaculture (particularly mussel rope culture) and other marine industries. It produces free swimming short duration 'tadpole'-like larvae when the water is warmest during the summer, but free-floating fragments of colonies can also re-attach and continue growth.

CCW has undertaken surveys of Holyhead Marinaand the wider harbour area, along with various other marinas around Wales to look for the sea squirt. To date it has only been found growing on the pontoons, mooring chains and a few boat hulls in the marina in Holyhead Harbour and has not been found anywhere else in the commercial side of the harbour, or in any other marinas in Wales (Holt *et al* 2009; Irving 2009).

CCW commissioned Dr Sarah Kleeman to review methods of eradication and critically assess their chances of success (Kleeman, 2009). Her report concluded that the sooner steps are taken to remove the species from Holyhead marina, the greater the chance of preventing its spread. The results of the surveys undertaken in February and July suggest that the sea squirt was in the initial 'lag phase' of the infestation with mainly scattered small colonies found throughout the marina area. However, recent investigations in early September have shown a rapid increase in size, coverage and number of colonies which would suggest that there has been accelerated development during the warmer late summer months and that it may be entering the 'explosion' or 'established' phases of the infestation model. Time is now critical to eradicate it, or at least keep it at low levels, before it spreads into the wider marine environment and to mussel cultivation areas in the Menai Strait and a smaller mussel farm at the east side of Holyhead Harbour area.

## 7.2 Method for eradication
### Background

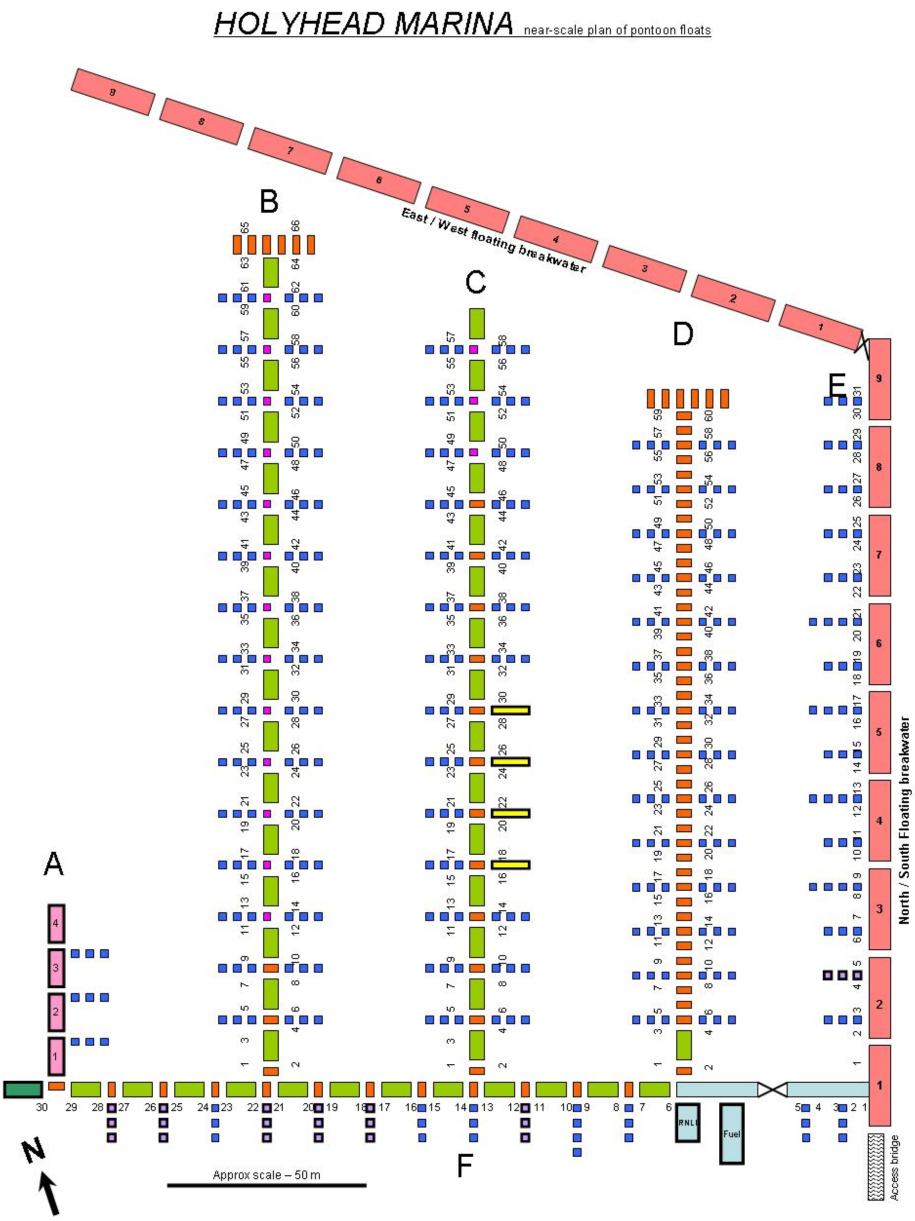
The methods used for eradicating this species in other parts of the World (e.g. Coutts, 2006; Pannell and Coutts 2007) rely on encapsulating the structures on which the colonies are growing with silage wrap or other sheet plastic materials to isolate the animals from the water flow they require for feeding and oxygenation. Without an oxygenated water supply the sea squirt and any other fauna inside the wrapping start to die which in turn accelerates anoxia (stagnation) which quickly kills the remaining colonies. An accelerant, for example acetic acid or bleach granules, can be added to the inside of the wrap to kick-start the stagnation process. Once the wraps are on the process can take a week or so to kill the sea squirt and by using an accelerant this can be achieved in two or three days. It is worth noting here that this species cannot simply be scraped off in an attempt to eradicate it as the fragments will disperse and spread further than even the larvae can swim. It is therefore important to keep physical disturbance of infected structures, including boats, to a minimum.

## 7.3 Materials and methods for eradication in Holyhead marina

Holyhead Marinacomprises over 500 floating pontoons that support walkways and births. These range in size from just over 3 $m^2$ on the pontoon fingers, to over 100 $m^2$ on the breakwater sections.

The eradication will take place in a series of waves. The first will be a pilot study to develop materials and methods on a subset of these pontoons i.e.:
Six pontoon fingers (i.e. 18 small floats)
Two walkway pontoons (two medium-large floats)
One 'internode' float (between the two walkway pontoons)
One large breakwater pontoon (on the east or east-west breakwaters)
All chains associated with the above structures
Two mooring buoys and chains to the west of the marina

(see Figure 1 below for layout of marina and target area for pilot study)



# KEY

| Pontoon type – Size in m | L | W | H | Submerged height | Free board | Girth | # units | Unit area to be treated m² | Total area to be treated m² |
|---|---|---|---|---|---|---|---|---|---|
| Breakwater | 20.03 | 4.0 | 1.0 | **0.70** | 0.33 / 0.18* | | 18 | 113.76 | **2047.68** |
| Walkway B, C, F | 7.37 | 3.25 | 0.67 | **0.40** | 0.30 | 4.50 | 44 | 32.45 | **1427.80** |
| Walkway A | 9.58 | ~3.20 | - | **~0.5** | - | - | 4 | 43.44 | **173.76** |
| Walkway – west end F | 7.45 | 3.13 | 0.72 | **0.42** | 0.30 | 4.40 | 1 | 32.21 | **32.21** |
| Walkway D / internode / ends | 1.24 | 3.04 | 0.70 | **0.45** | 0.30 | 4.30 | 80 | 7.62 | **609.60** |
| Walkway F - east end | 20.00 | 3.00 | - | **~0.70** | - | - | 2 | 92.2 | **184.40** |
| Internode | 1.07 | 1.65 | 0.95 | **0.67** | 0.32 | 2.88 | 26 | 5.41 | **140.66** |
| Finger – solid | 7.42 | 1.04 | 0.95 | **0.70** | 0.30 | 3.00 | 4 | 19.56 | **78.24** |
| Finger – std | 1.23 | 1.02 | 0.80 | **0.55** | 0.25 | 2.46 | 323 | 3.73 | **1204.79** |
| Finger – type 2 | 1.88 | 1.09 | 0.70 | **0.40** | 0.32 | 2.26 | 21 | 4.43 | **93.03** |
| Fuel jetty - concrete | 15.01 | 4.00 | | **~0.70** | | | 1 | 86.65 | **86.65** |
| RNLI jetty - concrete | 10.00 | 4.04 | | **~0.70** | | | 1 | 60.06 | **60.06** |
| | | | | | | Totals | **525** | | **6,138.88 m²** |

## Notes:

Height = measurement from base to top of float
Freeboard = measurement from water surface to top of float * and lower edge of wooden batten on breakwater sections
Girth = measurement around narrowest section of each float taking into account space taken by marine growth

Long solid concrete pontoon with chains around N and E side of marina forming breakwater. Adjoining ends of each section are chamfered. No internodes

Main walkways on section B and C plus some of F and D

Newly developed section of walkway A with no fingers yet, no internodes

Odd sized walkway single section at W end of F

Internode and walkway D. Used in various orientations between larger floats and pontoon B and D ends

Concrete sections similar to breakwater near access bridge

Small internode floats – used on parts of B and C. Limitations of Powerpoint don't allow central alignment on drawing.

Single long floats of GRP covered polystyrene – two with lip at top and two without. Hard butted up against internodes.

Single small floats used on majority of fingers in groups of 3 (occasionally 4). Float sits under metal box with waist top suitable for drawstring topped bags.

Similar to above but GRP coated polystyrene. Metal case at top.

Fuel
RNLI
Similar in construction to other concrete sections

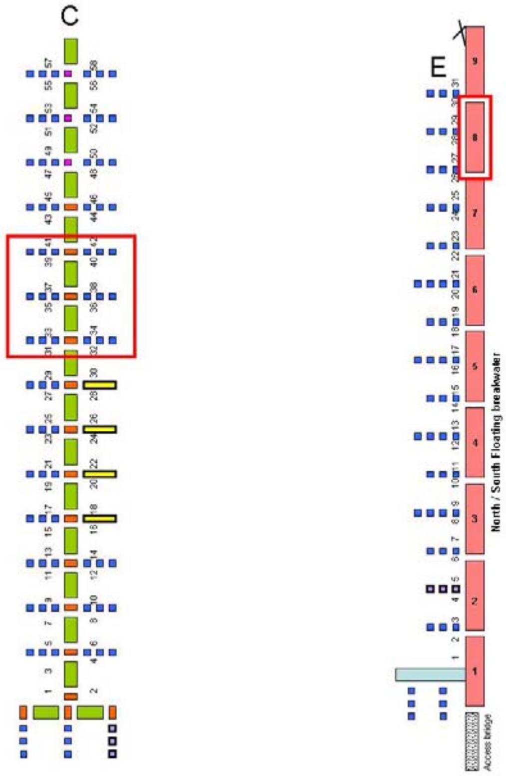



**Figure 1 ctd – Areas selected for pilot study on pontoons C and E**

The results of the pilot will then indicate how quickly the rest of the marina can be treated limited by cost, time, weather etc. but we hope to increase the scale of the operation by creating enough bags and wraps to be able to treat the whole marina in a total of 5-10 waves (i.e. approximately 100 bags/wraps of varying sizes will be deployed at each wave).

**A. Creating a physical barrier to water circulation.**

To eradicate *D. vexillum*, each pontoon will be wrapped in a purpose-built re-usable bag made from either PVC coated 'tarpaulin' fabric or waterproof sail cloth (heavy duty close weave nylon). The larger pontoons, which are too large to cover with one large bag, will be wrapped using several overlapping bags/sheets sealed at the seams. Then water will be pumped out of the bags using a petrol-driven seawater pump leaving little more than the water trapped amongst the fouling organisms and the residue between the inner wall of the bag and the pontoon surfaces. The biota trapped within the bag will soon deplete the oxygen content of the water and create localise hypoxic conditions. This will in turn lead to death and initial decomposition of the biota which will further accelerate anoxic conditions resulting in 100% mortality of fauna inside the bag. The rate of this process is largely temperature dependant but at around 12$^{o}$C should be complete in 10-21 days.

The decomposition and stagnation process will be entirely confined to the bags and wraps, but there is likelihood of sulphurous / rotting odours escaping which would be noticeable at close range. This is less likely to be problematic during the cool autumn-spring period when the pilot and full eradication are running.

Chains and mooring ropes will be treated similarly using durable sheet plastic or tarpaulin-type material wrapped around and tied to the structure to form an impermeable tube-shaped barrier to water exchange.

Once this process has been completed the bags / wraps will be removed for cleaning and redeployment and the decayed debris will be allowed to sink to the seabed below the pontoons. This may result in localised short-term anoxia on the seabed immediately below the pontoons, but this will also serve to eradicate fragments of *D. vexillum* that might have dropped off the pontoons earlier.

**B. Using chemical treatment to accelerate the eradication process.**
Weak bleach solution (e.g. 0.025% NaClo final concentration – for example diluted 5%-8% 'household bleach') will be used on a small proportion of the pontoons in the pilot study and can be used if necessary on the other structures to cause 100% mortality of *D. vexillum* in as little as 48 hours. Quantities required to reach the desired concentration vary between the different sized pontoons but, for example, the largest pontoons with over 100 $m^2$ of treatable surface area will require around 50 to 60 L of bleach each and the smallest 2 L. After approximately 48 hours the treated bags and wraps will be removed and the content allowed to disperse – the heavier debris will drop to the seabed below the pontoons. Although bleach will be rapidly consumed by reacting with the organic material within the wraps there will be a low concentration of bleach-contaminated seawater and decaying biological material released into the water around the pontoons as the wraps are undone.

**NOTE** Chemical treatment to accelerate the eradication process will only be tested during the pilot (mid to late October) on a few of the smaller pontoon floats and will only be used in the full eradication process when it is impractical to leave bags and wraps on for more than a few days (e.g. when strong winds from the north and east are forecasted and the wraps need removing from the pontoons to avoid damage to the materials and working in dangerous conditions). For a worst-case scenario (i.e. having to use accelerant on ALL the pontoons see spreadsheet below– NOTE - this action is highly unlikely to be required). The calculations for the amount of bleach required are based on an estimate of the volume of water enclosed in the bags and wraps. The values below are given for water layer options of 100 mm and 50 mm 'thick / deep' surrounding the pontoons enclosed within each bag but these values may well be smaller if the water volume can be reduced significantly by the use of a seawater pump.

| | Volume $m^3$ to be treated (area x typical water layer enclosed) | | Vol Sodium hypochlorite for 0.025% net per pontoon | | | Total bleach required (litres) for all pontoons of each size | |
|---|---|---|---|---|---|---|---|
| **Pontoon type (see Fig 1 above for sizes)** | **Assume Depth 100mm ave** | **Assume Depth 50mm ave** | **Industrial 14% bleach** | **Bleach 5%** | **No Units** | **Industrial 14% bleach** | **"Bleach" 5%** |
| Breakwater | 11.70 | 5.85 | 23.4 | 58.5 | 18 | 421 | 1,053 |
| Walkway B, C, F | 3.24 | 1.62 | 6.5 | 16.2 | 44 | 286 | 714 |
| Walkway A | 4.34 | 2.17 | 8.7 | 21.7 | 4 | 35 | 87 |
| Walkway – west end F | 3.22 | 1.61 | 6.4 | 16.1 | 1 | 6 | 16 |
| Walkway D / internode / ends | 0.76 | 0.38 | 1.5 | 3.8 | 80 | 122 | 305 |
| Walkway F - east end | 9.22 | 4.61 | 18.4 | 46.1 | 2 | 37 | 92 |
| Internode | 0.54 | 0.27 | 1.1 | 2.7 | 26 | 28 | 70 |

| | Volume $m^3$ to be treated (area x typical water layer enclosed) | | Vol Sodium hypochlorite for 0.025% net per pontoon | | | Total bleach required (litres) for all pontoons of each size | |
|---|---|---|---|---|---|---|---|
| Finger – solid | 1.96 | 0.98 | 3.9 | 9.8 | 4 | 16 | 39 |
| Finger – std | 0.37 | 0.19 | 0.7 | 1.9 | 314 | 234 | 586 |
| Finger – type 2 | 0.44 | 0.22 | 0.9 | 2.2 | 21 | 19 | 46 |
| Fuel jetty - concrete | 8.67 | 4.33 | 17.3 | 43.3 | 1 | 17 | 43 |
| RNLI jetty - concrete | 6.01 | 3.00 | 12.0 | 30.0 | 1 | 12 | 30 |

**Section 8**
**Details of deposit**

The marina is situated within the larger Holyhead Harbour area which is a semi-enclosed area of water partially cut off from open water by the Holyhead breakwater. See Figure 2 aerial image below.

The marina is situated at 53° 19'.130 N 004° 38'.340 W – the pontoons can be clearly seen at the western side of the harbour on the aerial photograph. See above for estimates of quantities and date of application.

**Section 9**
**<u>Method of deposit</u>**
Most of the work in the marina involving treatment of the pontoon floats will be carried out from the pontoons themselves. Bags and wraps will be secured into position using divers with support from personnel on the pontoons. Chemical treatment, if used, will be carried out from the pontoons.

Mooring buoys and chains and any other structures away from the pontoons that require treatment by divers will be supported by CCW's survey vessel Pedryn (11.7 m cabin RIB). Biosecurity measures to prevent the spread of non-native species will be observed by the boat operators.

**Section 10**
**Conservation bodies / other authorities / users consulted with regard to the eradication process so far:**

| **Authority / organisation** | **Person(s) / contacts** | **Notes** |
|---|---|---|
| Countryside Council for Wales | Rohan Holt, CCW, managing field eradication programme for CCW.<br>CCW N Region contact John Ratcliffe j.ratcliffe@ccw.gov.uk<br>HQ / N region: Tim Jones t.jones@ccw.gov.uk | Project funded by WAG |
| Environment agency | Bangor office first contact Mark Medway mark.medway@environment-agency.wales.gov.uk<br>Bangor office – Euryn Roberts (01248 484079) Euryn.Roberts@environment-agency.wales.gov.uk | EA have acknowledged CCW's need to treat this as a 'biological emergency' but have no need themselves to initiate emergency procedures. |
| North Wales and North West Sea Fisheries Committee (NWNWSFC) | Bill Cook & R Houghton | Phoned and sent methods statement by email |
| WAG marine licencing | Paul Moyle<br>Paul.Moyle@Wales.GSI.Gov.UK<br>02920 801250 | Sent draft FEPA application by email |
| Port of Holyhead authority (Stena) | Harbour masters cpt Brian McCleery and Cpt Wyn Parry<br>01407 606775 | Have jurisdiction over the whole harbour and marina area. Work permits required from Stena. |
| Holyhead Marina | Susan Cooper, Ed Hughes, Geoff Garrod<br>01407 764242<br>susan.cooper@holyheadmarina.co.uk | Marina owners/staff kept updated on all processes and communications. |
| Marine and Fisheries Agency (DEFRA) MFA | Terry Allen<br>Terry.TG.Allen@mfa.gsi.gov.uk | Sent outline plans and reports and has commented verbally on need for FEPA licence |
| Local yacht clubs and mooring associations in Holyhead | Contact via Martin Sampson at Anglesey diver training college. | Verbally informed of what might need doing to their mooring lines and buoys to clear D vex infection |
| Maritime and Coastguard Agency | Jim Paton in Holyhead office<br>jim.paton@mcga.gov.uk | Phoned and sent methods statement by email. |
| Anglesey Council Maritime officers | Duncan Brown 01248 752300 (752320 direct)<br>dbxht@anglesey.gov.uk<br>John Owen<br>joxht@anglesey.gov.uk | Phoned and sent methods statement by email. |

**Section 11**
**Public register**

There is no information contained in this application that we consider should not be included on the Public Register or made available on request.

## Appendix IV Sample diving risk assessment for eradication project.

### Countryside Council for Wales

### Diving project plan

To: Dr David Parker (CCW named contractor) from Rohan Holt, Diving Project Manager
**CC**: all members of survey team listed below and Holyhead Port Authority

| | |
|---|---|
| **Title of Project:**<br>**Eradication and control of non-native seasquirt *Didemnum vexillum* in Holyhead Marina** | |
| **HSE Approved Code of Practice applicable to this diving project:**<br>**Scientific and Archaeological ACOP** | |
| **Diving Rules applicable**: Holt 1998. Joint Nature Conservation Committee, English Nature, Scottish Natural Heritage, Countryside Council for Wales. Diving Rules | |
| Dates: | 2nd November then ongoing as results dictate (all parties will be updated) |
| Location of diving operations: | Holyhead Marinaand Harbour |
| Diving Contractor: | Dr David Parker, Countryside Council for Wales |
| Diving Project Manager: | Dr Rohan Holt |
| Names of Supervisors required (with Oxygen administration and 1st aid qualifications): | Rohan Holt, Martin Sampson, Harry Goudge, Liz Morris |
| Names of possible divers / standby divers and qualifications: * see table at end | Divers will be chosen as available from a pool of suitably qualified divers listed in the table at the end of this document. |
| Names of other personnel required and their duties: | Dan Crook – CCW non-diving assistant (as available).<br>Wayne Watkins non-diving assistant (as available)<br>Paul Turkentine (skipper *Pedryn*) (as available). |
| Others: | Other CCW/marina staff may be present to assist with carrying equipment and collecting data. |
| Any other groups / persons to contact before diving ops take place. | Holyhead Marina01407 764242<br>**Capt. Brian McCleery / Capt. Wyn Parry** Harbour Master 01407 606775<br>**Holyhead Port Control** 01407 606700 24Hrs portcontrol@stenaline.com and CH 14<br><br>**PERMIT TO DIVE REQUIRED BEFORE DIVING OPS TAKE PLACE IN HOLYHEAD HARBOUR**<br><br>Holyhead coastguard – VHF ch16<br><br>Fuguro jack up crane 'Excalibur' in NW corner of the Harbour Contact Drew Ross (Barge Master) Phone: 07770 324 533 / 07732 543 666 |
| Decompression schedule | Nitrox diving using Uwatec Galileo Sol decompression computers and heart rate monitors or Uwatec/Suunto decompression computers. |
| Equipment required: | Standard SCUBA twin-7L cylinders or 12L with pony with full face masks and voice communications (or mouth masks with voice comms); delayed SMB reel with safety line; underwater stills and video camera; writing boards, measuring tapes. Half masks will be used by divers if required in low-risk areas. |
| Emergency Oxygen equipment | An emergency O2 kit will be on the shore/boat with backup oxygen |

| | |
|---|---|
| | (Nitrox 80%) in one or more 12L cylinder. All personnel will be shown it in working order prior to the start of the survey. |
| Special kit requirements | None |
| Any special competencies required from any personnel: | All divers qualified as scientific divers under DWR 1997. |

| **Site specific details:** | |
|---|---|
| Sea / water conditions anticipated: | No strong currents or significant wave action anticipated in the main body of the marina area or harbour, although slight currents may be experienced at the north end of the Tinto jetty and at the end of the breakwater. Most/all diving will be done on neap tides.<br>Most areas sheltered by the Holyhead Breakwater apart from wind directions from N and NE. |
| Tidal conditions: | Diving ops in Harbour to take place at any stage of the tide. |
| Transport to site | Normal road vehicles used to access marina or Harbour wall – on foot on pontoons. For any diving outside the marina *Pedryn* (12 m wheelhouse RIB) will be on station and kept close to the divers. |
| Anticipated minimum underwater visibility: | 0.5-1 m (beware of silt plumes stirred up from recent shipping movements). |
| Other hazards | **Construction work** in and around the marina. No diving to take place in any area where construction and maintenance work is being carried out.<br><br>**Vessel movements** within the marina and harbour. Monitor CH14 and keep lookout for vessel movements. Inform marina and port control of diving ops taking place and keep VHF radio in earshot at all times.<br><br>Tell any boat-owners or any vessels that might be intending to enter or exit the marina/harbour etc that diving ops are taking place. Port Control will broadcast a notice to mariners on VHF.<br><br>Do not dive in the fairway on the eastern side of the pontoons if any vessels that are there are running engines or intending to move. Beware of vessels approaching from the entrance to the harbour.<br><br>Beware of divers surfacing between moored vessels and the marina pontoon / harbour wall sides. Windy weather can bounce boats against the pontoons / walls etc risking crushing injury to the divers.<br><br>**Diving in the vicinity of the jack up barge Excalibur**. Ensure that the barge master is informed before hand and kept updated with any changes to plans. Ensure that there are no vessel or barge leg movements planned and that the tide is not going to restrict access to Pedryn or the divers during the dive. Ensure that the divers have adequate clear surface and do not stray far under the barge hull if they need to examine the seabed under the barge. |
| Pollution: | Possible minor effluent discharge from Yachts in the marina and low-toxicity pollutants from antifouling and petrochemical spills. Therefore recommend use of full face masks. |
| Depth: | Max anticipated dive depth – 15 m, but most diving will be taking place at the edges of pontoons, moorings and shallow harbour walls. |
| Temperature: | Approximately 13$^{o}$C |
| Access: | On foot on the floating pontoons. Standby and surface support available to assist with kit and divers. Pedryn elsewhere. |
| Breathing gas: | Nitrox 32% or air from twin manifolded 7L cylinders. |
| In-water and surface communications requirements: | Full face masks and wireless voice communications (or mouth masks with voice comms) ; SMB reel with safety line to tender for |

| | |
|---|---|
| | each diver.<br>All divers must also carry a delayed SMB. |

| **Emergency Information** | |
|---|---|
| Emergency procedures: | Obtain permit to dive from HHd Port Authority prior to commencement of diving ops.<br>Radio in CH 14 to HHd Port immediately prior to and after each diving operation and call immediately in the event of an incident, especially if this causes the divers / boat to restrict other vessel's movements.<br>Call in to Holyhead Coastguard prior to and following each day's diving (also mobile phone available).<br>(Talk to marina staff to check any work operations within the marina.) |
| HM Coastguard No. | HMCG Holyhead 01407 762 051 |
| Chamber No. | Hyperbaric Treatment and Training Services, BUPA Murrayfield Hospital, Holmwood Drive, Thingwell, Wirral.<br>**24 hour telephone queries 0151 648 8000**<br>Checked operational 27/10/2010 and need to check in again just before sequence of dives begins. No requirement for booking. |
| Duty Diving Medical Specialist HMS Nelson, Portsmouth | 01705 818 888 |
| Medical expertise: | RH, KR, LK, MS qualified as first aiders and have O2 administration certification. |
| Medical equipment: | Medical Oxygen and 1st aid kit to hand (in van, Pedryn or on pontoons). Additional 12L cylinders of 80% nitrox also available for use in an emergency for a conscious casualty able to breath from a standard demand valve. |
| Casualty evacuation plan: | Evaluate casualty condition and phone 999 and ask for coastguard OR<br>Contact Holyhead coastguard by VHF CH 16. Possible nearest ambulance pick up point is in the marina car park when in Holyhead area.<br>For a suspected case of decompression illness contact the hyperbaric centre at Murrayfield on **0151 648 8000** |


Annotated Google aerial view of Holyhead Harbour showing location of shipping terminals, moorings and marina.

# Methods for deploying divers.

**AIM**
To survey the marina, jetties, harbour walls and other man-made surfaces for the presence of the non-native carpet sea squirt *Didemnum vexillum*. This species is currently growing on the pontoon structures in the marina and possibly on some of the mooring chains to the west of the marina. We do not know whether this summer's growth has produced larvae that have managed to settle elsewhere beyond the boundaries of the marina. We also need to investigate the hulls of any vessels / barges that have remained stationary for lengthy periods and the seabed under such vessels.

**Diver ops**
Pairs of divers will search the various structures around the harbour. There will be need to access most areas on the Aluminium/Tinto Jetty as well as Terminals 1-5 and the fish dock as well as areas of rocky seabed near to the marina and along the harbour wall. We will also need to investigate any vessels that have been on moorings for extended periods of time (e.g. immediately north and NW of the marina) and any other commercial craft such as the jack up Fugaro crane 'Excalibur' which has been moored in the NW of the harbour against the harbour wall for several weeks. There will be at least one surface support person and a supervisor on the surface at all times to help with kit and entering and exiting the water plus at least two other people present who can assist with the diving operations and take notes of the divers' findings.

## Additional background information

This non-native, colonial seasquirt is known to smother man-made objects such as pontoons, pilings, ropes, fish cage nets and mooring blocks etc. It can also be highly invasive on mussel and oyster beds and will smother them causing widespread mortality – this has already happened in New Zealand, USA and Canada.
See http://www.biosecurity.govt.nz/didemnum.

This species appears to have just 'arrived' in the UK and Ireland and there are also reports of this species in Plymouth and the River Dart in Devon. It was found in Holyhead Marinajust before Christmas 2008 and specimens have been sent to experts in USA who have confirmed its identity as *Didemnum vexillum*.

**List of qualified divers: (personal details have been part-deleted in this report)**

| | HSE Med Exp Date | HSE Med Number | Dive Cert | Cert Number | Phone |
|---|---|---|---|---|---|
| Harry Goudge | 15/04/2011 | 85*** | HSE PSD | SC/099846*** | 07849490*** |
| Liz Morris | 13/04/2011 | 85*** | HSE PSD | SC/099848*** | 07868705*** |
| Andrew Johnson | 01/04/2011 | 85*** | HSE PSD | SC/09954*** | 07817784*** |
| Hanna Nuuttila | 26/10/2010 | 63*** | PADI OWSI | 951*** | 07554567*** |
| Ben Wray | 22/01/2011 | 82*** | PADI OWSI | 461*** | 07515960*** |
| Rowan Abel | 22/01/2011 | 82*** | HSE PSD | SC/10027*** | 07815933*** |
| Mandy Knott | 29/01/2011 | 82*** | BSCA Advanced | A691*** | 07789793*** |
| Steve Barnard | 26/01/2011 | 82*** | PADI IDCS | 642*** | 07515447*** |
| Rodrigo Reis | 29/01/2011 | 82*** | PADI DM | 980*** | 07530514*** |
| Jack Egerton | 13/04/2011 | 83*** | HSE PSD | SC/3989*** | 07940713*** |
| Ashley Cordingley | 31/03/2011 | 85*** | HSE IV/ PADI DM | SC/10072*** | 07948516*** |
| Philip Hart | 05/10/2011 | 85*** | BSAC Advanced | 3/10/2*** | 07967431*** |
| Lucy Kay | 04/04/2011 | 85*** | HSE IV | 4/095*** | 01248373*** |
| Jonathan Easter | 16/04/2011 | 85*** | PADI DM | *** | 07799753*** |
| Rohan Holt | 04/04/2011 | 83*** | HSE IV / BSAC 1st class | Part IV/011*** | 07788910*** |

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Version: 27th October 2010

## APPENDIX V *Didemnum vexillum* alert poster



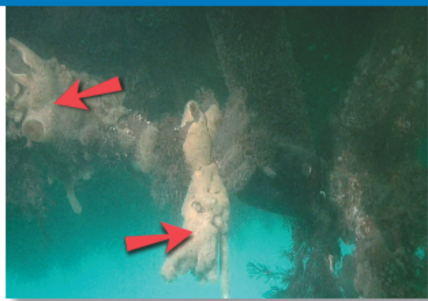
# Non-Native Marine Species Alert

## CARPET SEA SQUIRT

*Didemnum vexillum*

### What is *Didemnum vexillum*?

*Didemnum vexillum* (common name the Carpet Sea Squirt) is an invasive non-native species which has recently been found in North Wales. Thought to be originally from Japan it has become a pest in other countries because it grows very quickly and can smother habitats and species and interfere with fishing, aquaculture and other activities.

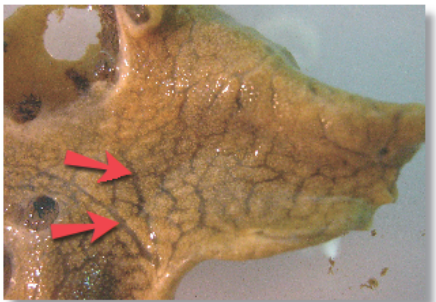


*Didemnum vexillum* (Carpet Sea Squirt) which has colonised a propeller shaft (Fig 1)

### What does it look like?

The Carpet Sea Squirt can be difficult to identify as it looks similar to some native species, but the following characteristics can help to identify it;

- The surface has a leathery texture (it is not slimy like other sea squirts) and has a noticeable veined surface (Fig 2).
- It has an orangey or mustard/tan colour which is fairly distinctive from our native species.
- It can grow either as thin flexible sheets, often overgrowing other species, or in long rope-like growths (Fig 1).



The visible distinctive water channels (fig 2)

### How does it spread?

The Carpet Sea Squirt releases larvae which can settle close to the parent colony or potentially be spread on marine equipment e.g. trailers and dinghies. It can also spread to new areas on the hulls of infected boats and fragments can easily reproduce.

### How can I help to stop it spreading?

- Keep your hull and marine/fishing equipment clean and free of fouling and treated with an appropriate anti-fouling paint.
- When travelling in the UK keep an eye out and report any sightings of this or other invasive species (see **www.thegreenblue.org.uk**) to the CCW enquiry line given below.
- If you are a berth-holder in North Wales please check your hull and fishing/marine equipment for signs of fouling.
- Use a closed loop or filtered wash down facility and/or steam clean your hull if needs be.
- If you do remove fouling i.e. weed, please dispose of it carefully and do not allow any to go back in the water.

Carpet Sea Squirt colony growing on the hull of a heavily fouled vessel

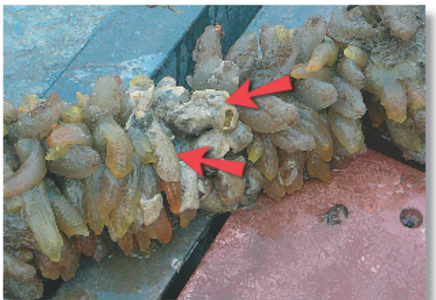
### What do I do if I think I have found the Carpet Sea Squirt?

Please contact the CCW enquiry line, **please do not try to remove any Carpet Sea Squirt while your vessel is in or near the water and do not move or take your boat out of the marina,** if you do the species may spread to other areas.

**For more information or to report any sightings please call the CCW enquiry line on**

## 0845 1306 229

**For a full fact sheet on invasive species visit our website www.thegreenblue.org.uk and follow links under 'You and your boat'**



Colonies of Carpet Sea Squirt growing on native species

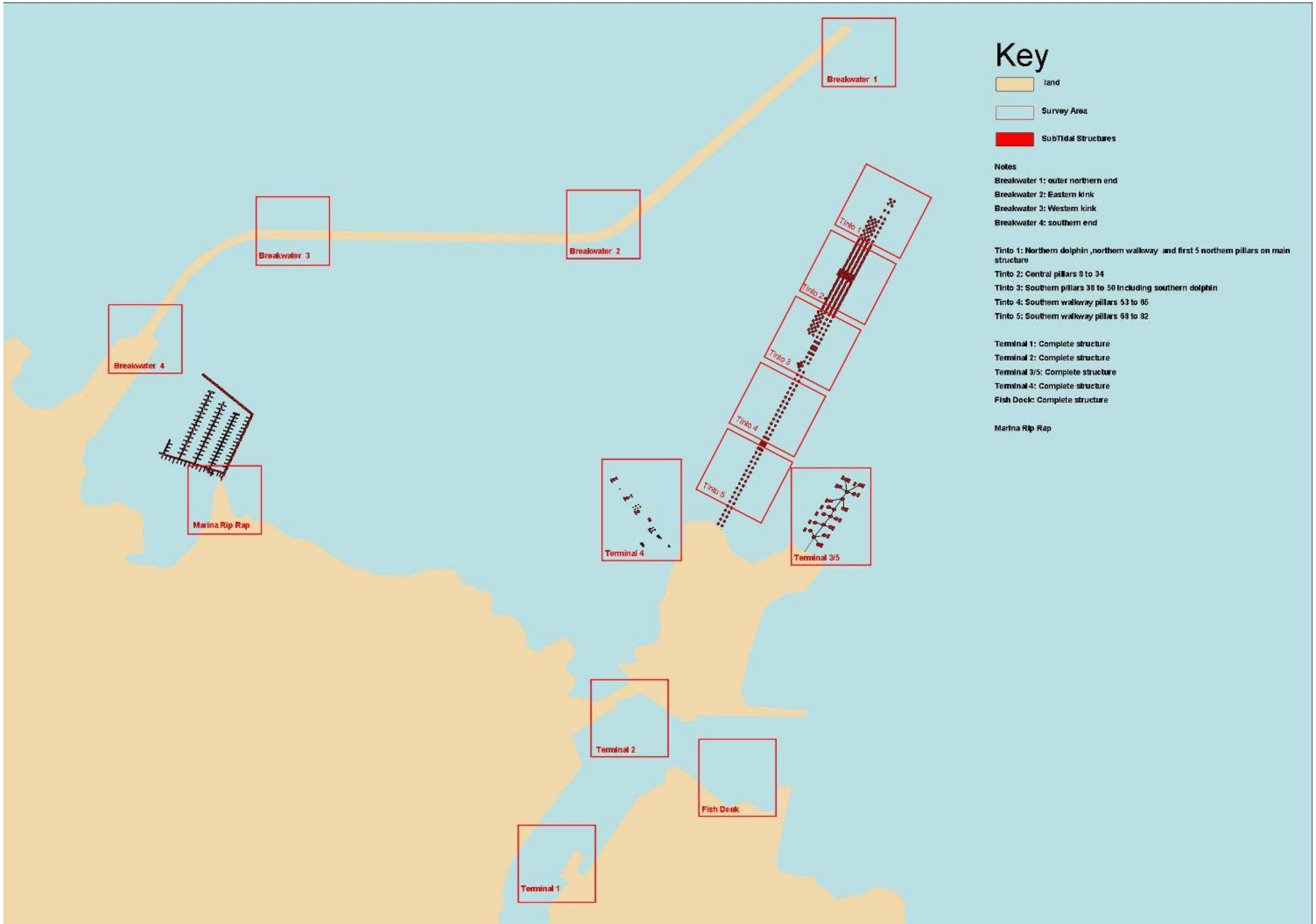
The Green Blue
Making the environment second nature

# APPENDIX VI DIVE LOGS

| Dive | | Diver | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | AC | RH | HG | LM | SB | RC | NH | HN | AJ | MK | RA | MS | KR | BW | JE | CL | Total |
| Date | Time | Total hrs | 67.7 | 40.0 | 26.6 | 10.3 | 43.0 | 16.2 | 14.3 | 12.6 | 21.3 | 4.8 | 3.0 | 1.5 | 1.2 | 15.8 | 5.3 | 3.1 | 286.5 |
| 23/09/2009 | Time | | | 120 | 154 | 19 | | | | | | | | 91 | | | | | |
| 09/10/2009 | Time | | 90 | 90 | | | | | | | | | | | | | | | |
| 23/10/2009 | Time | | 125 | 145 | | | | | | | | | | | | | | | |
| 30/10/2009 | Time | | 65 | | 75 | | | | 45 | | | | | | | | | | |
| 04/11/2009 | Time | | 65 | | | 110 | | 65 | 110 | | | | | | | | | | |
| 05/11/2009 | Time | | 155 | | | | | 155 | 140 | | | | | | | | | | |
| 06/11/2009 | Time | | 60 | | | | | | 60 | | | | | | | | | | |
| 09/11/2009 | Time | | 150 | | | | | 150 | 150 | | | | | | | | | | |
| 10/11/2009 | Time | | 100 | | | | | 100 | 100 | | | | | | | | | | |
| 11/11/2009 | Time | | 78 | | | | | 78 | 78 | | | | | | | | | | |
| 17/11/2009 | Time | | 140 | 45 | | | | 140 | 45 | | | | | | | | | | |
| 18/11/2009 | Time | | | 60 | | | | | 60 | | | | | | | | | | |
| 30/11/2009 | Time | | 45 | | | | | 60 | 15 | | | | | | | | | | |
| 01/12/2009 | Time | | 15 | | | | | | 15 | | | | | | | | | | |
| 09/12/2009 | Time | | | | 86 | | | 86 | | | | | | | | | | | |
| 10/12/2009 | Time | | 125 | 60 | | | | 120 | | | | | | | | | | | |
| 16/12/2009 | Time | | 38 | | | | | | 38 | | | | | | | | | | |
| 17/12/2009 | Time | | 20 | 20 | | | | 20 | | | | | | | | | | | |
| 13/01/2010 | Time | | | 46 | 46 | | | | | | | | | | | | | | |
| 21/01/2010 | Time | | 90 | 90 | 105 | | | | | 105 | | | | | | | | | |
| 28/01/2010 | Time | | 160 | 160 | 125 | 125 | | | | 135 | 135 | | | | | | | | |
| 04/02/2010 | Time | | 165 | 105 | 140 | | | | | | 100 | | | | | | | | |
| 16/02/2009 | Time | | 30 | 30 | | | | | | 70 | | | | | 70 | | | | |
| 19/02/2010 | Time | | 140 | 140 | | | | | | 145 | | | | | | 145 | | | |
| 25/02/2010 | Time | | 85 | 85 | | | | | | | | | | | | | | | |
| 05/03/2010 | Time | | | | | | 90 | | | | 90 | | | | | | | | |
| 04/03/2010 | Time | | 40 | 60 | | | 140 | | | | 140 | | 90 | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 08/03/2010 | Time | | | | 131 | | 131 | | | | | | | | | | | |
| 12/03/2010 | Time | | 90 | | | | 150 | | | 125 | 150 | 65 | | | | | | |
| 16/03/2010 | Time | | 125 | | | | 100 | | | 60 | | | | | | | | |
| 24/03/2010 | Time | | 35 | 105 | | | 105.00 | | | | | | | | | | | |
| 26/03/2010 | Time | | 126 | 85 | | 74 | 201 | | | | | | | | | 159 | | |
| 30/03/2010 | Time | | 90 | 75 | | | 75 | | | | 90 | | | | | | | |
| 06/04/2010 | Time | | 45 | 45 | | | 70 | | | | 70 | | | | | | | |
| 08/04/2010 | Time | | 65 | 80 | | | | | | 65 | | | | | | 80 | | |
| 09/04/2010 | Time | | 50 | | | | | | | | 50 | | | | | | | |
| 12/04/2010 | Time | | | | 155 | | 175 | | | | 175 | | | | | 155 | | |
| 13/04/2010 | Time | | 90 | | 175 | 175 | 90 | | | | | | | | | | | |
| 14/04/2010 | Time | | 165 | | 175 | | 205 | | | | | 160 | | | | | | |
| 15/04/2010 | Time | | 90 | 125 | 70 | 70 | 125 | | | | 90 | | | | | | | |
| 16/04/2010 | Time | | | | | | 125 | | | | 125 | | | | | | | |
| 13/04/2010 | Time | | | | | | 200 | | | | | | 90 | | | 115 | | |
| 20/04/2010 | Time | | 61 | | 61 | | 70 | | | | | | | | | | 70 | |
| 27/04/2010 | Time | | 155 | | | | 155 | | | | | | | | | | | |
| 29/04/2010 | Time | | 105 | | 100 | | | | | 50 | | | | | | 100 | | |
| | Time | | | | | | | | | | | | | | | | | |
| 04/05/2010 | Time | | 65 | 52 | | | | | | | | | | | | | 65 | 52 |
| 05/05/2010 | Time | | 95 | 76 | | | | | | | | | | | | | 95 | 76 |
| 06/05/2010 | Time | | 85 | 56 | | | | | | | | | | | | | 85 | 56 |
| 11/05/2010 | Time | | 96 | 96 | | | | | | | 60 | 60 | | | | | | |
| 14/05/2010 | Time | | 300 | 120 | | 45 | 190 | | | | | | | | | | | |
| 25/05/2010 | Time | | 75 | 110 | | | 110 | | | | | | | | | 75 | | |
| 26/05/2010 | Time | | 70 | 120 | | | 70 | | | | | | | | | 120 | | |

# APPENDIX VI DIVE SURVEY LOCATIONS



# APPENDIX VII MARINE NON NATIVE SPECIES IN WELSH WATERS APRIL 2010

| Species | Common name | Habitat | Distribution | Source of records | Grid ref for first record | Date of first record | Invasive? | Comments |
|---|---|---|---|---|---|---|---|---|
| **Plants** | | | | | | | | |
| *Anotrichium furcellatum* | A red alga | Shallow fully marine bay. | Milford Haven | Pembrokeshire Marine Species Atlas (1997), CCW survey of tidal rapids (2002) | SM942030 | 20 June 1996 | Spread from 2 records in 1996/7, to 6 sites in 2002 | |
| *Antithamnionella spirographidis* | A red alga | same | West Pembs, Pen Lleyn and North West Anglesey | OPRU survey, CCW Intertidal survey, NBN. | SM736050 (1m sq) | 1966 | Unknown. | First recorded in Pembrokeshire. Not recorded in North Wales until 1996 |
| *Antithamnionella ternifolia* | A red alga | same | West Pembs and Tremadog bay | Pembrokeshire Marine Species Atlas (1956), CCW Intertidal survey, NBN. | SM733066 | 25 Sept 1956 | Unknown | First recorded in Pembrokeshire in 1956. Not recorded in North Wales until 1999 |
| *Asparagopsis armata* | Harpoon weed | Infralittoral fully marine rocky cobbles | Pembrokeshire and Pen Lleyn | Various (1983) see NBN Gateway | SH214257 | 22/08/1983 | unknown | 1 records for north Wales and 1 vague record for South Wales |
| *Bonnemaisonia hamifera* | A red alga | same | Gower to NW Anglesey | Various see NBN Gateway | SH257756 | 1953 | unknown | |
| *Codium fragile* subsp. *atlanticum* | Green sea fingers | Lower shore and shallow subtidal | Glamorgan to NW Anglesey. | CCW Intertidal survey | SH12 | 1963 | Can displace native *Codium*, but not highly invasive. Maggs and John 2007 believe that this subsp. is considered native. | Unspecified grid reference. 1st detailed record occurs at Penmon (1977) |
| *Codium fragile* subsp. *tomentosoides* | Green sea fingers | Lower shore and shallow subtidal | Glamorgan to NW Anglesey. | CCW Intertidal survey | SH332313 | 01/07/1995 | Can displace native Codium, but not highly invasive. | |
| *Colpomenia peregrina* | Oyster thief | Intertidal rock pools | Swansea Bay, Gower, West Pembs, Pen Lleyn and NW Anglesey | Various inc. CCW Intertidal survey and NBN Gateway | SH257756 | 1953 | Not known to be | |

| Species | Common name | Habitat | Distribution | Source of records | Grid ref for first record | Date of first record | Invasive? | Comments |
|---|---|---|---|---|---|---|---|---|
| *Feldmannophycus okamurae* | A red alga (pom pom weed) | common in the midshore amongst *Osmundea pinnatifida* | West Angle Bay, south side, South Hook Point | CCW Intertidal monitoring survey | GR: SN 850032 and GR: SN 870054 | 8 September 2010 | ? | Found by Francis Bunker, Anne Bunker, Paul Brazier, Lucy Kay at West Angle and Tom Mercer at South Hook Point. |
| Grateloupia turuturu (= G. doryphora) | A red Alga | Lower shore rockpools | Milford Haven | New record for Milford Haven from Porcupine excursion (2008) | SN006068 | 1995 | Yes, locally | |
| *Heterosiphonia japonica.* | A red alga | subtidal | North Anglesey and Tremadog Bay | Seasearch 2009 | SH245833 | 04/04/2009 | unknown | |
| *Polysiphonia harveyi* | A red alga | same | West Pembs and Tremadog bay | Various see NBN Gateway & Marine Recorder | SM70 | 1956 | Can become very abundant, but hasn't spread widely in Wales | 2 early records with few details. 1st detailed record Sarn Badrig 15/08/1998 |
| *Sargassum muticum* | Jap weed | Lower shore rockpools and shallow subtidal | Swansea Bay round to East Anglesey | CCW Intertidal survey | SM859142 | 1997 | Yes, has spread very fast in Wales over the last 11 years. | |
| *Solieria chordalis* | A red alga | | Milford Haven | MNCR surveys 1985, CCW Intertidal survey | SM967043 | 11/10/1978 | unknown | |
| *Spartina anglica* | Common cord grass | Saltmarsh | Whole coast of Wales where suitable habitat. | Various see NBN Gateway | SS4090 | 1925? Definite record 21/07/1956 | Yes, but in recent years has become less vigorous. | |

| Species | Common name | Habitat | Distribution | Source of records | Grid ref for first record | Date of first record | Invasive? | Comments |
|---|---|---|---|---|---|---|---|---|
| **Animals** | | | | | | | | |
| *Balanus amphitrite* | A barnacle | Hard substrata | W. Pembs. | Pembrokeshire Marine Species Atlas | SM990121 | 06/06/1960 | unknown | 1 record only |
| *Bonamia ostreae* | Protozoan | Parasite of Oysters | Milford Haven | CEFAS monitoring (ref needed) | | 2005/6? | | Lethal to native Oysters, was/is widespread in the UK. |
| *Botrylloides cf. diegense* | A sea squirt | Mytilus edulis | Burry Port | Judith Oakley 17th July 2009 | SN445002 | 17/07/2009 | unknown | |
| *Botrylloides violaceus* | A sea squirt | Lower shore subtidal rocky boulder | Milford haven | Judith Oakley 2005 | SM967054 | 12/07/2005 | Fairly high abundance where found in Neyland marina Milford Haven | |
| *Bugula neritina* | bryozoan | Hard substrata intertidal and subtidal | Milford haven and Holyhead | CCW report Survey of North Wales and Pembrokeshire Tide Influenced Communities"and R.Holt pers com | SM741094 | 21/06/1995 | ? | |
| *Caprella mutica.* | Amphipod | Shallow subtidal | Milford Haven marina | 23rd September 2009 new record from Judith Oakley 2003 Anglesey identified by MPA project | SM901057 | 23/09/2009 | unknown? | |
| *Corella eumyota* | A sea squirt | Hard substratum subtidal | Milford Haven | New record by John Ryland, Porcupine excursion 2008 | | 2008 | No | |
| *Corophium sextonae* | An amphipod | Shallow subtidal | W. Pembs, Llyn Peninsula, East Anglesey | Various inc. Pembrokeshire Marine Species Atlas | SM794314 | 06/06/1973 | unknown | |

| Species | Common name | Habitat | Distribution | Source of records | Grid ref for first record | Date of first record | Invasive? | Comments |
|---|---|---|---|---|---|---|---|---|
| *Crassostrea gigas* | Portugese oyster | Lower shore and shallow subtidal | Severn, Milford Haven and Anglesey. | CCW Intertidal survey | SN013059 | Pre 1900 | Yes, Although not previously thought to be invasive this species has started forming razor sharp reefs in northern Europe overgrowing native species. | |
| *Crepidula fornicata* | American slipper limpet | Lower shore muddy gravel and mussel beds. | Milford haven, Carmarthen Bay. | CCW Intertidal survey. Marine Recorder, Ad-hoc records | SN006107 | 1906/06/06 (1 record until 1953 onwards) | Yes, has been found in South Wales since at least the 1960s, was recently introduced into North Wales via mussel lays, but an eradication programme here is ongoing. The species threatens oyster and mussel beds by overgrowing them. | |
| *Didemnum vexillum* | A colonial sea squirt | Shallow subtidal | North Anglesey | Stuart Jenkins pers comm. October 2008 | SH224835 | 2008 June | Yes, A highly invasive sea squirt that has recently been found in North Wales. It is known to overgrow native species very rapidly. | |
| *Dikerogammarus villosus* | Killer shrimp | Fresh and brackish water | South Wales | Environment Agency | ST1921573501 | 25/11/2010 | Yes, will kill native gammarid species | |
| *Elminius modestus* | Australasian barnacle | Intertidal open coast and estuaries on rock/cobbles. | Whole coast of Wales | PMSA & Marine Recorder,<br>CCW intertidal survey | SN5520 | Pre 1900<br><br>10/08/1957 | Yes (?) Now widespread around Wales, has displaced native barnacles in some habitats but in others such as estuaries has colonised new areas. | |

| Species | Common name | Habitat | Distribution | Source of records | Grid ref for first record | Date of first record | Invasive? | Comments |
|---|---|---|---|---|---|---|---|---|
| *Eriocheir sinensis* | Chinese mitten crab | River banks and shallow off shore areas. | Dee Estuary and River | Huw Jones (2005) EA Wales pers comm.. EA Fisheries – Max Gooch | SJ238756 | Oct 2010 | Yes, this species has recently spread into Wales via the Dee Estuary. It is known to cause damage to flood banks and feeds on fish eggs (amongst other things). | |
| *Ficopomatus enigmaticus* | A tubeworm | Shallow subtidal on hard substrata | Severn, Swansea Bay and Pembs | CCW intertidal survey, NBN | SM809068 | 06/06/1960 | Not known to be | |
| *Goniadella gracilis* | A polychete | Subtidal | Offshore from Anglesey down to Pembs. | Various see NBN Gateway | SM685576 | 12/07/1989 | unknown | |
| *Haliplanella lineata* | Orange striped anemone | Lower shore pools and shallow subtidal | Severn, Pembs and Anglesey | CCW Intertidal survey, NBN Gateway, CCW Marine recorder | SM794314 | 1973/06/06 | unknown | |
| *Mercenaria mercenaria* | American hard shelled clam | Muddy gravels and sandy mud, lower shore and subtidal | Western Pembs and Anglesey | Various see NBN gateway | SH302598 | 19/04/1973 | Not known to be | |
| *Mya arenaria* | Sand Gaper clam | Estuaries | Most of the estuaries in Wales, no records for the Severn. | CCW Intertidal survey | SN327089 | Pre 1900<br><br>05/05/1899 | unknown | |
| Mytilicola intestinalis | parasitic copepod | Parasitic in mytilus edulis | Milford Haven | PMSA | SN006107 | 06/06/1906 | Not known to be | |
| Mytilopsis leucophaeta | False dark mussel | ? | Cardiff Docks | Welsh Inverts Database record | ST17 | 1998 | Not known to be | |
| *Mytilus galloprovincialis* | mollusc | ? | Milford Haven, Saundersfoot | PMSA 1960's records, also on the NBN. | SN144048 | 05/05/1899 | ? | Hybridises readily with *M.edulis* |
| *Perophora japonica* | sea squirt | subtidal | Milford haven | CCW report Survey of North Wales and Pembrokeshire Tide Influenced Communities" | SM831056 | 09/04/2006 | ? | |

| **Species** | **Common name** | **Habitat** | **Distribution** | **Source of records** | **Grid ref for first record** | **Date of first record** | **Invasive?** | **Comments** |
|---|---|---|---|---|---|---|---|---|
| *Petricola pholadiformis* | American Piddock | Clay and Peat exposures. | Gower East Anglesey, Dee | Various see NBN Gateway | SS4189 | Pre 1900.<br><br>06/05/1978 | unknown | |
| *Potamopyrgus antipodarum* | Jenkin's spire shell | Freshwater and upper estuaries | Widespread around Wales | Various see NBN Gateway | SN5520 | Pre 1900<br><br>02/07/1949 | Not known to be | |
| *Rhithropanopeus harrisii* | Dwarf crab | | Roath Docks Cardiff | Eno et al | ST2075 | 1996 | ? | |
| *Styela clava* | Leathery seasquirt | Lower shore rockpools and shallow sublittoral. | Swansea Bay, Milford Haven, Tremadog Bay and Anglesey. | CCW Intertidal survey,<br>Marine Recorder, Ad-hoc | SM921033 | 109/09/968 | Yes (?) will compete with native species for food and space, but not spreading fast in Wales ? | |
| *Tiostrea lutaria* | New Zealand oyster | Lower shore, shallow subtidal | Menai Strait. | CCW Intertidal survey | SH491662 | 1960?<br>20000913 | Not known to be | |
| *Tricellaria inopinata* | Bryozoan | Hard substratum subtidal | Milford Haven | New record by John Ryland, Porcupine excursion 2008 | SM9605 | 2008 | Possibly? | |