

**Survey of a non-native ascidian (sea squirt)
Didemnum vexillum in Holyhead Harbour
February 2009**

**Rohan Holt, Kirsten Ramsay, Stephen Mowat,
Flora Kent and Kate Griffith**

CCW Marine Monitoring Report No. 67



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

Recommended citation for this volume:

Holt, R.H.F., Ramsay, K., Mowat, S., Kent, F.E.A. and Griffith, K. (2009). Survey of a non-native ascidian (sea squirt) *Didemnum vexillum* in Holyhead Harbour. **CCW Marine Monitoring Report No: 67.**

Report series: Marine Monitoring
Report number: 67
Publication date: February 2009
Lead officer: **Rohan Holt**
Title: Survey of a non-native ascidian (sea squirt) *Didemnum vexillum* in Holyhead Harbour.
Authors: Rohan Holt, Kirsten Ramsay, Stephen Mowat, Flora Kent and Kate Griffith
Series editors: Bill Sanderson
Restrictions: None

Distribution list (core):

CCW HQ Library, Bangor
CCW North Region Library, Mold
CCW North Region Library, Bangor
CCW West Region Library, Aberystwyth
CCW West Region Library, Pembroke Dock

National Library of Wales, Aberystwyth
NH Museum, London
University of Aberystwyth Library
University of Swansea Natural Science Library
University of Cardiff Library
PML Library, Plymouth
SAMS Library, Dunstaffnage

Welsh Assembly Government Library
Joint Nature Conservation Committee Library
Scottish Natural Heritage Library
Natural England Library
British Library

B. Cook, NW & NWSFC
Phil Coats, SWSFC

Summary

In the summer of 2008 a student carrying out a survey of non-native species in Holyhead Marina observed an unfamiliar colonial ascidian covering algae, mussels and other submerged substrata such as pontoons and ropes. A taxonomic expert confirmed the colonial ascidian or sea squirt as *Didemnum vexillum*, making it the first reported presence of this species in the UK.

D. vexillum is an invasive fouling species with rapid growth and mat-forming capabilities that colonises artificial and natural hard substrata. It has been reported in temperate waters worldwide with significant conservation and economic consequences through the alteration of marine habitats and damage to mariculture and fisheries, especially shellfisheries.

In December 2008, the Countryside Council for Wales undertook a diving survey of Holyhead Marina. *D. vexillum* 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 when it was introduced. Two vessel hulls were fouled with *D. vexillum*. These vessels have been scheduled for removal from the water and will be cleaned to prevent the dispersal of *D. vexillum*.

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 habitat suitable for *D. vexillum* within the harbour. Rapid assessment surveys of other marinas and ports across Wales found no other areas of colonisation.

Within Holyhead Marina *D. vexillum* has not yet exhibited the highly invasive and damaging colonisation that has been experienced in other temperate regions but there remains a threat that *D. vexillum* will proliferate through asexual and sexual reproduction. Recreational vessels may act as vectors for dispersal throughout the UK and should it spread to the commercial side of the harbour, beyond the marina, it increases the risk of fouling the hulls of trans-global shipping.

The survey has highlighted the need for the continued monitoring of the marina and harbour area to detect any increase in abundance or spread of *D. vexillum*.

Crynodeb

Yn ystod haf 2008, gwelodd myfyriwr a oedd yn cynnal astudiaeth ar rywogaethau anfrodorol ym Marina Caergybi chwistrell fôr a orchuddiai alga, misglod ac is-haenau tanddwr eraill, fel pontynau a rhaffau. Cadarnhaodd arbenigwr mewn tacsonomeg mai *Didemnum vexillum* oedd y chwistrell fôr. Dyma'r tro cyntaf i'r rhywogaeth hon gael ei chofnodi yn y DU.

Rhywogaeth ymledol sy'n tyfu ar strwythurau yn y môr yw *D. vexillum*. Mae'n tyfu'n gyflym, gan ffurfio carpedi sy'n cytrefu ar is-haenau caled naturiol ac artiffisial. Mae'r rhywogaeth wedi'i chofnodi mewn dyfroedd tymherus ar draws y byd ac mae wedi esgor ar ganlyniadau cadwraethol ac economaidd sylweddol trwy addasu cynefinoedd morol a thrwy niweidio môr-amaeth a physgodfeydd, yn arbennig pysgodfeydd cregyn.

Fis Rhagfyr 2008, cynhaliodd Cyngor Cefn Gwlad Cymru arolwg deifio ym Marina Caergybi. Daethpwyd o hyd i *D. vexillum* drwy'r marina mewn cytrefi bach gwasgaredig; ond gan na welwyd unrhyw 'ganolbwynt' amlwg lle gallai fod wedi cytrefu'n gyntaf, nid oedd modd darganfod pryd y cafodd y rhywogaeth ei chyflwyno. Gwelwyd *D. vexillum* ar gyrff dwy o llongau. Bydd y llongau hyn yn cael eu symud o'r dŵr cyn bo hir, a byddant yn cael eu glanhau er mwyn rhwystro *D. vexillum* rhag ymledu.

Yn ystod Ionawr 2009, cadarnhaodd arolwg deifio yn ardal ehangach Harbwr Caergybi – gan gynnwys terfynellau fferi a dociau llongau rhyngwladol – fod y rhywogaeth *D. vexillum* wedi'i chyfyngu i'r marina. Fodd bynnag, dangosodd yr arolwg fod cynefin mawr addas i'w gael ar gyfer *D. vexillum* o fewn yr harbwr. Hyd yn hyd, nid yw arolygon sydd wedi'u cynnal mewn marinas eraill yng Ngogledd Cymru wedi dod o hyd i gytrefi eraill. Ni ddaeth yr arolygon brysiog ar farinas a phorthladdoedd eraill ar draws Cymru o hyd i gytrefi eraill.

O fewn Marina Caergybi, nid yw *D. vexillum* wedi cytrefu yn y modd ymledol a niweidiol a welwyd mewn rhanbarthau tymherus eraill, ond ceir bygythiad o hyd y gall *D. vexillum* ymledu trwy atgynhyrchu anrhywiol a rhywiol. Gall llongau hamdden gynorthwyo'r rhywogaeth i wasgaru drwy'r DU i gyd. Pe bai'n ymledu i ochr fasnachol yr harbwr, y tu hwnt i'r marina, fe allai dyfu ar gyrff llongau a fyddai'n teithio ar draws y byd.

Mae'r arolwg yn tynnu sylw at yr angen i barhau i fonitro'r marina a'r harbwr fel y gellir gweld a yw *D. vexillum* yn ymledu neu'n cynyddu mewn nifer yno.

Background / introduction

Didemnum vexillum is an invasive colonial ascidian that has become established world-wide having been identified in Japan, North America, northern Europe, and New Zealand, following a global expansion since the 1970's (see Lambert, 2009 for review). It forms sheet like colonies on natural and artificial hard substrata as well as benthic organisms including other ascidians and algae. 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 Whitlatch, 2009) as well as rapid response, monitoring and management following introductions (Coutts and Sinner, 2004; Pannell and Coutts, 2007; Locke and Hanson, 2009).

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 *Didemnum vexillum*. A specimen was sent to a UK expert, who confirmed in November 2008 that it was very likely to be *D. vexillum*. Specimens were confirmed by Gretchen Lambert (pers. com. University of Washington, USA) as being *D. vexillum*.

Following a meeting between staff from CCW and Bangor University, we have developed a plan for assessing the scale of the problem and providing advice relating to the possible eradication and/or control of this species.

Part of that plan was to survey Holyhead Harbour and other harbours and marinas in the area and to work with an expert to confirm the taxonomy of the species. We have also commissioned a study outlining the possibilities for eradication and/or control of the spread of the species.

This field report is a summary of the results of a diving survey in Holyhead Marina on the 9th and 10th of December 2008 and a second wave of diving in Holyhead Harbour on 20th and 21st January 2009. The report also details the results of a series of marina/port assessment surveys carried out at locations around Wales between December 2008 and February 2009.

Winter weather - high winds and rough sea-state have prevented any diving work taking place outside the harbour area so far.

Method

Diving survey in the marina

Two divers were deployed in Holyhead Marina (see location map **Figure 1**) with the aim of viewing as wide a variety of habitats and substrata as possible. Each diver worked independently, each being attached by a safety line to their own personal ‘rope tender’. Each diver wore full face masks with through-water communications systems which were used to report back to a person recording the divers’ positions and data ‘live’.



OS base maps reproduced with permission of HMSO. Crown copyright reserved. CCW licence No. 100018813

Figure 1. Aerial view of Holyhead Harbour where **◆** indicates survey location.

The route the divers took around the marina is illustrated in **Figure 2**.

The divers aimed to record the presence and approximate abundance of *Didemnum vexillum* from:

- Sides of pontoons (no deeper than approx 1.5-2 m below sea level (bsl))
- Undersides of pontoons (approx 2 m bsl)
- Pontoon mooring chains and ropes (2-5m bsl)
- Concrete pontoon mooring blocks (3-5 m bsl)
- Hulls of vessels in the marina
- Seabed below the marina (3-6 m bsl)
- Rocky substrata under the walkway bridge at the entrance to the marina.

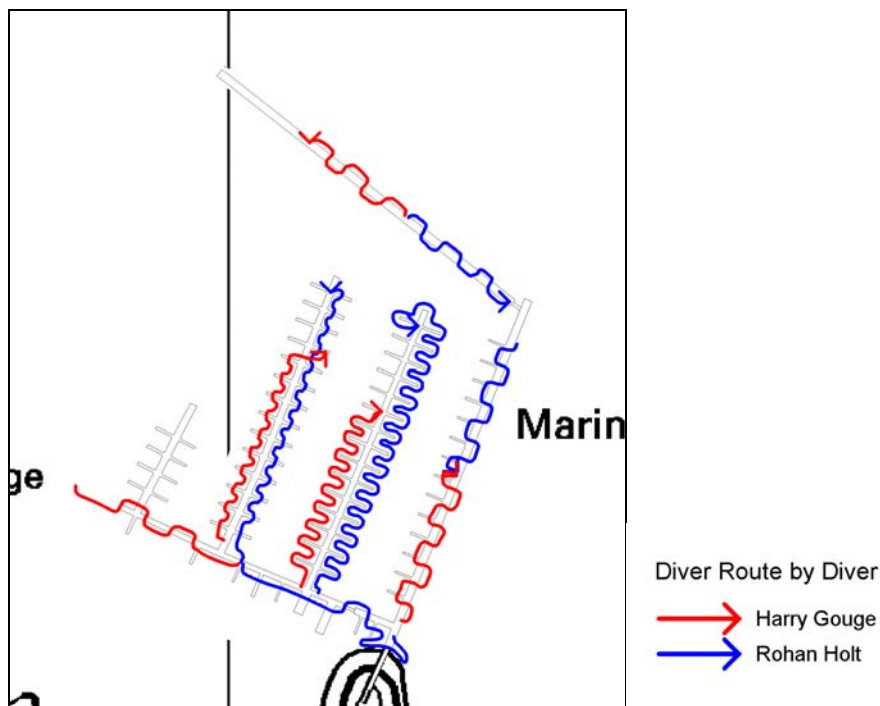


Figure 2: Route taken by the two divers around the marina

Diving survey of the ferry terminals, Fish Dock and large moorings

The other structures surveyed around the harbour included the large steel piles driven into the seabed that form the 'legs' of the ferry terminals. The piles are cylindrical, approximately 2-3 m in diameter, and have a chart datum to seabed depth range of between approximately 4 m and 10 m; much of which is potential *D. vexillum* habitat. All dives were carried out by pairs of divers using full face masks with diver to diver and diver to surface through water voice communications kit. Where possible the boat was moored to adjacent structures / buoys although in some case it was necessary to keep the boat mobile and close by.

Survey dives were carried out as follows (see Figure 1 and Table 1 results):

- Terminal 1 – sea walls and 2 piles at the HSS berth (2 pairs of divers)(21 in Fig. 1).
- Terminals 3 and 5 – three rows of piles form two adjacent terminals used by the large Stena and Irish Ferries. One large pile on each terminal was surveyed by a single pair of divers (19 in Fig. 1).
- Terminal 4 – two dives: piles on the far end and landward end of the jetty using one pair of divers (14 and 15 in Fig. 1).

- Aluminium Jetty – the long jetty used for unloading bulk aluminium ore carriers. Three stations were surveyed by one pair of divers: the seaward end, the landward end of the berth and the landward end of the jetty and adjacent hard substrata (16-18 in Fig. 1).
- Fish Dock – floating pontoon adjacent to the navigable channel into the inner harbour (20 in Fig. 1).
- Large-vessel mooring buoys that have vertical heavy chains to attach to mooring blocks and/or chains on the seabed. Four buoys surveyed by two pairs of divers in the outer harbour between the terminals and the marina (Photo 10) (10-13 in Fig. 1).

Yacht club moorings

The yacht moorings to the east of the marina were examined by hauling the mooring ropes and steel cables aboard Pedryn's stern deck by hand at low water. This allowed inspection of each mooring's buoy, the surface to seabed rope attached to the steel cable and approximately 3-4 m of steel cable mooring. When not in use the steel mooring cables lie on the seabed at low water. Eight moorings were examined (2-9 in Fig. 1).

Specimens

Divers retrieved specimens by hand from a range of different locations and substrata. Those easiest to collect were found overgrowing the abundant solitary ascidians such as *Ciona intestinalis*, *Asciella aspersa*, *Styela clava* (photos 1-4) on the marina pontoon mooring chains and the permanently submerged sides of the pontoons. In such cases the 'host' ascidians were collected whole. Thin sheets of *D. vexillum* were also removed from the marina pontoon sides using a flat-bladed knife.

Samples of colonial ascidians were also taken during the second wave of diving from the jetty piles, Fish Dock pontoons and mooring chains. These were examined prior to preservation as described below.

All specimens were labelled on site with the position in the harbour (e.g. berth number in the marina or pile position on a particular terminal) and substratum.

Samples were narcotised using a few menthol crystals dissolved in the seawater in each specimen bag soon after collection and the specimens stored overnight in at approx 4°C in a fridge. The samples were then transferred to a freezer at -20°C for approximately 45-90 minutes (sufficient time for ice crystals to form but not freeze solid) and then the seawater drained off. The samples were then fixed with 4% buffered formalin. After several days in formalin the samples were transferred to 99% IMS.

A separate batch of samples was collected from the marina during the second wave of diving (January) with the intent to send them away for DNA analysis. Approximately 30 separate colonies were collected by two pairs of divers on pontoon E (eastern side of the marina - at berth E17 and adjacent berth south). Each colony was very briefly rinsed in tap or distilled water to remove the seawater, blotted on paper towels, then placed in 95% ethanol (not denatured). The ethanol was changed after 24 hours.

Photographs

Photos of the *D. vexillum* were taken *in situ* using a Nikon D70s with a 60 mm macro lens in a Seacam underwater housing with twin Seacam Seaflash 250 strobes. Two sets of still images were taken underwater (see Figure 3 for locations and photos 1-4).

Photos were also taken on land to illustrate the layout of the marina and methods used. All images are archived on MMT image DVD number 17 (see photos 5-8, 10).

Photos were taken using an LCD compound microscope (Bresser) of larvae extracted from the *Didemnum vexillum* samples collected in December 2008. See photo 9a and 9b.

Video

Several video sequences were taken using a high-definition Sony HDV 1080i video camera in a Light and Motion Blue-fin underwater housing lit by twin Sunray HID video lights. See Figure 3 for location of the video recordings and stills taken from the video (photos 7 and 8).

Marina and Harbour survey

Figure 3 shows the marina/port sites surveyed including Holyhead Marina (Site 1) and a further ten sites initially considered susceptible to the introduction of *D. vexillum* (see Table 2 for results). Sites 2-7 (Fig. 3) were initially investigated in December 2008 and January 2009 due to their close proximity to Holyhead Marina and the frequent interchange of vessels from the marina. Sites 8-11 (Fig. 3), further afield in west and south Wales, were examined between the 9th and 13th of February 2009.

At least two people visited each site to determine the presence of *D. vexillum*. All subtidal substrate that was visible from the pontoons was examined including pontoon surfaces, ropes, buoys and fenders. An underwater colour video surveillance system (Videotech VT-21111) with attached Sunray HID light and flexible handle was used to survey surfaces that could not be observed from the pontoons, such as the underside of pontoons, marina walls and pilings to 3 m below sea surface. All surveys were timed to coincide with low water to optimise the area of marina structures that could be searched.



Figure 3. Distribution of marina/harbour sampling sites around Wales, see also Table 2 (Results section) for locations and position

Results

Figure 4 summarises the results of the divers' findings for the area of the marina surveyed.

Overall distribution of *Didemnum vexillum* in the marina

Colonies of *D. vexillum* were found throughout most of the marina apart from on and around the newer pontoons on the western side. Abundance ranged from less than 1% cover to a maximum of approximately 10% cover. On Figure 4 'rare' = 1-5 % cover and 'occasional' = 5-10 %. There was no single area found that could be described as an 'epicentre' of colonisation although there tended to be slightly higher abundance at the southern and central third of the marina (southern thirds of pontoons C and D) as well on the northern arm of pontoons known as the 'east-west breakwater'.

Of the different habitats surveyed *D. vexillum* colonised the flat pontoon sides and perimeter of the undersides of the pontoons. It was also found on the mooring chains and in particular on the tests of solitary ascidians growing on the shallower parts of the mooring chains but not on the deeper sections of chain that get immersed in mud at low water of spring tides. It was not found on the rocky sea defences below the entrance walkway nor on any of the concrete mooring blocks. All the 'natural' seabed under the pontoons comprised fine silty mud that did not support any conspicuous fauna apart from a few hermit crabs and terebellid worms.

Boat hulls

At the time of the survey the marina was only half full (130 boats out of a capacity of approximately 250 boats when full). Of those present, only two boats were found to have heavily fouled hulls. Both were investigated by the divers and video recordings made.

The density of flora and fauna colonising both vessels indicated that neither had probably moved far in the last 12 months. Both had large *Saccharina latissima* sugar kelp plants and other algae, mature mussels *Mytilus edulis*, feather stars *Antedon bifida*, large solitary ascidians including *Styela clava*, *Asciidiella aspersa* and *Ciona intestinalis* as well as other encrusting fauna such as barnacles and bryozoans. *Didemnum vexillum* was found encrusting parts of the propeller shaft and the rudder on a yacht (see video stills) and also encrusting some of the mussels on a motor vessel's hull.

Outer harbour, ferry terminals, Fish Dock and moorings

In summary *Didemnum vexillum* was not found anywhere in the harbour outside the marina area (See **Table 1** and **Figure 1** for overview)

Samples of colonial ascidians collected from the mooring chains, piles and harbour walls from all the locations outside the marina were either *Lissoclinum perforatum* or other native species of colonial ascidian (including *Didemnum maculosum*). These samples are currently being examined by ascidian taxonomists.

Cross Wales Marina and harbour survey

Didemnum vexillum was not recorded at any of the survey sites (Sites 2-11; Table 2) other than Holyhead. Ascidian species (e.g. *Ciona intestinalis*) were recorded within some marinas and these findings are detailed in Table 2.

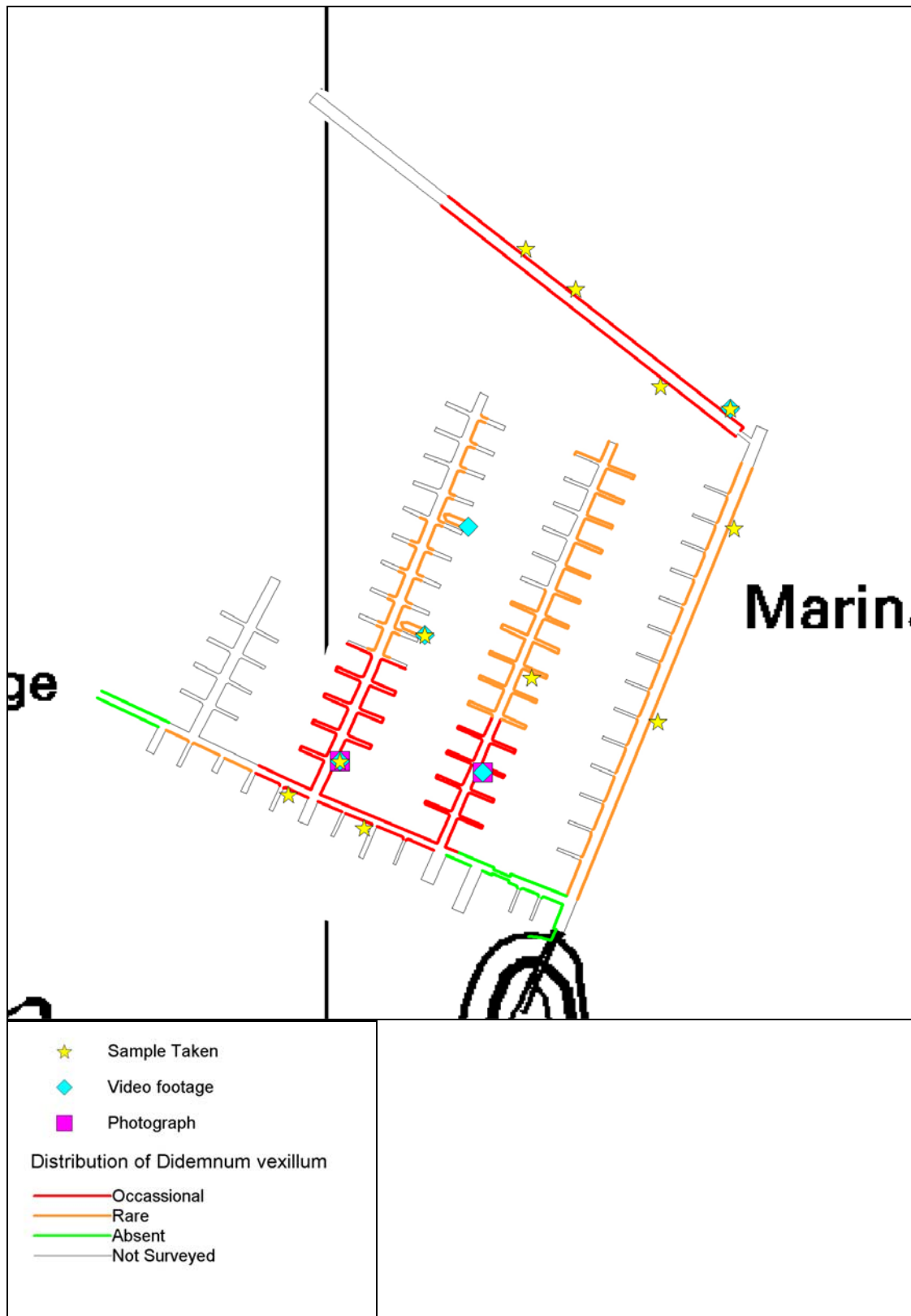


Figure 4. Summary of results from the diving survey in the marina

Survey site (Figure 1):	Location	Latitude decimal minutes (°N)	Longitude decimal minutes (°W)	Date	Divers	Didemnu vexillum Present/ Absent	Community	Substrate	Samples collected	Biomass on structure	Suitable for <i>D. vexillum</i> colonisation
1	Marina	53 19.173	04 38.488	10.12.08	RH HG	Present	Moderate-high species abundance and richness of filter feeding invertebrates and algae. Fringing kelp.	pontoons, chains, rope, boat hulls	Yes	High	Y
2	Yacht moorings	53 19.173	04 38.488	20.01.09	N/A	Absent	Sparse filamentous algae	wire cable		Low	N
3	Yacht moorings	53 19.152	04 38.488	20.01.09	N/A	Absent	Sparse filamentous algae	wire cable		Low	N
4	Yacht moorings	53 19.164	04 38.481	20.01.09	N/A	Absent	Sparse filamentous algae	wire cable		Low	N
5	Yacht moorings	53 19.194	04 38.411	20.01.09	N/A	Absent	Sparse filamentous algae	wire cable		Low	N
6	Yacht moorings	53 19.215	04 38.365	20.01.09	N/A	Absent	Sparse filamentous algae	wire cable		Low	N
7	Yacht moorings	53 19.215	04 38.262	20.01.09	N/A	Absent	Sparse filamentous algae	wire cable		Low	N
8	Yacht moorings	53 19.261	04 38.262	20.01.09	N/A	Absent	Sparse filamentous algae	wire cable		Low	N
9	Yacht moorings	53 19.285	04 38.333	20.01.09	N/A	Absent	Sparse filamentous algae	wire cable		Low	N
10	Large buoy	53 19.375	04 38.326	21.01.09	RH MB	Absent	Dense <i>Mytilus edulis</i> , <i>Saccharina latissima</i>	Heavy rope & chain	Yes	High	Y
11	Large buoy	53 19.116	04 38.114	20.01.09	BS KR	Absent	Abundant solitary and colonial ascidians	Heavy rope & chain	Yes	High	Y
12	Large buoy	53 19.116	04 38.028	20.01.09	MB KS	Absent	Abundant solitary and colonial ascidians	Heavy rope & chain	Yes	High	Y
13	Large buoy	53 19.175	04 37.952	20.01.09	BS KR	Absent	Abundant solitary and colonial ascidians	Heavy rope & chain	Yes	High	Y
14	Terminal 4	53 19.130	04 37.610	21.01.09	RH MB	Absent	Abundant solitary and colonial ascidians	metal piles & wood beams		High	Y
15	Terminal 4	53 19.204	04 37.734	21.01.09	RH MB	Absent	Abundant solitary and colonial ascidians	metal piles & wood beams		High	Y
16	Aluminium Jetty (shore end)	53 19.161	04 37.433	21.01.09	BS KL	Absent	Low density settlement	metal piles, mud and cobbles		Low	N
17	Aluminium Jetty (berth)	53 19.428	04 37.229	21.01.09	BS KL	Absent	Abundant solitary and colonial ascidians	metal piles	Yes	High	Y
18	Aluminium Jetty (seaward end)	53 19.038	04 38.776	21.01.09	BS KL	Absent	Abundant solitary and colonial ascidians	metal piles	Yes	High	Y
19	Terminal 3 & 5	53 19.186	04 37.172	21.01.09	BS KL	Absent	Abundant solitary and colonial ascidians	metal piles		High	Y
20	Fish Dock	53 18.764	04 37.331	20.01.09	RH KL BS MB	Absent	Abundant solitary and colonial ascidians	Floating concrete & metal pontoons	Yes	High	Y
21	Terminal 1	53 18.653	04 37.653	20.01.09	KR KL	Absent	Abundant solitary and colonial ascidians	rock, concrete and metal beams		High	Y

Table 1. Summary of Holyhead Harbour survey locations and results



Photo 1. *Didemnum vexillum* overgrowing *Styela clava* (R. Holt CCW)



Photo 2. *Didemnum vexillum* overgrowing *Ciona intestinalis* (R. Holt CCW)



Photo 3. Close up of *Didemnum vexillum* (R. Holt, CCW)



Photo 4. Arrangement of larger pores on surface of *Didemnum vexillum*



Photos 5 – 6.

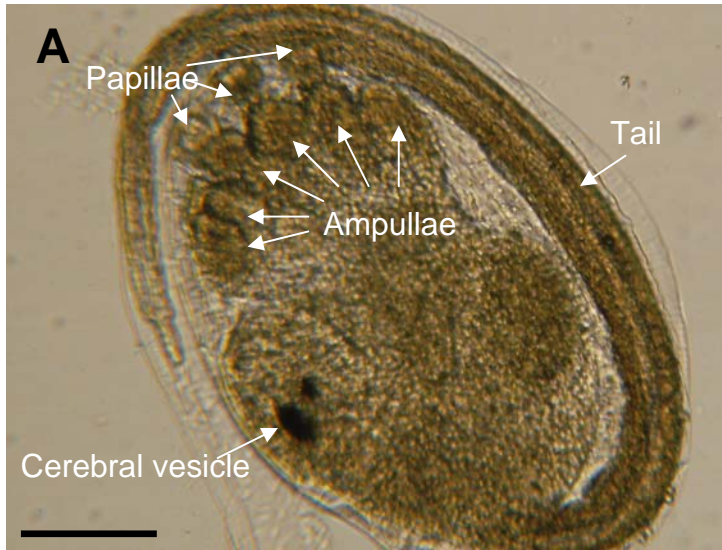
Heavily fouled yacht (photo: K Ramsay). Although everything above the waterline on this yacht appears to be pristine, large algae and kelp plants are visible from the surface and *Didemnum vexillum* was found on the prop-shaft and rudder as seen on the following stills taken from the video.



Photo 7. Large colonies of *Didemnum vexillum* on the prop-shaft and covering other solitary ascidians. The propeller is to the right of the picture (from video: H. Gouge).



Photo 8. Kelp and solitary ascidians *Styela clava* and *Ascidiella aspersa* attached to the rudder. *Didemnum vexillum* is the pale-coloured coating on some of the ascidians (from video: H. Gouge).

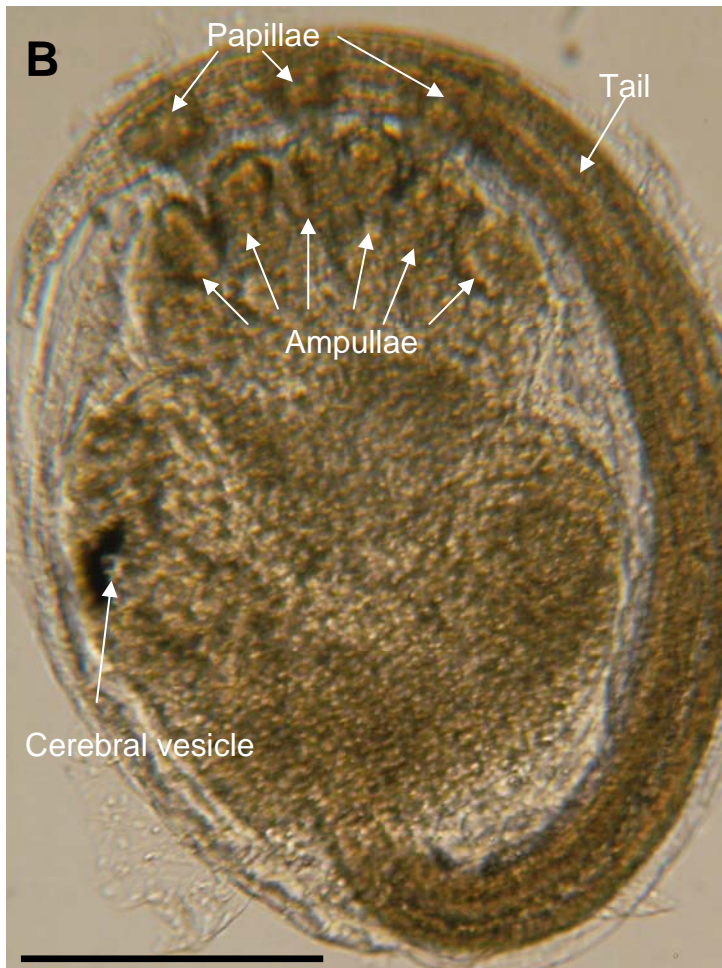


Photos 9 A & B

Didemnum vexillum brooded unhatched larvae. Removed from colony sampled during the December 2008 phase of the diving survey.

The appearance of the larvae in this sample matches those described by Lambert (2009): six pairs of lateral ampullae and three adhesive papillae.

Adult colony features also match: yellowish cream colouration; spicule-free bands between zooid groups; colony form - thin to thick encrusting to lobed.



Photos taken using LCD microscope. Scale bars are 0.2 mm



Photo 10. Divers KR and MB surface next to one of the large mooring buoys (photo: Flora Kent)

Survey site (Figure 3).	Location	Latitude decimal minutes (°N)	Longitude decimal minutes (°W)	Date of survey	Fouling Species Present	<i>Didemnum vexillum</i> Present or Absent
1	Holyhead marina	53 19.173	04 38.488	10.12.08	Moderate-high species abundance and richness of filter feeding invertebrates and algae. Fringing kelp.	Present
2	Deganwy marina	53 17.480	03 49.673	11.12.08	<i>Mytilus edulis</i> and filamentous algae, no ascidian species recorded	Absent
3	Conwy marina	53 17.458	03 50.286	11.12.08	<i>Mytilus edulis</i> and filamentous algae, no ascidian species recorded	Absent
4	Port Penrhyn	53 14.182	04 06.706	18.12.08	Mainly bare substrate and no ascidian species recorded	Absent
5	Port Dinorwic Victoria Dock - Caernarfon	53 11.178	04 12.582	12.12.08	<i>Mytilus edulis</i> , filamentous algae, occasional ascidian species (<i>Ascidella aspersa</i> , <i>Botryllus schlosseri</i>), <i>Botrylloides leachi</i> ,	Absent
6		53 08.562	04 16.669	30.01.09	<i>Mytilus edulis</i> , filamentous algae, <i>Ascidella aspersa</i> , <i>Mytilus edulis</i> , filamentous algae, <i>Ascidella aspersa</i> , <i>Ciona intestinalis</i> , <i>Botryllus schlosseri</i> ,	Absent
7	Pwllheli marina	52 53.191	04 24.389	16.12.08	<i>Botrylloides leachi</i> ,	Absent
8	Aberystwyth marina	52 24.595	04 05.202	09.02.09	Filamentous algae, no evidence no ascidian species recorded	Absent
9	Milford Haven marina	51 42.699	05 02.270	11.02.09 12.02.09	<i>Mytilus edulis</i> , filamentous algae, <i>Ascidella aspersa</i> , <i>Ciona intestinalis</i> , <i>Botryllus schlosseri</i> , <i>Botrylloides leachi</i> , <i>Styela clava</i> ,	Absent
10	Neyland marina Swansea marina and Swansea yacht club marina	51 42.632	04 56.544	12.02.09	<i>Mytilus edulis</i> , filamentous algae, <i>Ascidella aspersa</i> , <i>Ciona intestinalis</i> , <i>Botryllus schlosseri</i> , <i>Botrylloides leachi</i> , <i>Styela clava</i> , <i>Botrylloides violaceus</i>	Absent
11		51 36.984	03 56.048	10.02.09	Barnacles and tubeworm casts, filamentous algae, no ascidian species recorded	Absent

Table 2. Distribution of marina/harbour survey sites in Wales 2008-2009 with records of fouling species present and whether *D. vexillum* was present or absent at each site.

Discussion / conclusions

- **When did the marina become infected with *Didemnum vexillum*?**

The widespread, but fairly sparse and patchy distribution of *Didemnum vexillum* in Holyhead Marina could be interpreted as evidence of it having recently arrived and not had sufficient time to aggressively colonise all available substrata throughout the marina and wider harbour. It is evidently capable of rapidly smothering man-made structures with similar environmental parameters elsewhere around the world (Valentine *et al.*, 2007) and can reproduce both asexually and sexually through localised larval dispersion (Lambert, 2009). Larvae were found within some of the material from the marina.

The counter argument is that it arrived at least one, if not several years ago from more than one 'infection' event. Although its presence was confirmed in the summer of 2008 after other workers in Ireland alerted us to its presence in Malahide Estuary and Carlingford Lough in 2005/2006 (Minchin and Sides, 2006) it is possible that it arrived in Holyhead around or even before this time. The evidence in favour of this argument is that there appears to be no 'epicentre' for infection where the didemnid is abundant. Instead there appear to be two broad areas of scattered medium and small sized colonies in the marina where it is at slightly higher densities than others. The apparent 'slow' spread rate might also account for the lack of colonisation of new sections of marina that were installed in the winter of 2007-2008.

- **How did *Didemnum vexillum* reach Holyhead Marina?**

The two vessels found with *Didemnum vexillum* on their hulls in the marina are probably not the vectors responsible for bringing the species into the area. More likely they have been colonised during their (confirmed at least 1 year) long period of inactivity while in the marina. However, if these vessels were to move elsewhere, carrying their current 'fouling' burden with them when they go, they would be likely to act as vectors for *D. vexillum*.

Recreational boat users who do not regularly clean their hulls and only use their boats sporadically are more likely to spread *D. vexillum* than the more conscientious regular sailors who antifoul their boats. It would seem likely that *D. vexillum* arrived in Holyhead Marina by this method, possibly on yachts crossing from Ireland.

Contaminated recreational vessels should be brought ashore and jet-washed or scrubbed clean. Any debris should be allowed to dry and/or decompose but not be returned to the water.

- **Has it spread elsewhere in the harbour?**

Didemnum vexillum was not found outside the marina in the wider harbour area and on the commercial terminals. Judging by the vigorous growth of native and some non-native species of sea squirts on virtually all the structures surveyed there is considerable scope for *D. vexillum* to colonise huge areas of man-made and natural substrata. This would bring large international shipping in close proximity to *D. vexillum* colonies and further risk the spread of the species locally, nationally and internationally.

- **Has *D. vexillum* been found elsewhere in Wales?**

Outside Holyhead Marina, *D. vexillum* was not observed at any of the marina/harbour survey locations around Wales. However, it must be recognised that with surveys of this nature there is a possibility that small amounts of *D. vexillum* may have been

present at a site, but was missed during the survey. For *D. vexillum* to reach a new location it requires a vector, such as a recreational vessel, and suitable environmental conditions for colonisation. The marinas that already have populations of native and non-native ascidians (Pwllheli, Port Dinorwic, Milford Haven and Neyland) have the greatest potential to provide a suitable habitat for *D. vexillum*, should it be introduced via an infected vessel.

- **What next?**

There is an urgent requirement for establishing a programme of monitoring of the marina area at a resolution capable of detecting change/increase in size of colonies and rate of spread. Knowledge of *D. vexillum*'s growth rate and other ecological preferences at this particular location could also contribute to assessing the risks and potential damaging consequences of it spreading to other areas in North Wales. As part of the requirement for monitoring the species a selection of sites outside the marina in the commercial side of the harbour should be monitored frequently at regular intervals until more is known about its ability to spread in this area.

Delays in implementing a monitoring strategy and putting control measures into place (e.g. public awareness campaign, boat cleaning) and/or eradication (if feasible) could have serious ecological and financial consequences.

Acknowledgements

Many thanks to all the marina operators and staff for their assistance and providing us with access.

Also thanks to the following people for their help, often at short notice, in the running of the survey work and report production:

Robert Hardman, Stena Line, Holyhead Port Control.

Danny Mitchell, Stena Line, Holyhead Port Control.

Gretchen Lambert, University of Washington Friday Harbor Laboratories.

John Bishop, Marine Biological Association.

Stuart Jenkins, Bangor University

Sarah Kleeman, KCR Consultants Ltd.

Lucie Oliver, Environment Agency / CCW

Kate Smith, CCW

Bill Sanderson, CCW

Charles Lindenbaum, CCW

Monica Jones, CCW

Mark Burton, CCW, Skomer MNR team

Kate Lock, CCW, Skomer MNR team

Aethne Cooke, CCW

Kathryn Birch, CCW

Delyth Rowlands, CCW

Gabrielle Wyn, CCW

Paul Brazier, CCW

Natasha Lough, CCW

Harry Gouge, Marine Ecological Solutions

Liz Morris, Marine Ecological Solutions

Martin and Caroline Sampson, Anglesey Divers in Holyhead

References

- Biosecurity New Zealand (2009). Pests Website (<http://www.biosecurity.govt.nz/pests/didemnum>). Accessed January 2009
- Bullard S.G. and Whitlatch, R.B. (2009) *In situ* growth of the colonial ascidian *Didemnum vexillum* under different environmental conditions. *Aquatic Invasions* 4: 275-278.
- Coutts A. and Sinner J. (2004) An updated benefit-cost analysis of management options for *Didemnum vexillum* in Queen Charlotte Sound. *Cawthorn Report* 925
- Gittenberger A. (2007) Recent population expansions of non-native ascidians in The Netherlands. *Journal of Experimental Marine Biology and Ecology* 342: 122-126
- Lambert G. (2009) Adventures of a sea squirt sleuth: unravelling the identity of *Didemnum vexillum*, a global ascidian invader. *Aquatic Invasions* 4: 5-28
- Minchin D. and Sides E (2006) Appearance of a cryptogenic tunicate, a *Didemnum* sp. fouling marina pontoons and leisure craft in Ireland. *Aquatic Invasions* 1: 143-147
- Millar R.H. (1970) British Ascidians. The Linnaen Society of London. Academic Press London and New York.
- Pannell A. and Coutts A. (2006) Treatment methods used to manage *Didemnum vexillum* in New Zealand. *New Zealand Marine Farming Association INC.*
- USGS, 2009. Nuisance Species Website (<http://woodshole.er.usgs.gov/projectpages/stellwagen/didemnum/index.htm>). Accessed December 2009
- Valentine P.C., Collie J.S., Reid R.N., Asch R.G., Guida V.G., Blackwood D.S. (2007) The occurrence of the colonial ascidian *Didemnum* sp. on Georges Bank gravel habitat – ecological observations and potential effects on groundfish and scallop fisheries. *Journal of Experimental Marine Biology and Ecology* 342: 179-181

Annex A. Dive Logs.

Date	Divers / Standby	Boathandler	Supervisor	Gas Mix EANx/ MOD	Leaving surface	Arrive surface	Total time	Max depth
9/12/08	Rohan Holt		K. Ramsay	32	11.58	13.36	97	5.8
	Harry Gouge		K. Ramsay	32	11.59	13.39	100	5
	Kate Smith (Stby)		K. Ramsay	34				
	Rohan Holt		K. Ramsay	32	14.37	15.20	43	5
	Harry Gouge		K. Ramsay	32	14.40	15.20	40	5
10/12/08	Rohan Holt		K. Ramsay	24	11.03	11.54	51	5.5
	Harry Gouge		K. Ramsay	24	11.05	12.21	76	5
	Kate Smith (Stby)		K. Ramsay	34				
	Rohan Holt		K. Ramsay	24	13.29	14.15	46	3.9
	Harry Gouge		K. Ramsay	24	13.33	14.20	47	5
	Rohan Holt		K. Ramsay	24	14.48	15.09	21	3.4
20/01/09	Harry Gouge		K. Ramsay	24	14.50	15.15	25	3
	Rohan Holt		K. Ramsay	22	11.39	11.52	13	3.8
	Bill Sanderson		K. Ramsay	32	11.39	11.52	13	3.8
	Mark Burton		K. Ramsay	32	11.32	11.52	20	4.4
	Kate Lock		K. Ramsay	32	11.32	11.52	20	4.4
	Kirsten Ramsay	Rohan Holt	K. Lock	22	13.24	13.32	8	5.5
	Bill Sanderson	Rohan Holt	K. Lock	32	13.24	13.32	8	5.5
	Mark Burton	Rohan Holt	K. Lock	32	13.31	13.37	6	8.5
	Kate Smith	Rohan Holt	K. Lock	21	13.31	13.37	6	8.5
	Kirsten Ramsay	Rohan Holt	K. Lock	22	13.37	13.48	11	8.9
	Bill Sanderson	Rohan Holt	K. Lock	32	13.37	13.48	11	8.9
	Rohan Holt	Bill Sanderson	K. Ramsay	22	14.49	15.01	12	3.5
	Kate Lock	Bill Sanderson	K. Ramsay	32	14.49	15.02	13	3.5
	Mark Burton	Rohan Holt	K. Smith	32	15.29	15.40	11	8.7
Kirsten Ramsay	Rohan Holt	K. Smith	32	15.29	15.40	11	8.7	
Kate Lock	Rohan Holt	K. Smith	32	15.28	15.43	15	9	
Bill Sanderson	Rohan Holt	K. Smith	32	15.28	15.43	15	9	

Date	Divers / Standby	Boathandler	Supervisor	Gas Mix EANx/ MOD	Leaving surface	Arrive surface	Total time	Max depth
21/01/09	Rohan Holt	B. Sanderson	K. Lock	22	10.42	10.54	12	10
	Mark Burton	B. Sanderson	K. Lock	32	10.42	10.54	12	10
	Rohan Holt	B. Sanderson	K. Lock	22	11.04	11.12	8	10.2
	Mark Burton	B. Sanderson	K. Lock	32	11.04	11.12	8	10.2
	Rohan Holt	B. Sanderson	K. Lock	22	11.26	11.32	6	8.4
	Mark Burton	B. Sanderson	K. Lock	32	11.26	11.32	6	6.8
	Kate Lock	R. Holt	M. Burton	32	12.34	12.49	10	11.7
	Bill Sanderson	R. Holt	M. Burton	32	12.34	12.49	10	10.8
	Kate Lock	R. Holt	M. Burton	32	12.56	13.05	9	11.1
	Bill Sanderson	R. Holt	M. Burton	32	12.56	13.05	9	10.6
	Kate Lock	R. Holt	M. Burton	32	13.32	13.38	6	5.2
	Bill Sanderson	R. Holt	M. Burton	32	13.32	13.38	6	5.2
	Rohan Holt	B. Sanderson	K. Lock	28	14.40	14.54	14	11.4
	Mark Burton	B. Sanderson	K. Lock	32	14.40	14.54	14	11.4

Annex B: DATA ARCHIVE APPENDIX

The report and data collected internally by CCW (not under contract) is archived as Project 214 (Media 580, 592 & 593). This archive is maintained on backed-up server based storage at CCW headquarters.

The data archive consists of:

[A] Digital versions of the contract report: Microsoft Word document(s); and an equivalent Adobe Portable Document Format version. The report for publication has the names of boats and/or boat owners redacted.

[B] Excel spreadsheets containing GPS points taken in the field

[C] GIS data

Within the pontoon area

MapInfo tab files showing the distribution and density of *Didemnum vexillum* around the pontoons, the route taken by the surveyors and the location of any photos, video or live specimens taken.

In the wider harbour

MapInfo point layers showing point survey locations

[D] Still images and video

[E] Metadata is held on the CCW library catalogue metadatabase (OLIB), records no. [109566](#)

This is an ongoing project therefore a working copy of the data and report is held at *S:\Marine_Habitats_and_Species\Marine Monitoring\Didemnum vexillum*

28 Jan 2009