



Llywodraeth Cynulliad Cymru
Welsh Assembly Government
CORFF NODDEDIG SPONSORED BODY



Cyngor Cefn Gwlad Cymru
Countryside Council for Wales

***Didemnum vexillum* – Feasibility of Eradication and/or Control**

Dr S.N. Kleeman

CCW Contract Science report No. 875



Holyhead Marina. (image: Dr Sarah Kleeman).

© CCGC/CCW date

You may reproduce this document free of charge for non-commercial and internal business purposes in any format or medium, provided that you do so accurately, acknowledging both the source and Countryside Council for Wales's copyright, and do not use it in a misleading context.

This is a report of research commissioned by the Countryside Council for Wales. However, the views and recommendations presented in this report are not necessarily those of the Council and should, therefore, not be attributed to the Countryside Council for Wales.

Report series: CCW Contract Science report
Report number: No. 875
Publication date: March 2009
Contract number: 100 MFG 08
Contractor: Dr Sarah Kleeman, KCR Consultants Ltd.
Contract Manager: Kirsten Ramsay
Title: *Didemnum vexillum* – Feasibility of Eradication and/or Control
Author(s): Dr Sarah N. Kleeman
Restrictions: None

Distribution list (core):

CCW HQ Library, Bangor	Joint Nature Conservation Committee Library
National Library of Wales	Scottish Natural Heritage Library
British Library	Natural England Library
Welsh Assembly Government Library	

Distribution list (others):

Bill Somerfield, WAG	Niall Moore, GB non natives secretariat
Diana Reynolds, WAG	David Knight, NE
Graham Rees, WAG	Fiona Mason, SNH
Viv Collins, WAG	
Jessaby Battersby, JNCC	Kate Smith, CCW
David Thorpe, EA	Lucy Kay, CCW
Bill Cook, NWNWSFC	John Ratcliffe, CCW
Mark Diamond, EA	Rohan Holt, CCW
Phil Coates, SWSFC	Aethne Cooke, CCW
Susan Cooper, Holyhead Marina	Mike Camplin, CCW
Dan Minchin, MOI	Ziggy Otto, CCW
Stuart Jenkins, Bangor University	Adam Cole-King, CCW
John Bishop, MBA	John Hamer, CCW
Angela Robinson, Scottish Govt	Kirsty Lindenbaum, CCW
Huw Thomas, Defra	Catherine Duigan, CCW
Scot Mathieson, SEPA	David Parker, CCW
Tracy McCollin, FRS	Gabrielle Wyn, CCW

Recommended citation for this volume:

Kleeman, S. N. 2009. *Didemnum vexillum* – Feasibility of Eradication and/or Control. CCW Science report Report No: 875, 53 pp, CCW, Bangor.

CONTENTS

CONTENTS	<i>i</i>
LIST OF FIGURES	<i>iii</i>
LIST OF TABLES	<i>iv</i>
CRYNODEB GWEITHREDOL	<i>v</i>
EXECUTIVE SUMMARY	<i>ix</i>
1 Background	<i>1</i>
2 Description of <i>Didemnum vexillum</i> (<i>Dv</i>)	<i>2</i>
2.1 Taxonomy and identification	<i>2</i>
2.2 Habitat preferences and growth rate	<i>2</i>
2.3 Factors that may affect <i>Dv</i>	<i>3</i>
2.3.1 Temperature and salinity tolerances.....	<i>3</i>
2.3.2 Predators.....	<i>4</i>
2.4 Distribution	<i>4</i>
2.4.1 New Zealand	<i>5</i>
2.4.2 North America.....	<i>5</i>
2.4.3 Ireland.....	<i>5</i>
2.4.4 Netherlands.....	<i>6</i>
2.4.5 France	<i>6</i>
2.4.6 Japan.....	<i>6</i>
2.5 Reproduction and dispersal	<i>6</i>
2.5.1 Method of reproduction.....	<i>6</i>
2.5.2 Methods of dispersal	<i>7</i>
2.5.2.1 Dispersal via natural mechanisms	<i>7</i>
2.5.2.2 Dispersal via human mediated vectors.....	<i>7</i>
3 Risk analysis on the likelihood and consequences of <i>Dv</i> spreading to value areas in Wales 10	
3.1 How long has <i>Dv</i> been present at Holyhead Marina?	<i>10</i>
3.2 What is the likely extent of infestation at Holyhead Marina?	<i>11</i>
3.3 What are chances <i>Dv</i> could naturally disappear/die out?	<i>12</i>
3.4 What is the likelihood <i>Dv</i> will spread to all available habitats within Holyhead Harbour?	<i>12</i>
3.5 How long will it take for all available habitats to be occupied?	<i>13</i>
3.6 What is the likelihood <i>Dv</i> will be transported by recreational vessels to other Wales locations?	<i>14</i>
3.7 What is the likelihood <i>Dv</i> will establish in other marinas in Wales?	<i>16</i>
3.8 What is the likelihood <i>Dv</i> will establish in mussel beds in North Wales?	<i>16</i>
3.9 What is the likelihood <i>Dv</i> will establish in SACs in Wales?	<i>17</i>
3.10 What are the consequences if <i>Dv</i> were to spread to:	<i>18</i>
3.10.1 Marina and harbours.....	<i>18</i>
3.10.2 Mussel farming.....	<i>19</i>
3.10.3 Conservation areas	<i>19</i>

4	<i>Assessment of the feasibility of eradication in Holyhead Marina</i>	22
4.1	Stage of infestation:	22
4.2	Resources available:	22
4.2.1	Control methods	22
4.2.1.1	Pontoons	22
4.2.1.2	Pontoon anchor chains.	25
4.2.1.3	Vessel hulls	26
4.2.2	Capability	27
4.3	Cost effectiveness	28
4.4	Critical success factors	28
4.5	Probability of success	28
5	<i>What measures could be used to prevent reinfection of the marina?</i>	31
5.1	Monitoring / Surveillance	31
5.2	Stakeholder Communication / Outreach Programs	31
5.3	Vector Control	32
6	<i>What measures could be used to prevent spread if eradication is not considered?</i>	33
6.1	Option 1: Do Nothing	33
6.2	Option 2: Least control. Learn to live with the problems caused by the species and/or leave any control attempt to affected stakeholders.	34
6.3	Option 3: Monitoring the event and providing stakeholders with information....	34
6.4	Option 4: Containment of Dv, by controlling movement of vectors (e.g. vessels and moveable equipment) from affected areas within the Marina, to prevent incursion into other areas that are presently Dv-free	35
6.5	Option 5. Monitor spread beyond stage 1-3 and triggering containment of Dv by controlling movement of vectors (e.g. vessels and moveable equipment) from affected areas within the Marina, to prevent incursion into other areas that are presently Dv-free	35
7	<i>Cost Benefit Analysis</i>	37
8	<i>Recommendations</i>	38
9	<i>Acknowledgements</i>	40
10	<i>References</i>	41
	<i>Appendix 1: Questionnaire to assess the risk of Didemnum vexillum in Wales</i>	44
	<i>Appendix 2: Timeline of response to an incursion of the slipper limpet (Crepidula fornicata) in the Menai Strait in 2007.</i>	51

LIST OF FIGURES

Figure 2.1. Infestation curve showing responses from questionnaire participants.....	11
Figure 2.2. North West England Cruising Routes, December 2008.....	14
Figure 2.3. Wales Cruising Routes, December 2008.....	15
Figure 2.4. Marine SACs in Wales with reef features.....	17
Figure 3.1. Submerged substrates within Holyhead Marina.....	23

LIST OF TABLES

Table 2.1. Nomenclature for qualitative likelihoods.....10

Table 3.1. Scenario of total costs involved in eradicating Dv from Holyhead Marina
as well as undertaking a test case in developing capability in marine invasive pest responses ...30

CRYNODEB GWEITHREDOL

Crynodeb gweithredol

Yn ogystal ag amlinellu statws y chwistrell fôr, *Didemnum vexillum* (Dv), yng Nghymru, mae'r adroddiad canlynol yn cyflwyno nifer o strategaethau posibl ar gyfer rheoli'r boblogaeth bresennol, gan gyflwyno tystiolaeth a fydd o gymorth i ddewis y dull mwyaf effeithiol o reoli Dv yn y dyfodol agos. Mae Dv yn enghraifft o rywogaeth trefedigaethol a goresgynnol, ac felly'n peri gofid mawr i'r diwydiant acwafeithriniad ac i ecoleg gyffredinol yr ardal. Ym Mehefin 2008, darganfuwyd Dv ar swbstradau artiffisial ym Marina Caerdybi. Dyma'r unig gofnod o Dv yn y DU hyd yma.

Lledaeniad ac effeithiau posibl

Mae cryn ansicrwydd ynglŷn â'r sgîl-effeithiau posibl pe bai'r boblogaeth yn parhau heb ei monitro. Yn sgîl asesiad risg ansoddol, darganfuwyd tebygolrwydd uchel y bydd y boblogaeth yn lledaenu o Farina Caerdybi ac yn sefydlu ei hun mewn nifer o gynefinoedd newydd o amgylch y DU, gan beri gofid mawr i holl ardaloedd gwarchoddedig a physgodfeydd Cymru. Credir mai cychod hamdden yw'r fector mwyaf tebygol o hyrwyddo lledaeniad Dv yn yr ardal.

Mae'r effeithiau posibl yn sylweddol: Mae'n hysbys fod Dv yn peri niwed i nifer o safleoedd gwarchoddedig a physgodfeydd, yn enwedig y bysgodfa cregyn gleision. Mae goblygiadau cyfreithiol a chyllidol posibl pe na bai Dv yn cael ei fonitro.

Ymarferoldeb difa'r boblogaeth

Argymhellir yn gryf gan arbenigwyr ledled y byd y dylid rhoi pwyslais ar unrhyw ymgais i ddifa'r boblogaeth yn syth. Pe na bai ymdrechion o'r fath yn cael eu cyflawni ar frys, mae'n bur debygol y byddai'r boblogaeth yn cyrraedd lefel a fyddai'n ei gwneud hi'n anodd, os nad yn amhosibl, i'w difa.

Mae gwerthusiad o'r dulliau posibl o reoli a/neu ddifa Dv yn dangos fod dulliau addas ar gyfer taclo'r pla cyfredol yn bodoli. Mae asesiad cychwynnol yn dangos fod y dulliau yma yn gost effeithiol ac yn ymarferol:

Swbstrad	Dull	Dichonolrwyd d technegol	Tebygolrwydd o fethiant*	Amcan gost (deunyddiau a llafur yn unig)#
Strwythurau arnofio e.e. pontŵn.	Mewngapsiwleiddio mewn plastig gan ddefnyddio gorchuddiadau silwair neu ddeunydd gwrth-ddŵr arall. Ychwanegu cyflymydd (asid asetig neu glorín).	Eithaf hawdd i'w weithredu. Dim angen offer cymhleth.	0% os gweithredi r yn y dull priodol.	£186 am bob 1.5m ² o bontŵn. £244 am bob 3.75m ² o bontŵn. £540 am bob 22m ² o bontŵn. £1410 am bob 80m ² o bontŵn.
Cadwyni angor.	Lapio mewn plastig.	Hawdd i'w weithredu. Dim angen offer cymhleth.	0% os gweithredi r yn y dull priodol.	£84 y gadwyn – lleiheir y gost os oes modd cyfuno costau llafur gydag (1).
Cyrff cychod.	Mewngapsiwleiddio mewn plastig gan ddefnyddio gorchuddiadau silwair neu ddeunydd gwrth-ddŵr arall. Ychwanegu cyflymydd (asid asetig neu glorín).	Eithaf hawdd i'w weithredu. Dim angen offer cymhleth.	0% os gweithredi r yn y dull priodol.	£600 y cwch.

Nodiadau: * Gan gymryd nad yw swbstradau eraill ym Marina Caerdybi wedi eu heintio yn ystod y broses ddifa.

Amcan gostau yn unig yw'r rhain, a gallent amrywio'n sylweddol. Amlygir amser, y gallu i weithredu ar y fath raddfa, a'r posibilrwydd o ailgyflwyno Dv o ffynonellau eraill (e.e. Iwerddon), fel y prif ffactorau a fyddai'n cael cryn effaith ar lwyddiant y broses ddifa.

Dylid cwblhau'r broses honno cyn y cyfnod lle mae'r tebygolrwydd mwyaf o ryddhau larfa (Mehefin - Rhagfyr). Serch hynny, mae'r gallu o fewn y DU i weithredu ar y fath raddfa yn annhebygol. Opsiwn arall yw cynnal cyfnod treialu difa yn ystod 2009, dileu yn llwyr yn 2010, a gweithredu pellach yn 2011 lle bo'r angen.

Cymherer hefyd i ystyriaeth fod y gwerth cymharol mewn cyllido'r broses ddifa yn lleihau wrth i'r tebygolrwydd o ailgyflwyno Dv trwy ffynonellau eraill sydd heb eu rheoli gynyddu. Er fod hyn uwchlaw dibenion yr adroddiad canlynol, pwysleisir y galw am strategaeth gydweithiol ac amlddisgyblaethol wrth ddelio gyda rhywogaethau di-frodorol. Argymhellir yn gryf y dylid rhoi ystyriaeth lawn i gyflwyno ymgyrchoedd codi ymwybyddiaeth, rhaglenni monitro a chynlluniau rheoli, sy'n gosod seiliau i ddau gynllun difa posibl (E1 ac E2):

Disgrifiad o'r cynllun difa		Dull(iau) rheoli	Tebygolrwydd o lwyddo	Amcan gost (dros y 10 mlynedd nesaf)
E1	Difa'n llwyr yn 2009, gyda thriniaethau pellach yn 2010 a 2011 lle bo'r angen.	Dulliau rheoli: <ul style="list-style-type: none"> • Gorchudd plastig gyda neu heb gyflymydd. • Mygu gyda llain blastig. • Gwaredu. • Dŵr croyw. Monitro. Cyfathrebau. Rheolaeth fector gwirfoddol (Cod ymarfer – CY).	Siawns o 50% o ddifa'n llwyr yn 2009, yn codi i 95% erbyn 2010 a 2011. Llwyddiant yn ddibynnol ar sicrwydd yr arolygiadau a gyflawnwyd yn ystod 2008/2009. Ansicr ar gyfer CY.	£385,000 yn ystod y 3 mlynedd gychwynnol*. Tua £15,000 y flwyddyn ar gyfer monitro parhaol wedi'r 3 mlynedd gychwynnol. Costau wedi'r 3 mlynedd gychwynnol yn ddibynnol ar lwyddiant y broses ddifa. Os yw'n fethiant wedi 3 mlynedd, arolygu a/neu roi terfyn ar y cynllun.
E2	Treialu'r broses ddifa, gyda thriniaeth lawn yn 2010 a thriniaethau pellach yn 2011 lle bo'r angen.	Datblygu strategaethau Rhaglenni ymchwil Dulliau rheoli: <ul style="list-style-type: none"> • Gorchudd plastig gyda neu heb gyflymydd • Mygu gyda llain blastig • Gwaredu • Dŵr croyw Monitro Cyfathrebau Rheolaeth fector gwirfoddol (Cod ymarfer – CY).	Siawns o 50% o ddifa'n llwyr a 98% o reoli'r boblogaeth yn 2009. Siawns o 95% o ddifa'n llwyr mewn blynyddoedd dilynol. Yn dilyn monitro yn haf 2009, arolygu a/neu roi terfyn ar y cynllun. Ansicr ar gyfer CY.	£350,000 yn ystod y 3 mlynedd gychwynnol*. Tua £15,000 y flwyddyn ar gyfer monitro parhaol wedi'r 3 mlynedd gychwynnol. Costau wedi'r 3 mlynedd gychwynnol yn ddibynnol ar lwyddiant y broses ddifa. Os yw'n fethiant wedi 3 mlynedd, arolygu a/neu roi terfyn ar y cynllun.

Nodiadau: * Yn cynnwys cyfanswm costau difa, monitro, cyfathrebau a rheoli. Amcan gostau yn unig yw'r rhain, a gallent amrywio'n sylweddol

Lle na roddir ystyriaeth i ddifa'r boblogaeth, argymhellir yr opsiynau rheoli canlynol:

Opsiwn		Dull(iau) rheoli	Tebygolrwydd o lwyddo	Effeithiau amcangyfrifol (dros gyfnod o 10 mlynedd)
1	Gwneud dim	Dim	Isel	Colled i'r diwydiant cregyn gleision (tua 5%) = £1,375,125 yn dilyn heintio*. Colled i'r diwydiant cregyn gleision (tua 25%) = £6,875,625 yn dilyn heintio*. Rheolaeth gan y Marina a'r perchnogion cychod (ansicr ac yn ddibynol ar benderfyniadau ynglŷn â deddfwriaethau sy'n gysylltiedig â maeddu cyrff cychod) Rheoli'r ardaloedd gwarchoddedig sydd wedi eu heintio (costau ansicr i'r llywodraeth) Posibilrwydd o gostau cyfreithiol i'r llywodraeth (ansicr)
2	Rheolaeth leiaf	1.Cyfathrebau 2.Rheolaeth wirfoddol (cod ymarfer)	Cymhedrol i Isel	1.£20,000 dros gyfnod o ddwy flynedd, ond gallai barhau os yw'r sgîl-ffeithiau negyddol yn sylweddol. 2.Gweler Opsiwn 1.
3	Monitro ac ymwybyddi aeth	1.Cyfathrebau 2.Monitro 3.Rheolaeth wirfoddol (cod ymarfer)	Cymhedrol i Isel	1.£20,000 dros gyfnod o ddwy flynedd, ond gallai barhau os yw'r sgîl-ffeithiau negyddol yn sylweddol. 2.£45,000 yn y flwyddyn gyntaf (Caergybi + Sir). £30,000 y flwyddyn wedi hyn. Bydd y costau yn cynyddu ac yn parhau os yw'r sgîl-ffeithiau negyddol yn sylweddol. 3.Gweler Opsiwn 1.
4	Cyfyngiant	1.Cyfathrebau 2.Rheolaeth orfodol a/neu wirfoddol (cod ymarfer)	Isel	1.£20,000 per year over two years but may continue indefinitely if impacts are high. Gweler Opsiwn 1, gyda'r posibilrwydd ychwanegol y bydd angen gorfodaeth i reoli'r cyfarwyddiadau a osodwyd.
5	Monitro i bennu dechrau'r camau priodol a chyfyngiant	1.Cyfathrebau 2.Monitro 3.Rheolaeth orfodol a/neu wirfoddol (cod ymarfer)	Isel	1.£20,000 dros gyfnod o ddwy flynedd, ond gallai barhau os yw'r sgîl-ffeithiau negyddol yn sylweddol. 2.£25,000 yn y flwyddyn gyntaf (Caergybi yn unig). £20,000 y flwyddyn wedi hyn. Bydd y costau yn cynyddu ac yn parhau os yw'r sgîl-ffeithiau negyddol yn sylweddol. 3.Gweler Opsiwn 1, gyda'r posibilrwydd ychwanegol y bydd angen gorfodaeth i reoli'r cyfarwyddiadau a osodwyd. Mae'r costau hyn yn ansicr.

Nodiadau: Costau uniongyrchol = costau difa, monitro, cyfathrebau a rheoli. Amcan gostau yn unig yw'r rhain, a gallent amrywio'n sylweddol.

* Tybir y bydd ffermydd yn cael eu heffeithio trwy golledion cynhyrchu rhwng 5-25% ymhen 5 mlynedd. I'r diben hwn, ystyrir y bydd colledion cynhyrchu yn ystod 5 o'r 10 mlynedd nesaf

Gwerthusiad o'r effeithiau a'r ystyriaethau economaidd

O gofio'r ansicrwydd ynglŷn ag effaith Dv yn y DU, yr anhawster o ddadansoddi costau dewisiadau rheoli, a'r analluogrwydd i roi gwerth ariannol cyfatebol i'r effeithiau posibl ar ardaloedd gwarchoddedig, cyflwynir gwerthusiad economaidd o'r manteision a'r costau fel dadansoddiad 'cost a budd' i'r diwydiant cregyn gleision yn unig. Yn yr achos hwn, mae'r costau sy'n gysylltiedig ag ymgyrch di-wreiddiad lawn (£350-380,000 dros gyfnod o 3 mlynedd, gyda

thua £15,000 ym mhob blwyddyn ddilynol ar gyfer monitro ac archwilio) yn dipyn llai na'r amcan golled i'r diwydiant cregyn gleision (£1,375,125 - £6,875,625) dros y 10 mlynedd nesaf).

Mae'r dystiolaeth sy'n deillio o'r adroddiad presennol yn dangos fod difa'r boblogaeth yn bosibl, yn gost effeithiol ac yn amserol. O gofio fod y cyfle i ddifa'r boblogaeth yn bodoli nawr, ond yn debygol o gael ei gollu yn ystod y blynyddoedd nesaf, gellir dadlau felly y byddai diffyg gweithredu yn annerbyniol. O dan y 'Cyfarwyddyd Cynefinoedd', mae'n angenrheidiol i Aelod-wladwriaethau i ddilyn camau i osgoi dirywiad cynefinoedd naturiol a chynefinoedd rhywogaethau (Erthygl 6(2)). Mae Erthygl 22 o'r 'Cyfarwyddyd Cynefinoedd' hefyd yn rhoi gorfodaeth ar Aelod-wladwriaethau i sicrhau rheolaeth ar gyflwyniadau bwriadol o rywogaethau di-frodorol. Mae canlyniadau'r asesiad risg yn dangos tebygolrwydd uchel iawn y bydd cynefin riffiau a niweidiwyd yn cael ei effeithio yn ystod y 10 mlynedd nesaf pe na bai gweithredu yn digwydd nawr i reoli'r boblogaeth ym Marina Caergybi.

Argymhellion

Argymhellir y dylid gweithredu cynllun difa E2 ym Marina Caergybi cyn gynted â phosibl, a hynny'n cynnwys:

- Gweithredu cynllun E2 fel astudiaeth achos i brofi strategaethau posibl ar gyfer Bioddiogelwch Morol, yn ogystal â datblygu'r gallu yng Nghymru i weithredu ar y fath raddfa.
- Ffurio gweithgor Dv a datblygu rhwydwaith byd-eang o gysylltiadau.
- Cynnal profion triniaeth yn 2009 a datblygu dealltwriaeth o'r costau, adnoddau ac amserlenni angenrheidiol i ddifa'r boblogaeth.
- Monitro swbstradau cyfagos yng Ngorffennaf / Awst 2009 a gwerthuso cynllun gweithredu fydd yn ddibynnol ar y canlyniadau.
- Cyflawni difa'n llawn rhwng Ionawr - Mehefin 2009, gyda thriniaeth bellach yn 2011 ble bo'r angen.
- Datblygu rhaglen addysgu a chodi ymwybyddiaeth ymysg perchnogion cychod ym Marina Caergybi.
- Cydweithio gyda Marina Caergybi a'r perchnogion cychod er mwyn datblygu strategaethau glanhau cyrff llongau.
- Datblygu strategaethau ymchwil er mwyn astudio effeithiolrwydd y technegau rheoli yn ogystal â bioleg Dv (nodweddion gwasgariad, cyfradd twf ayyb).
- Datblygu rhaglen fonitro ar gyfer datblygu gwell dulliau o adnabod ardaloedd posibl ble mae Dv yn debygol o fodoli.
- Datblygu gwell dealltwriaeth o brif lwybrau fector - h.y. cychod hamdden.
- Cydweithio gyda gwledydd eraill er mwyn ceisio rheoli rhywogaethau sy'n maeddu cyrff cychod a'r fectorau sy'n bennaf gyfrifol am hwyluso lledaeniad y fath organebau i ardaloedd newydd.

EXECUTIVE SUMMARY

The objective of this report is to outline the status of *Didemnum vexillum* (Dv) in Wales, provide management options and recommend the most feasible action to take. Dv is an invasive colonial tunicate that causes serious risk to both the aquaculture industry and the general ecology of a region. In June 2008, Dv was found on artificial substrates in Holyhead Marina. This marks the only confirmed occurrence of Dv in the UK.

Potential spread and impacts

There is considerable uncertainty about the potential spread and impact of Dv if left unchecked. Results of a rapid qualitative risk assessment revealed a high likelihood that Dv would spread from Holyhead Marina and establish in other habitats around the UK, where virtually all conservation and fishery areas in Wales are at risk. The most likely vector is recreational vessels.

The potential impacts are significant: Dv is known to adversely affect a number of conservation sites and fisheries, in particular the mussel fishery. If left unchecked there are potentially significant legal and financial implications.

Feasibility of eradication

A pertinent recommendation from experts worldwide emphasises that any attempts at eradication should be undertaken immediately. If attempts are not made now, it is highly likely that infestation will increase to a level where eradication will become difficult if not impossible.

Evaluation of potential methods to control and/or eradicate Dv indicate that suitable techniques exist to tackle the current infestation. Initial assessment indicates these methods are both cost effective and able to be applied logistically:

Substrate	Method	Technical Feasibility	Risk of failure*	Estimated Cost (materials and labour only)#
Pontoons	Plastic encapsulation using silage covers or tarpaulins Addition of an accelerant (acetic acid or chlorine)	Relatively easy to apply does not require complex equipment	0% if applied correctly	£186 per 1.5m ² pontoon £244 per 3.75m ² pontoon £540 per 22m ² pontoon £1410 per 80m ² pontoon
Anchor chains	Plastic wrapping	Easy to apply and does not require complex equipment	0% if applied correctly	£84 per chain – costs reduced if labour can be combined with efforts in wrapping pontoons.
Boat hulls	Plastic encapsulation using silage covers or tarpaulins. Addition of an accelerant (acetic acid or chlorine)	Relatively easy to apply does not require complex equipment	0% if applied correctly	£600 per boat hull

Notes: * This assumes that other substrates within Holyhead Harbour are not infected at the time of eradication. # Costs may vary significantly and are an approximation only.

The major critical success factors are time, capability and the risk that Dv will be reintroduced from other sources (e.g. Ireland).

Eradication would need to be completed prior to high risk periods for larval release (June - December). In addition, capability within the UK is lacking, further increasing the chance of failure. An alternative option is to undertake a trial period of eradication in 2009, followed by a full scale eradication in 2010, with follow-up treatments in 2011 if required.

It is also considered that the relative value in devoting funds to eradication is lessened by the probability that reinfection will occur from other unmanaged sources. Although beyond the scope of this study, this highlights the need for an overarching strategy in dealing with invasive species.

At the very least, and for the purposes of this assessment, it is recommended that consideration is given to implementing awareness campaigns, monitoring and surveillance programmes and vector control plans. These form the basis for two suggested eradication plans (E1 and E2):

Eradication plan description		Method/s Tools for management	Likelihood of success	Estimated cost (over 10 years)
E1	Full eradication in 2009 with follow up treatment in 2010 and 2011 where necessary	Control tools: <ul style="list-style-type: none"> • Plastic wrapping with or without accelerant • Plastic smothering • Removal • Freshwater Monitoring and surveillance Communications Voluntary vector controls (Code of practice - CoP)	50% chance of eradication in 2009 with 95% in 2010 and 2011 Success is dependant on confidence of surveys conducted in 2008/2009 Uncertain for CoP	£385,000 in first 3 years* Ongoing monitoring after 3 years estimated to be £15,000 per year Costs after 3 years dependant on success of eradication. If eradication failed after 3 years review and/or terminate programme.
E2	Trial eradication with full eradication attempt Jan-June 2010 and follow up treatment where necessary in 2011	Strategy development Research programmes Control tools: <ul style="list-style-type: none"> • Plastic wrapping with or without accelerant • Plastic smothering • Removal • Freshwater Monitoring and surveillance Communications Voluntary vector controls (Code of practice)	50% chance of eradication and 98% chance population controlled in 2009 95% chance of complete eradication in subsequent years. Following summer 2009 monitoring, review programme and/or terminate Uncertain for CoP	£350,000 in first 3 years* Ongoing monitoring after 3 years estimated to be £15,000 per year Costs after 3 years dependant on success of eradication. If eradication failed after 3 years review and/or terminate programme.

Notes: * Includes total costs of eradication, monitoring, communications and management as well as strategy development where relevant. Costs may vary significantly and are an approximation only.

Where eradication is not considered, the following management options are suggested and provided below:

Option		Method/s Tools for management	Likelihood of success	Estimated impacts (over 10 years)
1	Do Nothing	None	Low	Loss of mussel production (est. at 5%) is £1,375,125 once farms are infected*. Loss of mussel production (est. at 25%) is £6,875,625 once farms are infected*. Management by marinas and boat owners (uncertain and dependant on future legislation regarding hull fouling) Management of infected conservation sites (uncertain costs to government) Potential litigation costs to Government (uncertain)
2	Least Control	3. Communications 4. Voluntary controls (Code of practice)	Moderate to Low	4. £20,000 per year over two years but may continue indefinitely if impacts are high. 5. Same as for Option 1
3	Monitoring and awareness	4. Communications 5. Monitoring 6. Voluntary controls (Code of practice)	Moderate to Low	3. £20,000 per year over two years but may continue indefinitely if impacts are high. 4. £45,000 in the first year (Holyhead + state-wide). £30,000 in subsequent years. Costs may increase and continue indefinitely if impacts are high. 5. Same as for Option 1
4	Containment	3. Communications 4. Enforcement and/or Voluntary controls (Code of practice)	Low	2. £20,000 per year over two years but may continue indefinitely if impacts are high. 3. Same as for Option 1 with the addition that enforcement may be required to regulate controls imposed - it is impractical to assess these costs.
5	Monitor to "trigger" stage and containment	1. Communications 2. Monitoring 3. Enforcement and/or Voluntary controls	Low	1. £20,000 per year over two years but may continue indefinitely if impacts are high. 2. £25,000 in the first year (Holyhead only). £20,000 in subsequent years. Costs may increase and continue indefinitely if impacts are high. 3. Same as for Option 1 with the addition that enforcement may be required to regulate controls imposed. These costs are uncertain.

Notes: Direct costs = costs of eradication, monitoring, communications and management. These costs may vary significantly and are given as an approximation only . * It is assumed that farms will be affected after 5 years with production losses ranging between 5-25%. For this purpose, it is considered that 5 years within the next 10 years will suffer production losses.

Evaluation of impacts and economic considerations

Given the uncertainty of the impacts of Dv in the UK; the difficulty in applying costs to various management options; and the inability to apply monetary values to impacts on conservation areas, the economic evaluation of benefits compared to costs are presented as a simplified account of the potential cost-benefit to the mussel industry only. In this regard, the costs to undertake a full eradication campaign (£350-380,000 over 3 years with approx. ~£15,000 each year thereafter for monitoring and surveillance) is greatly overwhelmed by the potential loss in value to the mussel industry (£1,375,125 - £6,875,625 in the next 10 years).

The body of evidence in conducting this report has indicated that eradication is both feasible and cost effective. It is also considered timely. It could be argued that given that the opportunity to eradicate exists now, but is likely to be lost in the next few years, lack of action would be deemed unreasonable. Under the Habitats Directive, Member States have an obligation to take steps to avoid the deterioration of natural habitats and the habitats of species (Article 6(2)). Article 22 of the Habitats Directive also places an obligation on Member States to ensure the regulation of the deliberate introduction of non native species. Results of the risk assessment indicate a very high probability that vulnerable reef habitat will be affected within the next 10yrs if action is not taken now to control the Holyhead Marina population.

Recommendations

It is recommended that the eradication plan E2 be implemented at Holyhead Marina immediately, which may include:

- Instigation of the E2 plan as a case-study to test potential strategies for Marine Biosecurity preparedness and response, as well as development of capability, in Wales.
- Formation of a Dv working group and development of a global network contact list.
- Develop education and awareness programmes targeted to Holyhead Marina boat owners.
- Conduct eradication treatment trials in 2009 and develop an understanding of the costs, resources and time lines required to eliminate Dv.
- Monitor surrounding substrates in July/August 2009 and review implementation plan depending on results.
- Undertake full scale eradication in Jan-June 2010 and follow up treatment in 2011 where necessary.
- Work with Holyhead Marina and boat owners to develop hull cleaning strategies.
- Develop research strategies to study the effectiveness of control techniques as well as the biology of Dv (dispersal characteristics, growth rates etc).
- Develop monitoring programmes for improved methods for identifying potential areas where Dv is likely to occur.
- Develop a better understanding of principle vector pathways – e.g. recreational vessels.
- Develop communication with other countries for cooperation in hull fouling/vector management.

1 BACKGROUND

In July 2008, an unidentified colonial ascidian was observed in Holyhead Marina, covering algae, other ascidians and submerged substrata such as pontoons, chains and ropes. A taxonomic expert identified the species as *Didemnum vexillum*.

D. vexillum, henceforth referred to as Dv, 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.

Diving surveys within Holyhead Harbour were undertaken by the Countryside Council for Wales in December 2008 and January 2009 (Holt *et al.*, 2009). Dv was found to be confined to the marina floating pontoons, anchor chains and two boat hulls moored at the marina.

This finding is the first reported presence of Dv in the UK. Rapid surveys of other marinas were carried out at locations around Wales between December 2008 and February 2009 and failed to detect the pest in areas outside Holyhead Harbour.

It is likely that the invasion has been fairly recent, at least within the last 5 years. Abundance ranged from less than 1% cover to a maximum of approximately 10% cover and morphological forms were sheet-like. Furthermore, additional findings from the survey show a large area of habitat suitable for Dv that has not yet been colonized, suggesting that the pest has not yet filled all available habitats within either the marina or the wider harbour area. This in turn presents the risk that the ascidian may spread further within the harbour, consistent with its behaviour globally, and thus present a problem as a source of infection for other habitats around Wales.

This report was commissioned to provide advice on the feasibility of eradication and/or control of Dv in Holyhead Harbour and to assess the potential for Dv to spread in Wales and the associated consequences if left unchecked.

2 DESCRIPTION OF *DIDEMNUM VEXILLUM* (DV)

2.1 Taxonomy and identification

Dv is a colonial ascidian. Until recently, the taxonomic relationship and geographic origin of *Didemnum* species discovered worldwide remained unresolved issues. Recent molecular data from colonies sampled from Europe, the east and west coasts of North America, Japan and New Zealand strongly indicate that *Didemnum vexillum* represents a single species, possibly native to the northwestern Pacific Ocean, that has become established globally (Stefaniak *et al.*, 2009).

Although molecular diagnostic tools are available, identification can be made by morphological examination, however a highly skilled expert is required for species confirmation. Colonies exhibit a wide range of morphological variation. The pinkish, tan, or pale orange colonies can be long and rope-like (up to about 1 m in length) or can form undulating, encrusting mats. Larvae are also able to be identified morphologically and are relatively large and visible with a hand lens.

2.2 Habitat preferences and growth rate

Throughout its current range, Dv is abundant at many nearshore and offshore sites, preferring salinities above 25ppt and temperate water conditions. It can grow at depths ranging from <1m to at least 81m. Dv is capable of rapid growth and dispersal and at many subtidal sites it is a dominant space holder.

Populations have invaded a variety of habitats and will grow on a wide variety of hard substrata. Dv tends to prefer substrate that has some degree of fouling present and is able to overgrow plants, invertebrates and algae. It is very common on pontoons, docks and pilings and is commonly found on boat hulls that have not been regularly maintained or cleaned. In aquaculture areas it is found on suspended mussel lines and salmon cages. However, unlike some introduced species that remain restricted to artificial substrates, Dv can quickly colonize and overgrow apparently healthy natural benthic substrates, including subtidal rock outcrops and gravel (pebbles, cobbles and boulders) in deeper water (30-80 m) as well as shallow intertidal rock pools.

Dv may in fact be more common in off-shore open water habitats than has been documented. Surveys of deep-water habitats are logistically difficult to perform and are conducted less frequently relative to surveys conducted in shallow, near-shore habitats.

Dv is considered an “ecosystem engineer”, capable of drastic modification of the habitats it invades (Wallentinus and Nyberg, 2007). In the majority of cases where it has been reported as a new introduction, it has grown extremely rapidly within a few years following first observation. This phenomenal growth rate can result in massive colonies that overgrow almost every other sessile species (Coutts and Forrest, 2007; Gittenberger, 2007; Valentine *et al.*, 2007a, b). On the Georges Bank fishing grounds it occupies areas that total more than 230 km², where colonies coalesce to form large mats that cover more than 50% of the seabed.

Various studies have reported extremely fast growth rates; e.g. in two weeks the mean biomass can increase by 60% at 2.5 m (Bullard and Whitlatch, 2009) and small colony fragments (5 to 9 cm²) can re-attach and grow rapidly by asexual budding, increasing in size 6 to 11 fold in the first 15 days (Valentine *et al.*, 2007a). Experience with Dv in New Zealand has also found extremely rapid growth rates: larvae released from colonies in Spring/early Summer settled and developed asexually very rapidly (e.g. reaching 30 cm diameter within 21 days). By the end of summer colonies had developed long tendrils (up to 2m in length) (A. Coutts, pers. comm.)

Because it can reproduce asexually, the only limiting factor determining the size the colony can achieve is the medium on which it is growing. Dv is known to undergo a “die back” stage in cold winter temperatures and low salinity following high rainfall and proximity to river outlets, however populations are known to recover from these events.

Growth form may be related to habitat type, current velocities, or space availability, as rope-like forms are common on vertical rock walls and floating surfaces (docks, ropes, boat hulls) in relatively quiet areas, while encrusting, warty mats that conform to the surface of the substrate are common on rocky seabeds where currents are strong. In deeper water areas with suitable cobble habitat, Dv forms extensive mats on the seafloor. There are few other resident benthic invertebrate species (e.g., sponges, bryozoans, coelenterates) capable of forming mat-like structures in this substrate type (See Mercer *et al.*, 2009).

Dv colonises gravel sea bed areas by essentially “gluing” small pebbles and cobbles together, thereby altering the seabed complexity from a complex three-dimensional system to a two-dimensional “mat”. These mats create a barrier to water flow at the sediment-water interface and have the potential to alter the flux of materials from the water-column to the sediment-column (Mercer *et al.*, 2009).

Dv does not inhabit soft-bottom habitats. Coutts (2002) noted that colonies dislodged from a barge moored in Marlborough Sounds, New Zealand, generally survived if they encountered hard substrata, but eventually died if they landed on muddy or sandy bottoms.

Photographs of Dv in its known habitat are presented on a web site dedicated to these organisms - <http://woodshole.er.usgs.gov/project-pages/stellwagen/Didemnum/>

2.3 Factors that may affect Dv

Dv is spreading worldwide in cool temperate areas. It has shown no lasting sign of dying out in areas it has successfully invaded, and new invasions continue to be reported. However, many of the invasions are recent, and their long term effects are not yet known. Despite this, there are various factors that may affect Dv:

2.3.1 Temperature and salinity tolerances

There is evidence that Dv is unable to tolerate low salinities, however mortality resulting from seasonal changes in salinity is unlikely to be a major factor controlling its distribution. In Southern California following heavy winter rains, complete mortality was suffered by ascidians in the uppermost 0.5 m of the water column, however recruits from adults living in deeper water re-infected these areas when normal conditions returned (Daley and Scavia, 2008).

Dv can tolerate temperatures that range from a low of -1 to -2°C to a high of at least 24 to 25°C (Valentine *et al.*, 2009) and daily changes of up to 11°C (Valentine *et al.*, 2007a). Water temperatures above 8 to 10°C are necessary for colony growth; however, colonies can survive extended periods of time below this temperature threshold as an overwintering form (Daley and Scavia, 2008).

The following summarises temperature tolerances of Dv:

1. Studies have shown the optimal growing temperature to be 14–18 °C
2. Water temperatures above 8 to 10°C are necessary for colony growth. The maximum temperature of survival of the species is not known but may be 25°C. Recent studies suggest that colony growth rates decline when temperatures exceed 21°C for 7 consecutive days (Daley and Scavia, 2008).
3. High temperature variability (~11°C) in the warm season may suppress colony development or recruitment. Water temperature variability may be due to the movement and interaction of strong tidal currents, tidal fronts, and the mixing of warm unstratified bank water with highly stratified cool water masses.
4. Cold temperatures, from 5°C to at least several degrees below zero centigrade, cause colonies to regress, but will often regenerate as temperatures warm. Colonies growing on live marine animals may be more resistant to cold conditions (Gittenberger, 2007).

5. The first occurrence of recruits is linked to annual temperature patterns rather than discrete temperature values (i.e. larval recruits will be released at the end of a developmental period as water temperatures warm, not necessarily when a particular water temperature is reached. E.g. warmer winter temperatures have been known to result in earlier recruitment of several invasive ascidians and ultimately higher summer abundances. See Stachowicz *et al.*, 2002):
- a. The length of time required by an overwintering colony to develop and release brooded larvae is affected by the severity of the cold period and degree of “die back” (i.e. the degree to which colonies degrade in the cool season influences the length of time they require to regenerate, reproduce sexually, and brood larvae).
 - b. This time period may depend on local temperature trends and on the condition of the colony at the end of the preceding cool season.
 - c. In general, however, recruits are likely at 14 to 20°C.
 - d. The time required for a colony to develop and release larvae and the length of the warm season probably affects the length of the recruiting period at a site;
 - e. Recruitment might be regulated chiefly by declining temperature and therefore could end at approximately the same temperature at all sites.
 - f. As waters cool later in the warm season, larvae continue to recruit at temperatures below the temperature of initial appearance. Evidence suggests that larvae ceased to recruit in the range of 9 to 11°C.
 - g. During the warm season, highly variable temperatures likely inhibit the reproductive process and successful colonization. In some locations, temperature variability is a few degrees in areas where the species is present; whereas it is high (11°C) where the species is absent (Valentine *et al.*, 2009).
 - h. At deeper water sites, where minimum temperatures are warmer than at shallow sites, it is possible that colonies are not as affected in the cool season (i.e., do not degenerate) and thus could have a longer recruiting season. This may explain the successful colonization of large areas of gravel habitat on Georges Bank (Valentine *et al.*, 2009).

It is relevant to note here that conditions in Wales, and indeed throughout the UK, are suitable for expansion of the species.

2.3.2 Predators

Few predators have been reported for Dv. Photographic evidence has suggested predation by large sea stars and sea urchins (Bullard *et al.*, 2007a; F. Poole pers. comm.). Littorine snails have been observed feeding avidly on dying colonies (Valentine *et al.*, 2007b) as well as live colonies (see Lambert, 2009). A chiton has been observed feeding on Dv in New Zealand (A. Coutts pers. comm.).

2.4 Distribution

Dv appears to be undergoing a rapid world-wide expansion, with most of the new records appearing in the past 10-15 years. In all cases where it has been recorded as a new occurrence it has undergone simultaneous population increases. It now occurs in temperate waters in many parts of the world, including: Japan; northern Europe (France, Netherlands, Ireland); the U.S. east coast (from Long Island, NY to Eastport, ME near the border with Canada); the U.S. and Canadian west coasts (California, Washington, British Columbia); and New Zealand (North and South Islands).

It is likely that Dv originated in Japan and spread to new locations either via hull or sea chest (water intake area) fouling, with subsequent local spreading by fouled recreational craft, barges,

commercial vessels etc., drifting and reattachment of dislodged fragments, and movements of fouled aquaculture stock and gear (Lambert, 2009).

2.4.1 New Zealand

Dv was first identified in 2001 smothering wharf piles and moorings in a northern harbour. A heavily-fouled barge then translocated the ascidian to an international shipping port some 500 km south, near the heart of the New Zealand mussel industry. From the barge's mooring area, Dv subsequently spread to the seabed beneath, and to nearby vessels and artificial structures (i.e., barges, recreational vessels, moorings, salmon cages and wharf piles). By July 2003, Dv had clearly become well-established. Following a cost-benefit analysis in 2003 (Sinner and Coutts, 2003) an attempt at eradication was made. Although a number of the response methods were completely effective, others were less so and the overall suite of measures failed to eradicate the ascidian. Hence, the structures and vessels from which it had been eliminated gradually became re-infected. By July 2004, Dv had reinfected 87% of the wrapped piles, 7 of 22 recreational vessel moorings, and both barges from which it had been eliminated using chlorine. Furthermore, an infected salmon farm pontoon that had been moved to an aquaculture area approximately 35 km away from the known infection site resulted in transfer of the pest to the remainder of the salmon farm, thus increasing the reservoir of larvae for its almost inevitable dispersal to adjacent mussel farms. Even though a subsequent benefit-cost analysis suggested further eradication efforts would have net benefits, uncertainty over the timeframe and costs, as well as the likelihood of success, undermined stakeholder confidence to the extent that they chose to abandon the program (Coutts and Forrest, 2007). Seven years later and to this date, it has now spread to around 353 hectares.

2.4.2 North America

Dv is highly invasive on both coasts of North America, where it continues to spread rapidly. The initially observed populations in the 1980s were isolated and small, however during the 1990s the species began a rapid population expansion. It now ranges along approximately 750 km of coastline on the east coast and 800 km on the west coast. All indications suggest that it is continuing to spread rapidly along the coasts of North America and is expected to spread into Atlantic Canada waters (Bullard *et al.*, 2007a).

Within its North American range, Dv has a highly disjunct distribution. For example, Bullard *et al.* (2007a) found that colonies were present at approximately 50% of the 190 sites surveyed along the east and west coasts of the U.S. from 1998 to 2005. Interestingly, no colonies were observed at five sites surveyed along the Oregon coast, eight sites south of Port San Louis, CA, or 14 sites surveyed south of Shinnecock, NY to Virginia Beach, VA (Daley and Scavia, 2008). As well as infecting manmade substrates, it is known to occur at deep subtidal sites on natural substrates (> 30 m).

2.4.3 Ireland

Dv was first discovered in on the east coast of Ireland in October 2005 in Malahide marina, north of Dublin on the east coast of Ireland, although it was photographed on the hull of a fouled yacht in June 2005. A similar form was found at Carlingford marina, ~70km to the north on 28 June 2006 (Minchen and Sides, 2006). The distribution appears disjunct, and infections were not found in Dun Laoghaire or Howth marinas to the south of Malahide. Dv has since been found on mussel longlines and has also been recorded from the west coast in Galway Bay on oyster trestles.

The species was first observed as an overgrowing carpet that occupied up to several hundreds of square centimeters and, from these, extensive flexible pendulous growths extended over 60cm in length (Minchen and Sides, 2006). Despite its massive form and possible rapid growth it is considered the invasion has been fairly recent. Minchin and Sides (2006), who between them have had over fifty years diving experience, consider that its abundance and form would make it

very unlikely that Dv would have been overlooked. However there is an equal chance the species remained at low and undetectable stages for many years prior to 2005.

In Malahide there is evidence of natural disappearance of Dv as well as other fouling ascidians. In September 2008, Dv, as well as other ascidians, were noted to be absent. It is suspected that a freshwater purge has taken place as a result of a salinity decline (Dan Minchin, pers. comm.).

2.4.4 Netherlands

Dv was first recorded along the Dutch coastline in 1991, where it remained rare until 1996 (Ates, 1998). Since that time it has become the most common colonial ascidian in the area with an ability to overgrow virtually all hard substrata. This includes rocks, stones, sand, algae and almost all sessile marine animals. The sudden population expansion of Dv from 1996 onward coincided with the cold winter of 1995–1996, which caused decreased population sizes of many marine animals.

2.4.5 France

Dv was first observed in December 1999 at a SW part of Bassin Vauban as a moderate but dense population. There was no Dv on other quays in the SW part, however it was subsequently observed at the NW quay of the same basin which was found to have up to 100 % coverage on all heights of the quay, and covering extensive areas (hundreds of meters long). The infestation was found to completely smother other ascidians and mussels. Dv subsequently spread to nearly all constant-level basins that were examined in the port of Le Havre.

In 2002 and especially 2003, Dv was found to regress. The present state is that Dv is present in all the basins that have been examined in the ports and marinas of Le Havre, Brittany and Brest. Colonies are found attached to a brick quay approximately 3m water depth, barnacles, mussels and tunicates, ropes and floating docks. It is presently known to inhabit tidal basins, especially under pontoons (G. Breton pers. comm.)

2.4.6 Japan

Although likely to be native to Japan (see Lambert, 2009), Dv has increased in prevalence in the country due to the provision of new niches made available by aquaculture development. Oysters were grown primarily in intertidal beds until the 1980's, so it is unlikely that Dv gained a foothold on the stock until the various culture methods involving complete submersion such as rack and tray and longline became common. Miyagi is an area of Japan where Dv has been common at least since the early 1980's.

2.5 Reproduction and dispersal

2.5.1 Method of reproduction

Dv undergoes both sexual and asexual reproduction. Larvae are brooded and spawning typically occurs over warmer water periods between 14 to 20°C. The time required for a colony to develop and release larvae is not fully understood, but the reproductive season can be long, with colonies releasing huge numbers of larvae over several months (see Lambert, 2009).

Larvae have a very short free-swimming stage prior to settlement, where larvae are only competent of settlement between 10 minutes and a few hours after being liberated from a colony (Kott, 2002). Larvae are negatively phototactic and tend to settle on the underside of pontoons and boats. The presence of a consistent, mild wave action or 'swash zone' appears to favour their establishment (Connell, 2000).

Colonies can also reproduce asexually and consist of thousands of small individuals or zooids (approximately 0.2 mm wide and 1 mm long) embedded in a tough outer covering or tunic which varies in colour from pale pink to yellow or pale orange (Kott, 2004). Optimal asexual growth occurs at temperature between 14-18°C, however growth can occur between 11°C and 25°C.

Rapid budding of zooids to produce large mats or pendulous growths, depending on the environment. Budding enables the species to spread via fragmentation.

2.5.2 Methods of dispersal

Dv has the potential to be dispersed by larval release or fragmentation from adult colonies via:

1. Natural processes:

- Currents
- “Hitchhiking”

2. Human vectors:

- Hull fouling
- Ballast water/sea chests
- Fishing and dredging
- Aquaculture

2.5.2.1 Dispersal via natural mechanisms

Although the spread of Dv has primarily been attributed to human mediated processes, other dispersal mechanisms need to be investigated. For example, larvae and colony fragments could be passively transported to new areas by water currents, or released by colonies that have colonized the carapaces of crustaceans or other mobile, hard shelled organisms.

Throughout the *Didemnum* genus, larvae produced in sexual reproduction are generally short-lived, swimming for only a few hours before attaching to substrate. This short, free-swimming larval stage is not considered to be able to last long enough to be carried great distances by ocean currents and it is likely that larval dispersal of Dv contributes only to local spread.

However currents may spread Dv via fragments from which new colonies can form asexually. This risk becomes even more apparent if the pest develops to the large pendulous stage. In its rope-like growth form, long flaccid lobes extend from the central portion of attached colonies that easily break off. There are anecdotal reports of divers observing lobes breaking off substrates and becoming lodged on surrounding substrata, reattaching and over the course of several months thriving in their new locations. It has been observed that during suspension, fragments adapt to the water habitat by changing their gross morphology into spheres (Carman, 2008) and that 60% of fragments are capable of surviving suspension for 18 days while 15% can survive in suspension for 30 days. Furthermore, Bullard *et al.* (2007b) found that fragments can re-attach within six hours after being in contact with the substrate. Thus, fragments are viable for a considerable amount of time and may tolerate being transported great distances before settling and reattaching.

Dispersal via fragmentation may have two significant advantages. First, reattached lobe fragments may be less susceptible to competition or predation than small newly settled larvae. Second, brooded larvae contained in fragments could be released before or after reattachment and further increase dispersal capability. It has been suggested that the widespread distribution of Dv in Georges Bank may be a result of the constant disturbance and fragmentation by scallop dredging operations, resulting in colony fragments floating away and reattaching (Lengyel *et al.*, 2009).

2.5.2.2 Dispersal via human mediated vectors

Vessels – hull fouling and ballast/sea chests:

The most probable trans-oceanic vector is shipping, either via hull fouling or contaminated sea chests in large oceanic vessels. Rapid regional and local dispersal can result from many modes of

transport, with slower moving recreational vessels and barges, moving between marinas, ports, and harbours, likely to be one of the most significant vectors (Lambert, 2009).

Sea-going trade continues to increase globally, and the networks of movements have increased in range. For example, in Ireland up till the early 1900s, trade via sea was mainly confined to Britain, Northern Europe, and the eastern coast of North America. Today links exist with all continental regions.

Any vessel or structure of any size may accumulate fouling, and if mobile, can serve to transport fouling organisms. The following outlines the relative likelihood of the various vessel types introducing Dv.

Ships and barges

Dv produces larvae that have a short planktonic stage. As such, the likelihood of survival in ballast water is very low (Carlton and Geller, 1993).

Long distance survival of Dv fouling the hulls of fast-moving ships is unlikely, but they could survive on the hulls of slow-moving barges as is thought to have been a possible vector for Dv in New Zealand (Coutts and Forrest, 2007). Certain regions of hulls can support fouling, allowing colonies to develop in areas such as the propeller shaft housing and also in sea chests (Coutts and Dodgshun, 2007).

Ferries

Fast moving vessels have a lower likelihood of acting as a vector, although no studies have looked at this risk. However, the speeds generated by Ferries travelling between Ireland and North Wales, and the length of time spent in each port at any one time suggest they are an unlikely vector.

Commercial fishermen

In North America, commercial fishermen were not considered likely to act as high risk vectors. While this requires study here, discussions with the Marine and Fisheries Agency indicate that commercial vessels from Holyhead Harbour do not travel extensive distances. The risk is uncertain.

Recreational vessels

The proliferation of marinas for recreational vessels over recent decades is a worldwide phenomenon (Minchin *et al.*, 2006). In theory, recreational vessels were considered less likely to support extensive fouling accumulations, however it is becoming more apparent that hull fouling on recreational vessels presents a high risk for transfer of pest species, including Dv, especially on a local scale (following personal communication with global experts).

In Scotland, 59% of yachts surveyed in a recent study were found to have macrofouling attached to their hulls (Ashton *et al.*, 2006). In addition, a study undertaken by a Masters student at Bangor University on boats moored at 5 marinas along the Welsh coast found that a high percentage of the yachts surveyed had some degree of macrofouling; the greatest percentage being at Pwllheli and Conwy with 84%, followed by Deganwy with 72%, Holyhead at 71% and Victoria Dock with 65% (Kate Griffith, pers. comm.). However, the movements of heavily fouled vessels relative to clean vessels is unknown, complicating any assessment of risk.

Increased age of the antifouling paint, as well as long stationary periods and reduced sailing activity are thought to be responsible. In addition, in the UK recreational yachts frequently travel short distances and there is a high probability, therefore, that the source and recipient areas will be within the same climatic region, and that the fouling species will survive in the receiving habitat if similar environmental conditions exist.

In New Zealand, however, only 15 % of yachts surveyed had evidence of macrofouling (Floerl *et al.*, 2005). Recent discussions with Biosecurity New Zealand concur with this. In both NZ and Australia the government has been active in communicating the risks of hull fouling, promoting the good practice of keeping hulls clean and in some cases enforcing conditions that hulls are not fouled with certain risk species before entry into particular areas. In New Zealand, hull cleaning guidelines have been introduced (<http://www.biodiversity.govt.nz/seas/biosecurity>) and developed. The Australian Quarantine and Inspection Service (AQIS) has been trialing Biofouling Management Requirements for international yachts less than 25 metres in length since 2005, with a view towards applying risk-based Biofouling Management Requirements to all international vessels arriving in Australia. To assist boat owners, AQIS produced a Biofouling Fact Sheet providing information about how the protocol will impact them as well as 'Biofouling maintenance guidelines and log book' to assist in recording biofouling maintenance work performed on vessels (see <http://www.daff.gov.au/aqis/avm/vessels/less-25m/biofouling-protocols>).

While cleaner hulls in NZ and Australia are undoubtedly due to the effort devoted in each country to addressing this issue, the lower incidences in internationally travelling vessels may also be a result of the mode of use of yachts in these areas. In order for international ships to arrive at New Zealand, they must cross oceanic waters where macrofouling organisms are likely to be dislodged, prevented from feeding, or are unable to survive changes in environmental conditions experienced during oceanic voyages (Carlton and Hodder, 1995). Despite this the risk is still apparent. In any case, it cannot be ignored that international movements occur more frequently and via shorter distances throughout the EU.

Dredging and fishing

It is considered highly likely in North American situations that Dv may have been subsequently introduced offshore on Georges Bank via contaminated scallop dredging gear and boats from their home ports (see Lambert, 2009).

Dredging and fishing can have an influence on:

1. Making the environment more susceptible to invasion: In terms of growth forms, colonial ascidians are more successful than solitary ascidians in occupying primary space following disturbance (Altman and Whitlatch, 2007).
2. Dispersing fragments of Dv if already present: Fish and scallop trawls pulled through areas infested with Dv can fragment colonies and suspend them in the water column. If viable fragments survive in suspension they may be transported via ocean currents to other habitats.

Lobster and rock crabs may also represent a vector for the spread of Dv. Natural movement may contribute to regional spread and commercial shipment may spread Dv on a global scale (Bernier *et al.*, 2009). Lobster pots may also be a vector of concern.

Aquaculture

It is possible that Dv was introduced into the Gulf of Maine with oyster aquaculture in the Damariscotta River, ME, with the vectors likely to be the Pacific oyster (*Crossostrea gigas*) (Dijkstra *et al.*, 2007). Likewise in France in the late 1960s the introduction of huge quantities of *C. gigas* seed stock on shell from Japan and large quantities of adult *C. gigas* brood stock from British Columbia may have resulted in translocation of the pest. In Ireland, Dv has spread to oyster farms on the west coast and aquaculture operations have been implicated (D. Minchin, pers. comm.).

In New Zealand the movement of a fish farm net heavily fouled with Dv to an uninfected mussel growing area resulted in extremely rapid large scale fouling of the mussel lines within weeks (see Lambert, 2009).

3 RISK ANALYSIS ON THE LIKELIHOOD AND CONSEQUENCES OF DV SPREADING TO VALUE AREAS IN WALES

A series of questions were presented in a questionnaire (Appendix 1) and sent by email to contacts sourced from the WHSC website: <http://woodshole.er.usgs.gov/project-pages/stellwagen/Didemnum/>. Where participants were asked to assess the likelihood of an event, the following nomenclature was provided as a guide (Table 2.1):

Table 2.1. Nomenclature for qualitative likelihoods

Likelihood	Descriptive definition
High	The event would be very likely to occur
Moderate	The event would occur with an even probability
Low	The event would be unlikely to occur
Very low	The event would be very unlikely to occur
Extremely low	The event would be extremely unlikely to occur
Negligible	The event would almost certainly not occur

11 responses were received and are summarised in the assessment below.

The following presents an analysis of the level of infestation present at Holyhead Marina and a subsequent qualitative assessment of the likelihood of Dv spreading and establishing in value areas around the Welsh coast and the relative consequences if this was to occur.

3.1 How long has Dv been present at Holyhead Marina?

In the absence of any baseline data, it is difficult to determine if the observed population is a recent occurrence (i.e. 2008) or one that has remained at undetected levels over a longer period of time.

The stage at which a new invasive species is detected depends on a number of factors including (1) how conspicuous it is in terms of visibility and habitat, (2) frequency and mode of monitoring and sampling within a watercourse, (3) environmental conditions at time of sampling and (4) pure chance events.

In the current situation, Dv was observed by a skilled scientist actively surveying the marina in July 2008. The marina had not been surveyed previously prior to this event and the pest was not overly conspicuous so would not have been detected by an untrained eye.

The confinement to marina pontoons, chains, and 2 boat hulls and the sparse and patchy distribution on these substrates, lack of tendrill formations as well as absence from other suitable habitat could be interpreted as evidence of it having recently arrived, i.e. within the last 12 months. However, for this to occur, it is likely that the initial inoculum source was relatively large (A. Coutts, pers. comm.). The counter argument is that it arrived several years ago, from more than one 'infection' event, where environmental conditions and inoculum pressure have resulted in an apparently slow rate of development.

It is impossible to state how Dv entered Holyhead Marina. While recreational vessels are implicated, an interesting comment by one respondent suggested that the observed infestation may be the result of a stochastic event, where the initial inoculum source was large, such as the employment of a specialised vessel (e.g. a dumb barge) to install new structures in the harbour (A. Coutts, pers. comm.). Further research into vectors is required.

Questionnaire results:

2 respondents indicated it has arrived within the last 12 months, 5 responses considered it most likely that the pest arrived within the last 1-2 years, 3 responses considered 2-5 years more likely while 1 response was unable to make any firm statement.

3.2 What is the likely extent of infestation at Holyhead Marina?

Regardless of how long ago Dv established in Holyhead Harbour, coverage is relatively low, confined to manmade substrates within the marina and pendulous growth forms typical of long established populations are not apparent.

Questionnaire participants were asked to estimate the level of infestation using an infestation curve (Figure 2.1), derived from population dynamics studies, which has three distinct phases:

- the initial lag phase (stages 1-3) when the organism is establishing itself and becoming apparent
- the explosion phase (stages 4-7) when the organism's population and distribution increases rapidly
- the widespread phase (stage 8) when the organism's population has stabilised and filled most of the habitat suitable to it.

The lower Dv is situated on the curve, the more cost effective it will be to control it. If elimination is possible at a modest cost and the pest has the potential to cause adverse effects, then the cost benefit evaluation in favour of control action would be significant.

The higher Dv is on the curve, the more difficult and costly it will be to control. If control is attempted there will be greater uncertainty about the costs and benefits, and a greater risk of failure.

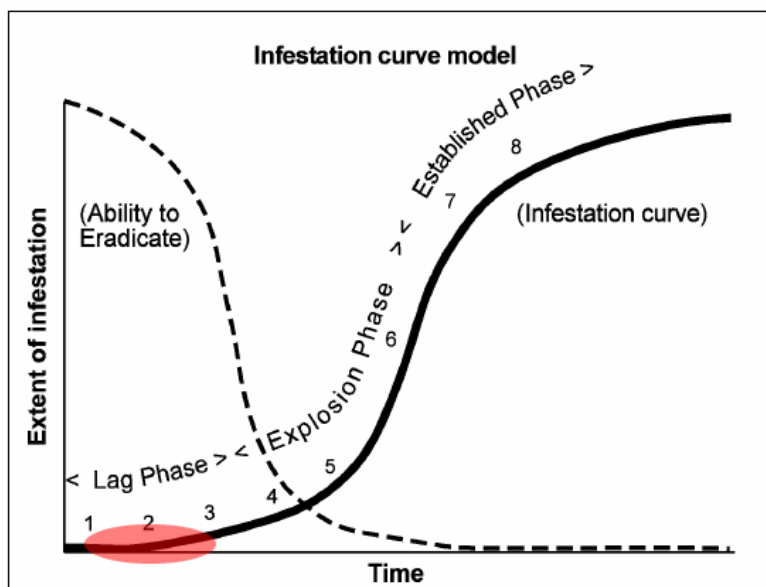


Figure 2.1. Infestation curve showing responses from questionnaire participants highlighted in red.

Questionnaire results:

It was considered by all respondents that Dv in Holyhead Marina is at the initial lag phase, when the organism is establishing itself and becoming apparent. It was considered likely by 3 participants that the pest was towards the higher end of the scale and may spread quickly in the near future.

3.3 What are chances Dv could naturally disappear/die out?

In general, the temperature conditions in Holyhead Marina range between 5°C and 22°C throughout the year. These are suitable growing conditions for Dv.

However, Dv is known to be susceptible to low salinity conditions, and evidence in Ireland has suggested that infestations on pontoons have disappeared at Malahide estuary and that this may be due to the large lagoon and freshwater that can accumulate and so pass by the marina purging not just *Didemnum* but also other tunicates (D. Minchin, pers comm.).

Despite this, mortality resulting from seasonal changes in salinity is probably rarely, if ever, a major factor controlling its distribution over long temporal scales. In Southern California, ascidians in the uppermost 0.5 m of the water column suffered complete mortality following heavy winter rains but were quickly replaced by recruits from adults living below the affected area after the rains had subsided (Daley and Scavia, 2008). Furthermore, Dv colonies situated on other organisms are less susceptible to environmental stressors.

There are 2 river outlets that feed into Holyhead Harbour, and while it is not known whether significant changes in salinity occur due to high rainfall, the discharge is minimal and unlikely to cause massive variations.

Questionnaire results:

Given that salinity changes are unlikely to have a lasting influence on DV populations, it was considered by most participants that this event would be extremely unlikely to occur, with the reminder considering the likelihood to be negligible. Indeed, the suitability of habitat and water temperature indicate that it will continue to develop within the marina.

3.4 What is the likelihood Dv will spread to all available habitats within Holyhead Harbour?

Holyhead harbour is located on Holy Island and is protected from the Irish Sea by a 2.4 kilometre breakwater sheltering an area of 260 hectares comprising the Inner, Outer and New Harbours. The approaches to Holyhead are beset by strong tidal currents (up to 6 knots), however Holyhead Bay is relatively quiet with less than 2 knots. Within the harbour it is virtually slack water, although with some wave movement at times. The tidal amplitude within the bay is 6m. Depths within the harbour range from 15m at the entrance to 6m approaching the marina. The Marina has 230 berths and is frequented by numerous vessels. The natural substrata of the estuary varies from gravel deposits at the shallow entrance to fine muddy deposits in the shallows beneath and adjacent to the marina, with rocky sea defences below the entrance walkway.

Surveys conducted in December 2008 and January 2009 revealed that Dv was not found anywhere in the harbour outside the marina area (Holt *et al.*, 2009).

Habitats currently infested:

1. Pontoons
2. Ropes and buoys
3. Chains supporting mooring pontoons
4. Boat hulls

Potential habitats within Holyhead Harbour:

Although Dv was not found outside the marina in the wider harbour area and on the commercial terminals, the dive survey undertaken in January 2009 assessed the potential for other substrates to be suitable for colonisation (Holt *et al.*, 2009). While the small mooring buoys were not considered suitable habitats, judging by the vigorous growth of native and some non-native species of sea squirts on virtually all the other structures surveyed there is considerable scope for Dv to colonise huge areas of the harbour.

Furthermore, there is potential for Dv to spread into natural areas including the rocky sea defences and the gravel patches in deeper waters.

The means of spread are likely to occur via larval dispersal via currents and fragmentation once populations reach the pendulous stage at the marina.

Potential habitats that have not yet been exploited are:

1. Seabed beneath marina: Low probability of infestation:
2. Rocky sea defences: High probability of infestation.
3. Yacht moorings: Low probability of infestation.
4. Large mooring buoys: High probability of infestation.
5. Terminals 2, 3, 4 and 5 and Aluminium Jetties: Moderate probability of infestation.
6. Fish dock: Moderate probability of infestation.
7. Gravel seabed: High probability of infestation.

Questionnaire results:

Given the dynamics of the Harbour, and the known global behaviour of DV, it was considered highly likely by the majority of participants that DV will continue to extend into all available habitat within Holyhead Harbour, where it will likely form pendulous type growths, given the sheltered nature of the marina.

3.5 How long will it take for all available habitats to be occupied?

It was considered difficult to determine the time for Dv to spread within the harbour with any certainty as the spread will be highly influenced by the vectors involved. Natural spread by non-feeding, short-lived larvae will be slow if the larvae must make their way buoy by buoy (hull by hull; dock by dock) to other parts of the harbour. Spread will be faster if sexual reproduction occurs all year round, however it is uncertain if this is occurring. There is some evidence to suggest that colonies in Holyhead Marina have regressed slightly during the colder months, but in general, water temperature conditions remain optimal (14°C to 18°C) for both growth and larval release for at least 6 months of the year.

Questionnaire results:

The majority of participants considered that spread within Holyhead Harbour would be considerable within the next 2 to 5 years.

3.6 What is the likelihood Dv will be transported by recreational vessels to other Wales locations?

Hull fouling presents the most likely vector for local dispersal of the pest at the current level of infestation. The potential vector group most likely to transport Dv to other locations around the UK coastline are recreational vessels moored to the marina pontoons. However, as infection spreads to occupy all available habitat within the harbour, all vessels hulls are considered potential vectors, with the exception of ferries which travel at high speeds and thus present a low probability of risk. In addition, natural dispersal by fragmentation may occur if colonies become extensive.

It is beyond the scope of this study to fully assess the movements of vessels in and out of the marina, however information taken from the recently published Atlas of Recreational Boating (RYA, 2009) demonstrates the movements of recreational vessels within the Irish Sea (Figures 2.2 and 2.3) as well as some indication of the intensity of activity in certain regions.

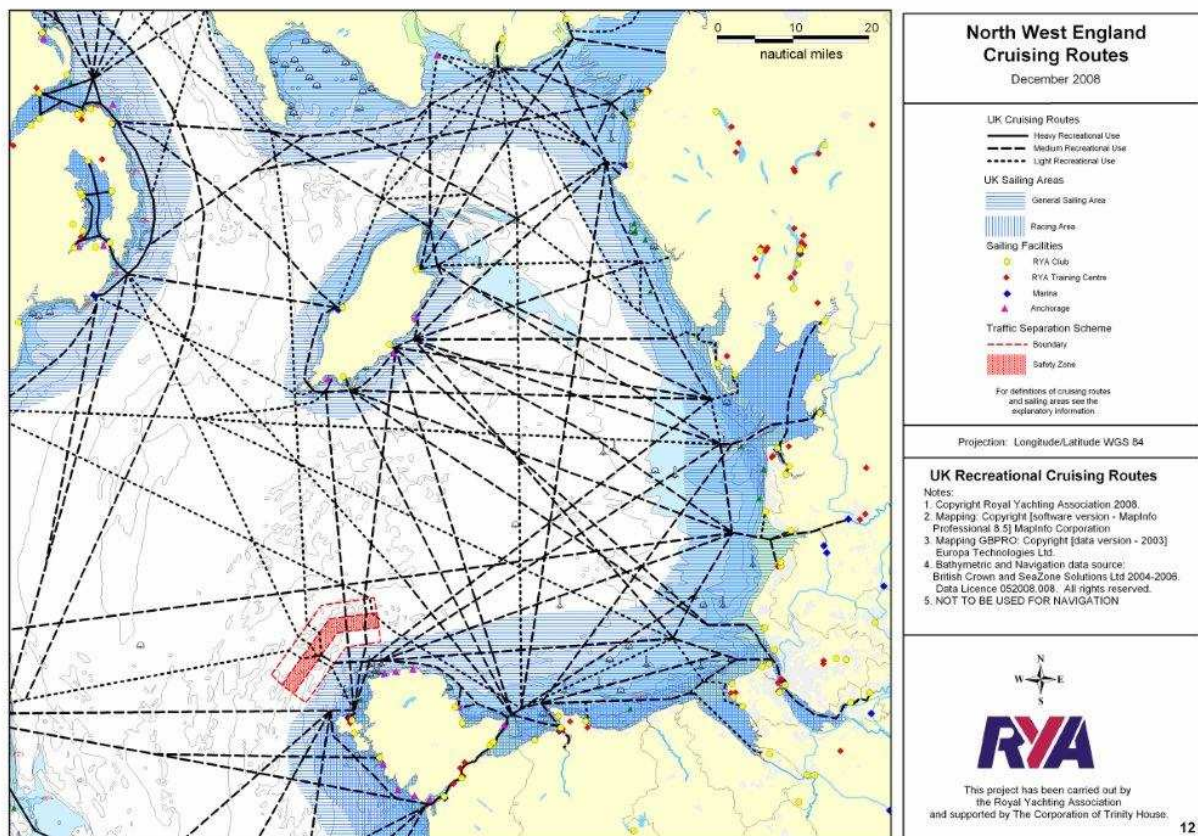


Figure 2.2. North West England Cruising Routes, December 2008. Image reproduced from the Atlas of Recreational Boating (RYA, 2009) with the permission of the RYA.

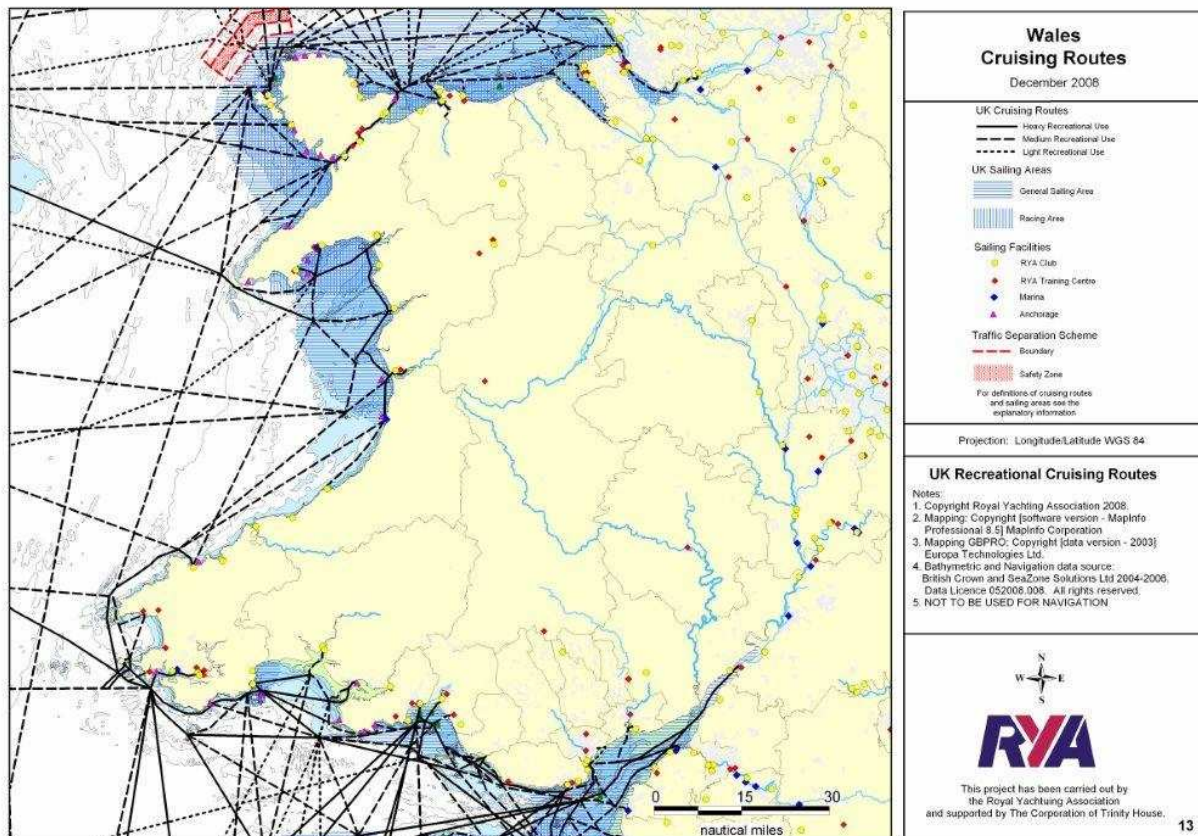


Figure 2.3 - Wales cruising routes, December 2008. Image reproduced from the Atlas of Recreational Boating (RYA, 2009) with the permission of the RYA.

The following information is provided from a report for the Department of Trade and Industry by the Royal Yachting Association (RYA, 2005): Holyhead is one of the UK's busiest ferry ports. There are about 8,000 conventional and fast ferry movements a year and over 500 calls from bulk carriers, cruise liners, coasters and large fishing vessels. Countless numbers of small fishing vessels and leisure craft call at the port. Holyhead harbour, being a harbour of refuge, may be entered in all weather conditions and at all states of the tide. Holyhead marina supports around 230 recreational vessels. In the winter months the marina is usually half full (around 150 vessels). The Irish Sea is a popular recreational boating area with routes to and from the English, Welsh, Scottish and Irish coasts as well as to and from the Isle of Man. The relative close proximity of these different and varied locations makes cruising in the Irish Sea particularly popular. Data from 2004 estimated the total moorings available to recreational boating in the area at just under 13,000.

The majority of routes tend to follow the coastline. Due to the tidal nature of many of the ports, such passages tend to be either very short distances, between neighbouring ports for example, or relatively long distances so that arrival times can coincide with the next tide. Areas such as the Menai Strait frequently require a temporary anchorage or stop at a nearby port due to limiting tidal constraints (RYA, 2005).

In summary, vessel movements are frequent along almost all areas of the coastline and often occur at times when larvae are seeking to settle: Overnight stays are likely and activity is most frequent over the summer period and up until September, a time that coincides with likely spawning periods of Dv.

Evidence suggests that Dv is most likely to spawn in temperatures above 14°C, with the spawning period extending into the cooler months to temperatures as low as 11°C. Sea surface

temperature data provided by the Irish Sea Observatory <http://coastobs.pol.ac.uk/cobs/sat/> over the last 3 years indicates that temperatures around Holyhead Harbour begin to exceed 14°C around June and July and do not fall below 11°C until late December. This suggests that the period available for spawning may be as long as 8 months, although the period of risk in acquiring infection from Holyhead may actually be from June to October, given that recreational vessels do not tend to have much activity between October and March. At any rate, the risk may extend through to December in the marina where the vessel overwinters as long as sea temperatures remain above 11°C.

Questionnaire results:

The majority of participants considered it highly likely that recreational vessels will transport infective stages of Dv from Holyhead Marina to other locations around Wales. Recreational vessels often remain stationary for long periods of time, many have poorly-maintained hulls, are relatively slow-moving, and move along the coast in places that are inaccessible to larger vessels.

It was also mentioned that while at this stage pendulous growth forms are not present, and thus spread via fragmentation is unlikely, it is considered highly likely that pendulous growth forms will develop within the next 5 years. In this regard, the harbour thus becomes a very high risk source in the translocation of DV via fragmentation and as such may function as a stepping stone to promote further dispersal (see Darbyson *et al.*, 2009): “*It is the local, secondary spread within a country that will ultimately determine the extent of the economic and environmental impact of a non-native species (Lodge et al. 1998).*”

3.7 What is the likelihood Dv will establish in other marinas in Wales?

Given our current understanding of the biology of Dv, areas most susceptible to invasion are those with growing season of 14-18°C of 6 months or longer and where mean monthly water temperatures are below 25°C.

Most coastal areas in Wales have temperatures above 14°C and not exceeding 22°C for at least 6 months of the year (May to November), however some of the Wales mainland coastal areas demonstrate very low temperatures (5-6°C) from November through to April.

There are numerous marinas along the coastline. Data for recreational yachts from 2005 listed around 11,600 club members and 1650 berths within the North Eales area alone (RYA, 2005).

Questionnaire results:

Given the plethora of recreational vessels, their movements, and environmental matching, the majority of participants considered it highly likely that Dv will establish in other marinas along the Welsh coast. It was considered that locations with low temperatures of 5 to 6°C might temporarily halt sexual reproduction, but that this will not eliminate establishment of Dv; i.e. the colonies might regress, but they will return as temperatures warm.

3.8 What is the likelihood Dv will establish in mussel beds in North Wales?

The Menai Strait is the UK’s most important mussel producing area. Mussels are grown to market size on the sea bed and the fishery is dependant on seed mussel, sourced from eroding mussel beds in sites both within North Wales and from around the UK coast. Mussel beds may

be subtidal and situated on sand and gravel substratum or intertidal and often concentrated on areas of hard substratum but may occur on mud or sand.

There is currently no commercial-scale line culture however there is growing interest in developing this in some offshore locations around North Wales, with a series of small-scale ‘trial’ operations currently underway. One of these is situated near Holyhead.

Questionnaire results:

The results were highly variable and reflect the lack of information and experience regarding the potential invasiveness of Dv on cultured mussel beds. Globally, the majority of mussel production is on hanging culture lines, where it is considered highly likely that Dv would establish. With regard to mussel beds, it is well documented that Dv will grow on mussels and it is also known to succeed on gravel substratum, and on sand as long as it is not moving in strong tidal and storm currents. An equal number of respondents considered the likelihood to be either high or moderate that Dv could establish in subtidal beds but unlikely (low likelihood) that intertidal beds would be affected.

3.9 What is the likelihood Dv will establish in SACs in Wales?

Special Areas of Conservation (SACs) are protected sites designated under the EC Habitats Directive. Reef areas present within these sites (Figure 2.4) were considered to have the highest potential for infestation.

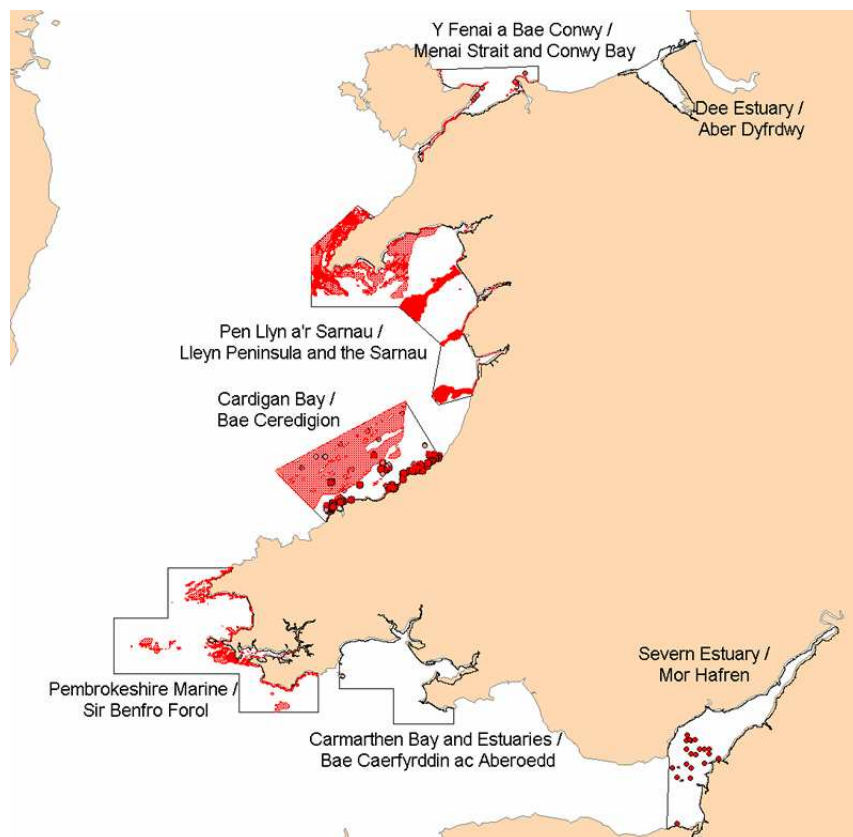


Figure 2.4 Marine SACs in Wales with reef features indicated as darker red areas and possible reef indicated as paler areas. (This map is reproduced from Ordnance Survey material with the permission of HMSO. Crown copyright reserved. CCW licence No. 100018813.)

The following general groups of types of reef present in SACs and considered suitable habitat for Dv are:

1. Rocky intertidal reefs
2. Rocky subtidal reefs (bedrock, boulders, cobbles, mixed)
3. Extensive boulder and cobble subtidal reefs – e.g. the Sarnau
4. Biogenic reefs
 - a. Horse mussel (*Modiolus modiolus*) reef
 - b. *Musculus discors* mussel reef
 - c. Honeycomb worm (*Sabellaria alveolata*) reef
 - d. *Mytilus edulis* mussel reef
5. Carbonate reef formed by methane gas leaking from the seabed.

Questionnaire results:

4 respondents considered the likelihood to be high while a moderate likelihood was given by another 4 respondents. One respondent considered the likelihood to be possible but low. In general, it was considered that given the documented occurrence of Dv establishing in such habitats around the world, it is likely that Dv would colonize reef areas within SACs. The main impediment to Dv colonizing reef areas would be turbid or muddy conditions and exposure at low tide. While there has been no evidence of infestation of natural reefs in NZ, the widespread coverage of gravel beds in Georges Bank indicate it can dominate these communities.

3.10 What are the consequences if Dv were to spread to:

3.10.1 Marina and harbours

Leaving Dv unchecked would probably not generate any major costs in the port itself. It would be considered a nuisance but no more hazardous than other foulers. It would, however, pose a significant risk in terms of transporting Dv and frustrating management efforts elsewhere.

Impacts will depend on future regulations. If legislation is brought in to ensure marinas and boats are clean there will be ongoing costs to achieve pest free status. Recent discussion with Biosecurity New Zealand has revealed that movement towards international hull fouling regulations are presently being discussed with the International Maritime Organisation in London (N. Parker, pers. comm.). The IMO, with MAF Biosecurity's leadership have initiated a biofouling correspondence group to try to fast-track international measures for biofouling. While it took over 10 years for ballast water to get to the current ratification stage, they are hoping to accelerate this process for hull-fouling measures. Hull fouling will likely be an issue in the future for Wales.

Questionnaire results:

The majority of responses considered the consequences to be low with the ascidian being considered a nuisance in these areas.

3.10.2 Mussel farming

In NZ, Dv readily attaches itself to lines on which mussels are also growing. Likewise in Canada, heavy infestations of ascidians in aquaculture operations have increased handling and processing costs. Lines and cages weighed down by ascidians require cleaning before they can be retrieved, and ascidians need to be removed from shells before they are marketable.

Furthermore, presence near spat and immature seeding mussels is a potential problem as it may force the need to eradicate Dv before mussels can be transported to other locations for growing and harvest. Presence of the pest would also restrict vessel movements to pest free locations.

The North Wales mussel industry is the most productive fishery in the UK. For the year ended March 2008, 8701 tonnes were taken from the fishery with a total value of £5,500,500 (Figures obtained via personal communication with the North Western and North Wales Sea Fisheries Committee).

It is impossible to determine with any accuracy the potential impact to production volumes, however if it is considered that subtidal beds (which account for 70% of production) would have a 90% chance of infestation, with a potential coverage of 40%, the impact to the fishery could be as high as 25% in loss of production (if it is assumed that the pest would smother mussels and result in poor growth or mortality). In addition to lost production, costs would be incurred through increased processing costs, potential loss of export markets as well as restriction to certain seed beds.

As an simplified estimate, a loss in production of 25% would equate to a loss in production value of £1,375,125 per year. A loss in production of 10% would equate to a loss in production value of £550,050 per year, while a loss in production of 5% would equate to a loss in production value of £275,025 per year.

This does not take into account loss of export markets and potential loss of seed sourcing areas which, in a worst case scenario, may result in total loss of natural seed collection and potential closure of the industry until alternative seed sourcing means are provided (e.g. hatchery production).

Questionnaire results:

While it was considered difficult to predict the extent of the potential impact in mussel bed culture, it would be expected that in general, fouling of cultured mussels would cause higher labour costs, mussel mortality and decreased growth rates in mussels as well as restriction to certain seed beds and export markets.

3.10.3 Conservation areas

It is now internationally recognized that invasive species are one of the major threats to world species biodiversity, second only to habitat destruction. However, predictions on the impacts of Dv are difficult to determine as changes to biodiversity may be initially insidious and in the

long-term highly detrimental. Whether non-native taxa will drive species loss is hard to predict (Gherardi, 2007).

Certainly, Dv has been shown to have an effect on natural communities, both at the inter tidal range and in deep water benthic communities, however the exact nature of these impacts are difficult to ascertain with any real certainty.

In NZ to date, effects on natural areas have not been a problem, as Dv has not been able to accumulate sufficient biomass to spread on and across gravel sea beds which are key feeding and reproduction areas.

Experience in the USA led to the assumption that Dv has a major impact on natural communities where it was found to have spread onto the seabed in Georges Bank (off the eastern coast) and expanded from six square miles to 100 square miles in the space of 4 years. However, despite the widespread distribution of Dv in the US, no dramatic environmental impacts, such as species extinctions, have been reported.

Indeed, despite predicting that the presence of the ascidian mats would reduce benthic species richness and abundance, Mercer *et al.*, (2009) found that these parameters were either not different or were significantly higher in samples taken inside *Didemnum* mats compared to samples collected immediately outside the mats. The presence of the mats did however result in subtle shifts in community structure and functional group dominance (Mercer *et al.*, 2009).

Although many studies are finding few direct consequences of Dv in natural areas, in Georges bank, detailed analysis suggested that Dv is able to out-compete other epifaunal and macrofaunal taxa. Anemones were one of the few groups of animals that appear able to resist overgrowth by Dv (Valentine *et al.*, 2007a). In some instances there was an increase in the abundance of two polychaete species which suggested that Dv is acting as a facilitator by creating a habitat that is more favourable to these two polychaete species (Lengyel *et al.*, 2009).

Further to this, additional studies are needed to assess whether the ascidian mats are providing a predator refuge for some benthic species and how subtle shifts in macrofaunal functional group composition caused by the presence of the mats may influence higher trophic levels in coastal ecosystems. An increase in prey availability has the potential to increase predator abundance that can, in turn, affect community-wide interactions.

The annual shift in colonial ascidian abundance that has occurred over the past 26 years is interesting. Historically (1979 to 1980) colonial ascidians in North America were most abundant during the late fall and winter. More recently (2003 to 2004) they are most abundant during the summer and early fall. This shift may have an impact on benthic communities where the increased abundance could inhibit recruitment of native species and reduce space available for other species. This in turn could lead to declines or shifts in species composition (Dijkstra *et al.*, 2007).

While it is impossible to predict the extent to which conservation sites are affected should Dv spread within the UK, the possible effects on natural areas (including SACs) could be:

- Potential to smother other organisms or deny them access to areas necessary to feed or reproduce.
- Inhibit settlement of other organisms (*Didemnum* is one of the few species which regularly recruits on other species but few species recruit on it (Osman and Whitlatch, 1995)
- Reduction of the spatial complexity of benthic habitats which would indirectly increase the risk of predation on shelter-seeking juvenile fishes and other organisms (Valentine *et al.*, 2007b).

In addition to the direct ecological impacts of the species, its presence has further implications for habitat management. If Dv were to spread to marine SACs in Wales (those most at risk are

probably the Menai Strait and Conwy Bay and the Pen Llyn a'r Sarnau SACs, followed by the Pembrokeshire Marine and Cardigan Bay SACs) it could cause ecological damage by overgrowing resident species and thus altering the structure of the biological community. If this were to happen then the habitat at the site could no longer be considered to be in favourable condition, as the 'typical species' and 'structure and function' conservation objective would not have been met (for more information on conservation objectives see <http://www.ccw.gov.uk/landscape--wildlife/managing-land-and-sea/marine-policies/policy,-legislation--guidance/draft-regulation-33-advice.aspx>).

A spread of Dv out of the marina and into the wider marine environment could also have consequences for BAP/Section 42 habitats and features of intertidal Sites of Special Scientific Interest (SSSIs). Those most likely to be at risk would be tidal swept channels, fragile sponge and anthozoan communities on subtidal rocky habitats, subtidal mixed muddy sediments, *Musculus discors* beds, Blue mussel beds and Horse mussel beds and various intertidal biotopes which constitute features of SSSIs.

The Water Framework Directive (WFD) is currently the principal legislation covering the management of inland, transitional and coastal waters in the EU. The current approach to its implementation regarding ecological assessment using biological elements is to develop assessment systems tailored to detect a response to a specific pressure. Although the WFD does not specifically mention invasive alien species (IAS), discussion has commenced on how to incorporate them into ecological assessment owing to their ability to significantly alter the structure and functioning of aquatic ecosystems.

A potential framework has been suggested whereby IAS are treated as both a pressure and as part of a biological element to be monitored, where densities and distribution of IAS in water bodies are matched to normative definitions for quality classes in the WFD by expert groups at EU level. The aim would be to allow a rapid and consistent assignment of ecological status on the basis of IAS abundance and distribution in a water body.

Thus there may be a requirement for the provision of a separate report of the ecological degradation resulting from IAS so that specific management measures may be designed. Thus the presence of Dv may result in the lower classification of a water body. For example, a high abundance of IAS would indicate high pressure and poor or bad ecological status (Cardoso and Free, 2009).

Questionnaire results:

In general the impacts were considered moderate (see Table 2.1), but that the "value" of these resources deemed by a country affects the allocation of any sensible assessment. In basic scientific terms, however, the majority of respondents considered it highly likely that Dv would cause alteration of reef ecosystems through overgrowth of epibenthic and infaunal species:

"... occupies space otherwise utilized by non-didemnid invertebrates and marine algae, blocks algal growth on boulders and marine plants for grazers such as snails, provides acidic secondary substrate that cannot be utilized by epibionts and macro-epiphytes, blocks sunlight from reaching host plants causing reduced biomass or death, and blocks nutrients from getting to corals" (M. Carman, pers. comm.)

4 ASSESSMENT OF THE FEASIBILITY OF ERADICATION IN HOLYHEAD MARINA

In a recent report providing advice to the European Commission on determining and prioritising future areas of Community action with respect to invasive alien species (IAS) (Miller *et al.*, 2006), it is stated that where feasible, eradication is often the best course of action to deal with the introduction and establishment of IAS.

Feasibility is a reflection of the stage of infestation, the resources available to undertake the eradication, the cost effectiveness of the process and the probability of success.

4.1 Stage of infestation:

Evidence to date suggests that Dv in Holyhead Marina is a relatively recent occurrence. Results from the risk assessment survey indicate that the current level of infestation is low on the infestation curve (Figure 2.1) and therefore a high likelihood exists that control can be implemented in a cost effective manner.

The pest is restricted to pontoons, chains and 2 boat hulls. All of these structures have potential to be treated using existing control methods.

4.2 Resources available:

4.2.1 Control methods

Control techniques have been developed and tested extensively in New Zealand (see Coutts and Forest, 2005; 2007 and Pannell and Coutts, 2007) and are presented below with cost estimations for implementing in Holyhead Marina. It should be noted that these costs may vary significantly and are given as an approximation only.

The main techniques to control Dv include:

1. Wrapping/enveloping infected underwater structures (pontoons) in plastic film, which prevents supply of clean water to the Dv and smothers it through lack of oxygen. An accelerant (acetic acid or chlorine will reduce application time). If applied correctly this is considered 100% effective.
2. Removal of infected matter from the water and air drying for 48 hours, i.e. ropes, bouys etc. 100% effective.
3. Cleaning hulls of infected vessels and applying anti-fouling chemicals (which also prevents Dv from re-attaching itself for the duration of the anti-fouling effectiveness).
4. Temporary exposure of infected matter to freshwater is also a possible treatment.

The following treatments are suggested below for infected substrates in Holyhead Marina including 1) pontoons, 2) anchor chains and 3) boat hulls. It should be noted that costs are a rough estimation only and that actual costs may vary considerably from the values given below.

4.2.1.1 Pontoons

Key considerations

- The marina is currently supported by approximately 520 pontoons, ranging in size from 1.5 to 80 m² (Figure 3.1).
- Although not all pontoons are infected, it may require that all are pontoons are treated to ensure eradication is successful.
- Successful treatment of these structures would significantly reduce inoculum pressure/further spread.

- pontoons cannot be cost-effectively removed from the water, hence require in situ treatment.
- High probability of Dv fragmentation and release into the surrounding water if not treated correctly.
- Require owners' permission to treat.
- Treatment ideally cannot interfere with public access.
- Given the high number of pontoons, it is considered not logistical to treat all structures at the same time. Hence, a set of up to 18 "sweeps" are proposed, using recycled wrapping material for each "sweep".

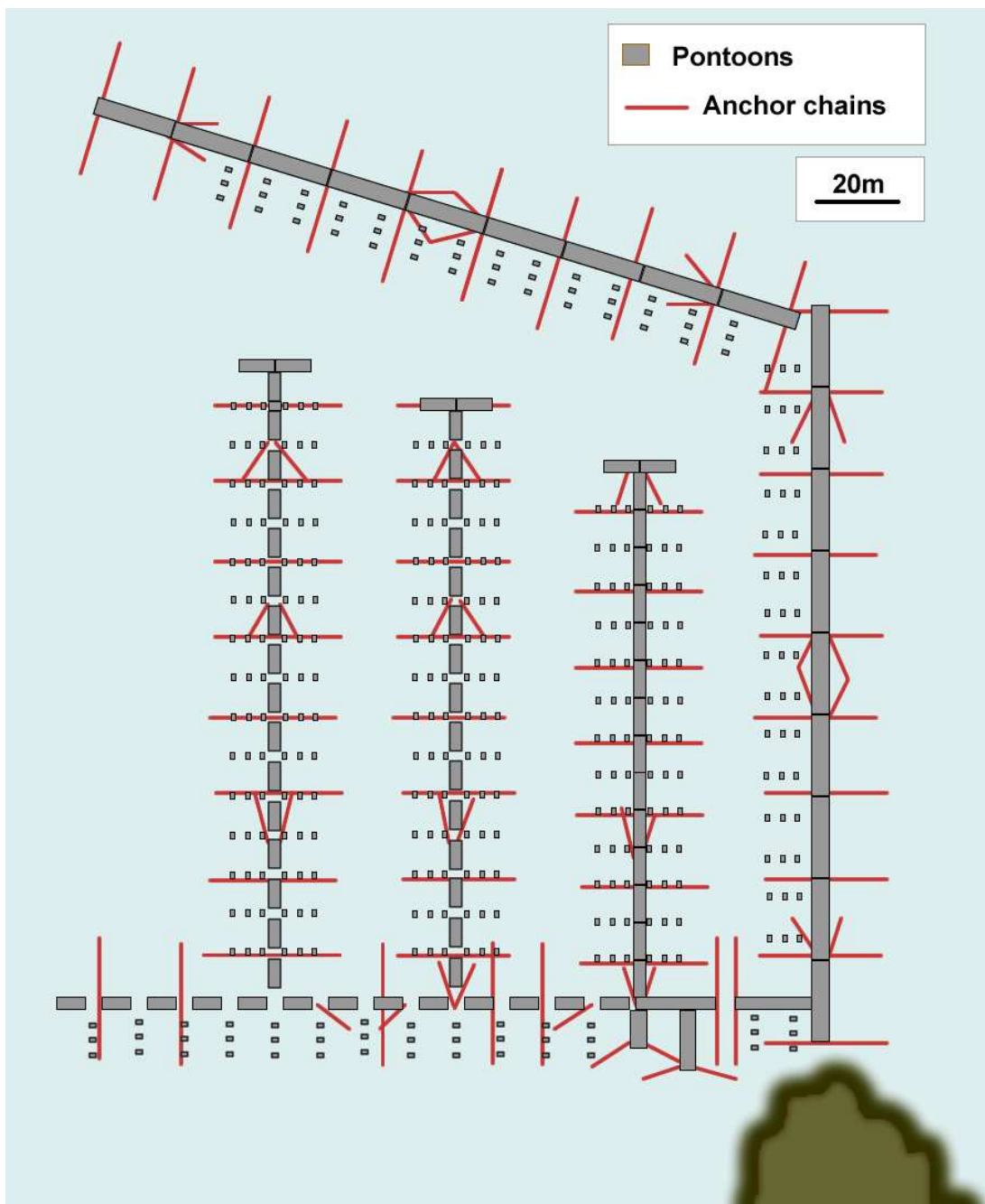


Figure 3.1 – submerged substrates within Holyhead Marina.

Materials and methods

Two encapsulation techniques would be suitable for treatment of submerged pontoons:

1. “Set-n-forget” encapsulation using tarpaulins or plastic silage covers

- PVC Truck Tarpaulins (see www.allplas.co.uk) could be used as covers to encapsulate pontoons of various sizes and similar to methods described by Coutts and Forrest (2005).
- At least 2 above water personnel would be required to fix the plastic to the smaller pontoons and for the larger pontoons, possibly 4 personnel in addition to two divers would be required to deploy the covers underneath the structures.
- Topside operators would be involved in pulling one side of the cover above the water line and securing it to the pontoon using either PVC cellotape, ropes or a staple gun.
- Divers would displace as much of the water between the covers and the pontoons as topside operators secured all remaining sides.
- Covers would be removed after one month.
- Defouled material would be released to the surrounding environment to break down naturally or sent to landfill (depending on assessment of risk), while covers would be recycled to treat subsequent pontoons or where damaged, removed to landfill.
- Recycled covers would be used to treat subsequent “sweeps” of up to 30 pontoons per sweep.

2. Pontoons in high demand and/or requiring rapid treatment could be treated as above, but with an accelerant added (i.e., ~ 5% acetic acid or a suitable concentration of granulated chlorine mixed with seawater).

- Approximately 20% acetic acid would be pumped into the encapsulated area at a rate of about 50L to every 1000 L of seawater to achieve an approximate 5% working concentration. Or bleach can be effective. Accelerant can be applied by securing the edges of a tarp to the pontoon then pouring a suitable volume of granulated chlorine mixed with freshwater
- Covers removed after 48 hours.

Costs

- The size of covers used will depend on the size of the pontoons including an additional ~ 500 mm to 1000mm margin to account for the sides.
- The quantity of covers used will depend on the number of “sweeps” considered practicable.
- It is considered feasible that ~50 small pontoons (1.5m²) could be treated in one “sweep”. The cost in materials is estimated to be around £4,800.
- It is considered feasible that up to 30 medium pontoons (3.75m²) could be treated in one “sweep”. The cost in materials is estimated to be around £2,800.
- It is considered feasible that up to 15 medium to large pontoons (22m²) could be treated in one “sweep”. The cost in materials is estimated to be around £3,600.
- It is considered feasible that up to 10 large pontoons (80m²) could be treated in one “sweep”. The cost in materials is estimated to be around £9,600.
- The cost for labour for each sweep will depend on the number of personnel deemed to be required for deploying the covers over different sized pontoons. It is assumed that, during

each sweep, at least 2 divers will be present in the water. Regulations for diving in marinas state that divers are roped with full voice communications. Therefore at least 5 topside operators would be required: 2 rope handlers, 1 diving supervisor, 1 comms operator and 1 rescue diver. An additional 2 topside operators may be required to secure the pontoons. Therefore the cost for labour for each individual sweep (~18 sweeps in total) would be ~£4,500.

- The total cost for materials and labour to undertake 18 sweeps of the marina is estimated at £101,800. This is assuming that the majority of plastic covers are able to be recycled for each “sweep”.

Justification for methods

The above process was considered cost effective, relatively easy to apply without requiring complex equipment, and 100% effective in NZ studies (Pannell and Coutts, 2007). Furthermore the process was environmentally friendly and had a low probability of damage to private property.

Potential problems in application

Problems associated with the success of the process may include damage to the plastic covers due to boats abrading the plastic and letting in fresh sea water as well as the offensive smell of rotting material that must be endured during the removal process and could result in complaints from marina users. The possible environmental impact of accelerants used will need to be assessed.

Following discussions with Holyhead Marina, it is considered negligible that boats will touch the pontoons and offensive smells were considered a minor problem. The main concern voiced by the marina related to the safety of the 30m nylon anchor ropes. The Marina would require assurance that these ropes would not be abraded in any way.

All wrappings will need to be inspected frequently and fixed if compromised. This is considered feasible during the “sweeps” scenario.

The main logistical issue relates to the high number of pontoons to be treated. It is logistically not feasible to treat all (~520) pontoons at one time. It is estimated that 18 sweeps could be conducted, each sweep requiring one week duration if an accelerant is used. The cost effectiveness of the method is further enhanced by conducting sweeps, where recycled covers can be reused. For example, if tarpaulins were purchased to treat each individual pontoon at the same time, the cost of materials would be around £85,000. A sweep strategy would reduce the cost of materials to around £20,800. This is dependant of course on the resilience of the tarpaulins, which can only be assessed by conducting trials.

The criticism of this process lies in the risk of untreated pontoons releasing larvae back onto treated pontoons, however strategies could be developed so that applications are applied over the colder water months where larvae are not being released. It is likely that the number of sweeps could be reduced following the initial trials. Evidence suggests that a 4 month period from February to June would be suitable for conducting this process.

4.2.1.2 Pontoon anchor chains.

Key considerations

- The floating jettys within the marina are anchored with a chain (7m) / nylon rope (30m) / chain (7m) system. Approximately 140 anchor chain systems support the marina (see Figure 3.1).

- Survey findings revealed that the nylon rope and bedding chain had no fouling present, while the upper 7m chain was fouled
- High probability of Dv fragmentation and release into the surrounding water if not treated correctly.
- Wrapping is considered most effective when applied from the top towards the bottom of the chains rather than vice versa.
- Methods will need to provide assurance that no damage is suffered by the nylon rope.
- Wrappings will need to be checked on a regular basis

Materials and methods

- Wrapping chains with industrial clingfilm or plastic wrap.
- Wrapping could be left indefinitely.
- Plastic could be applied by divers and topside operators during pontoon sweeps.

Costs

- If it is possible for divers to wrap chains during the same working day as pontoon wrapping operations, the costs for labour can be ignored. While this is considered possible, it may not be considered feasible in trials. In this regard, the cost in labour may be an additional 5 days, if it is considered that one diver can wrap 1 chain in 10 minutes. This would equate to around £11,250.
- Materials are not considered to be overly expensive. In New Zealand, balage wrap was used to successfully wrap wharf piles, with wrapping material sealed with PVC tape (48 mm x 30m). The cost of each wrap equates to around £90.00 per roll (0.75 x 1500m x 25um) of plastic and £4 per roll of tape. Three rolls of plastic and 30 rolls of PVC tape could feasibly treat 140 chains of 7m length, when considering a 10cm overlap when wrapping. This cost equates to around £390 in materials to treat 140 chains.

Justification for methods

The above process is considered cost effective, relatively easy to apply without requiring complex equipment, and if applied correctly is considered 100% effective both in killing the pest and avoiding release of fragments into the surrounding seawater. In addition, should the outside of wrappings become re-infected, their removal provides a second treatment option.

Potential problems in application

Loose wrappings can be an environmental hazard and wrapping methods may involve undesirable repetitive bounce diving. All wrappings will need to be inspected frequently and fixed if compromised. This is considered feasible during the “sweeps” scenario. Costs may rise if it is considered that a team of divers should be used to share the workload to elevate the effects of bounce diving.

4.2.1.3 Vessel hulls

Key considerations

- Two vessels ranging in size from approx 7 to 10m in length require treatment.
- Boats will need to be treated in situ as removal will likely result in fragmentation of existing populations.
- Require owners’ permission to treat.

- Treatment must not cause any damage to vessel or anti-fouling coating.
- Treatment must ideally not inconvenience owners.

Materials and methods

- An in situ plastic set-n-forget encapsulation technique could be adopted similar to Pannel and Coutts (2007).
- Plastic covers of suitable size will need to be sourced.
- Covers can be cut to size to suit the vessel.
- Two divers are needed to guide the covers underneath the vessels assisted by two topside operators who subsequently secure the plastic to the vessel using either PVC cellotape or ropes.
- Divers displace as much of the water between the cover and the hull as possible as topside operators secure all remaining sides to the vessel.
- An accelerant using approximately 20% acetic acid can be pumped into the encapsulated area at a rate of about 50L to every 1000 L of seawater to achieve an approximate 5% working concentration.
- The covers should remain on the vessels for a minimum of 7 days.
- Covers can be removed by hand from the surface and all plastic retained onboard a suitable barge or vessel. The defouled material and acetic acid can be released to the surrounding environment to break down naturally and retained plastic disposed of in landfill or recycled.

Costs

- The estimated cost to treat each vessel is £600.

Justification for method

100% effective if applied correctly.

- Cost-effective.
- Does not require vessel to be removed from the water for treatment.
- Set-n-forget method.
- Fast acting (i.e., treatment occurs within 7 days).
- Easy to apply and does not require complex equipment.
- High probability of containment, therefore low risk of spread.
- Low probability of damage to private property.

4.2.2 Capability

While methods exist to eradicate Dv, there is little to no capability in the UK in undertaking these methodologies.

Suitable materials will require sourcing, numbers of personnel required to undertake the techniques will need to be determined and the effectiveness of the techniques, while considered 100% effective in NZ situations, may not be as certain in the current situation. In addition, there is insufficient time in 2009 to undertake the required number of sweeps before periods where larvae are likely to be released (June/July).

4.3 Cost effectiveness

It is considered highly likely that eradication techniques can be developed that are both cost effective and reusable. The total cost of one single eradication attempt (materials and labour only) is expected to be around £120,000. Subsequent attempts would be expected to be less than this value as experience derived should shorten application times and may reduce numbers of required personnel. Furthermore, it may be revealed that much of the pontoon wrapping materials can be reused from year to year.

4.4 Critical success factors

It is likely that Dv is bordering on the explosion phase (Figure 2.1). As such, timing is crucial and action must be taken immediately if eradication is to be attempted. It is considered highly likely that the pest will extend onto other available habitats within Holyhead Harbour, after which time there will be greater uncertainty about the costs and benefits, and a greater risk of failure.

If Dv in Holyhead is left to spread then eradication of large established colonies, particularly in natural reef areas, is highly unlikely to be feasible, or desirable given current methods. For example bleach and smothering are effective, but have negative effects other native species. While complete mortality of all benthic organisms may be acceptable at small spatial scales, the environmental cost will eventually outweigh the benefit of eradicating Dv.

4.5 Probability of success

In general, eradication in the marine environment is extremely difficult. The few successful efforts that have been recorded in the marine environment have several common elements:

- early detection and correct identification of the invader;
- the pre-existing authority to take action;
- the pest could be sequestered to prevent dispersal, or else had very limited dispersal capabilities;
- there was political and public support for eradication, and acceptance of some collateral environmental damage;
- follow-up monitoring verified the completeness of the eradication.

Another common element of successful eradications was a high degree of certainty that a lack of action would have major consequences, usually based on knowledge of the adverse effects of the same or closely related species (Loche *et al.*, 2009). The reaction by the Mussel Industry, in cooperation with CCW, to an introduction of the Slipper Limpet (*Crepidula fornicata*) with mussel seed into the Menai Strait in 2007 presents an excellent example where the known adverse consequences to the industry prompted a rapid coordinated response that has thus far been shown to be successful (see Appendix 2).

The current situation, however, while having the potential to meet many of these requirements if action had been taken immediately on discovery of the pest in 2008, has undoubtedly lost a considerable amount of time already in ensuring a successful response. The reasons for this lie simply in both the lack of any response protocols for invasive marine species as well as any overarching strategy.

Despite this, the majority of respondents in the questionnaire considered that given the localised distribution and early stage of infection, an eradication attempt **should** be made immediately. If an attempt to control infestation levels does not occur now, the pest will quickly spread and result in a situation where eradication will simply no longer be feasible.

However, the likelihood that eradication can be successfully achieved in 2009 is considered to be moderate (~50% chance of successful eradication). Although techniques have been shown globally to be 100% effective on similar substrates, there is little to no capability in the UK in deploying these techniques, especially in the time required prior to larval release (likely to be June 2009).

Furthermore, too much time has been lost already since the first observation of the pest (summer 2008), and it is likely that larvae have already been released and may have been transported to other habitats adjacent to the marina. These individuals would not have been able to be observed in the December and January surveys as their growth would be considered minimal in the colder temperatures. However, when temperatures begin to elevate in June/July, growth of these populations would be expected to occur and visible forms more likely to be observed. If this has occurred, cleaned substrates within the marina would simply be re-inoculated from larva released from other, as yet unknown, substrates.

An alternative option is to undertake a trial period of eradication in 2009, followed by a full scale eradication in 2010, with follow-up treatments in 2011 if required.

Capability and methodologies developed in 2009 would, at the least, maintain infestation levels to stage 1-3 (see Figure 2.1) and may in fact serve to eradicate the pest, however the risk exists that larval release would re-inoculate cleaned surfaces thus reducing this likelihood. To account for this, a full scale eradication attempt could then be applied from Jan-June 2010 when colonies are likely to be stagnant and not releasing larvae.

This scenario is considered to have a very high probability of success:

- 50% chance of eradication in the first year of trials and 98% chance of ensuring the population remains at a low level of infestation
- 95% chance of complete eradication in subsequent years following trials if it is revealed that no other substrates are infected.
- Success depends on the confidence of surveys conducted in 2008/2009 (see below)

There are 2 major uncertainties in this approach:

1) It is possible that other substrates, found to be uninfected in the surveys conducted in December 2008 and January 2009, are in fact infected but that growth has been insufficient to observe with the naked eye. One strategy to “catch” this event would be to conduct monitoring in July/August 2009 when new growths have had sufficient time to develop and be observed. If Dv is found on other substrates that prove impractical to clean, then the programme can be reviewed and/or halted.

2) Another major criticism of this option is that while trials are being conducted there is a risk of larvae being transported from Holyhead marina to other habitats in Wales. However, at the current stage of infestation, this risk is considered equal to or lower than the risk from Ireland. At this stage it is not considered cost effective to severely impose control measures on high risk vectors. However, it is likely that this issue will need to be addressed. In any case, if Dv is left

unchecked at Holyhead Marina, infestation levels will bloom and the relative risk of spread will increase significantly.

In summary, a successful eradication is considered possible, but this option relies on the development of an eradication strategy to ensure full value is obtained from the process.

Although beyond the scope of this study, it is considered that an excellent opportunity exists to use Holyhead Marina as a test case to develop protocols and capability in dealing with invasive species, and all for a relatively cheap price tag (see possible scenario of costs in Table 3.1).

For example, monitoring of the harbour in July/August 2009 would reveal whether spread had occurred to other substrates and may provide a “check” to re-evaluate control measures. Associated research into larval release of existing populations could be undertaken at the same time, thus enhancing the knowledge base. Communications exercises could be undertaken with the marina and recreational vessel owners to encourage hull cleaning.

Table 3.1 – scenario of total costs involved in eradicating Dv from Holyhead Marina as well as undertaking a test case in developing capability in marine invasive pest responses.

Activity	Cost (£)		
	2009	2010	2011
Purchase of control materials	21190	5000	1000
Labour in field	50000	92250	50000
Development of an implementation strategy	8000	1000	500
Project management	3000	3000	1000
Development of monitoring manual	2000		
Monitoring and surveillance	20000	20000	20000
Communications strategy for vector control	15000	5000	5000
Research and analysis	10000	5000	5000
Total	131199	133260	84511

5 WHAT MEASURES COULD BE USED TO PREVENT REINFECTION OF THE MARINA?

There is a very high probability that Dv could be eradicated from Holyhead Marina if action is taken now. However, the relative value in devoting funds to eradication is lessened by the probability that reinfection will simply occur from other unmanaged sources, such as Ireland. Indeed, the knowledge available on Dv paints a picture of very wide distribution within the EU alone. Furthermore, it is unknown whether deep sea gravel beds, known to be extensive throughout the Irish Sea, are infested (see Pantin, 1991).

However, the key message that emerges is that, aside from Holyhead Harbour, the species is not known to be present in other areas in the UK, despite the UK being surrounded by infected countries. This would tend to support the recommendation that an eradication attempt is worthwhile.

Even so, it is considered probable that re-infection will occur again within the next 5 to 10 years. However, by implementing basic strategies in preparedness, future incursions could be managed efficiently, further aided by the capability developed in the initial eradication.

Furthermore, efforts applied to vector control would help to lessen the risk of not only Dv but other invasive marine species. Unless active effort is devoted to controlling high risk vectors, such as recreational vessels, these situations will continue to occur: i.e. invasive species will continue to appear and difficult and costly processes will need to be undertaken to make a decision on whether or not to do anything about it.

It is emphasised that a marine biosecurity strategy is required and at the very least, response protocols be developed. This is an extremely complex issue and beyond the scope of this report, however, as a start, a brief account of processes necessary to accompany the eradication attempt is presented below:

5.1 Monitoring / Surveillance

A monitoring program would greatly compliment efforts to control the spread Dv. This would include the development of monitoring programs to detect changes in the abundance of existing populations of Dv as well as the establishment of new colonies outside its current distribution. Tracking changes in the size of existing colonies as well as the abundance of larvae would help identify locations and seasons where vector control efforts should be closely observed. Monitoring in areas where colonies of Dv have not yet appeared but are at risk of an introduction would provide a way of measuring the success. Also early detection increases likelihood of eradicating newly established populations.

Developing a monitoring program requires identifying monitoring sites, determining the length of time to monitor (e.g. twice yearly monitoring over 3 years) and developing a network of university and agency researchers who can assist in establishing standardized monitoring protocols and data collection.

5.2 Stakeholder Communication / Outreach Programs

Educating the public, private industry and policy makers about potential environmental and economic costs of Dv, and how their actions can reduce the risks and impacts of this nonindigenous species would be invaluable to the program. Human activities play an important role in spreading Dv, and preventing further introductions will require changing individual behaviours and industry practices.

In North America, a number of invasive species websites already contain information on *Didemnum*, including sites maintained by the U.S. Geological Survey (woodshole.er.usgs.gov/project-pages) and are excellent resources for stakeholders. In New Zealand, the lead Biosecurity agency has been pushing the clean boats message for the past few

years. Pamphlets outlining why to keep your boat clean and how to do it have been produced, signs are displayed at marinas and slipways and an advertising programme was undertaken in yachting/fishing magazines. This led to a wider awareness of biosecurity and it appeared more people kept their boats clean. There was also an arrangement with a paint manufacturer where they promote a clean boats/living seas message (Biosecurity New Zealand, pers. comm.).

5.3 Vector Control

One of the main concerns in devoting effort and funds to attempt to eradicate Dv in Holyhead is the argument that the risk from other infected areas (e.g. Ireland) will continue regardless. Under the same rationale, devoting effort to controlling vectors in Holyhead Harbour appears to be impractical unless vector control programs are implemented in neighbouring areas also. This issue is beyond the scope of this study but can simply not be ignored.

A range of strategies for minimizing invasive species introductions through vessel fouling have been proposed or adopted by various countries, states/territories and regions. New Zealand and Australia have devoted effort to this cause.

In late 2005, Australia announced the implementation of national regulations to prevent invasive species introductions via fouling on internationally travelling vessels under 25m. This represents the first legal regime aimed towards the prevention of invasive species introductions via hull fouling (see <http://www.daff.gov.au/aqis/avm/vessels/less-25m/yachts>). The rule became mandatory in October 2006 after a one year voluntary period, and requires that vessel gear and seawater systems are clean of marine pests and growths. In addition, operators must perform one of three antifouling measures:

- Clean the hull one month prior to arrival
- Apply antifouling paint within one year before arrival
- Book the vessel to be slipped and cleaned within one week of arrival

In Wales, practices requiring boats to “clean hulls” can be implemented but not enforced. This is only an effective practice if there is state-wide co-operation. High level decisions need to be made if this will be a UK-wide or EU-wide venture. As mentioned previously, movement towards international hull fouling regulations are presently being considered by the International Maritime Organisation and it is likely that hull fouling will be an issue in the future for Wales.

While there is little information on the efficacy of management strategies for this vector, management strategies have potential to be developed and will likely involve voluntary co-operation by vessel owners and marinas. In general, the primary impetus for management is not fouling prevention, but the minimization of toxic antifouling paint release into the water as residues are scraped off during hull cleaning. There is, however, potential to combine management strategies to address both issues.

It is recommended that at the very least, a communication and awareness strategy be developed for Holyhead Marina, with a view to encouraging vessel owners to keep their hulls clean. Furthermore, in discussions with Holyhead Marina, potential exists for the development of an in-water, contained, cleaning system. This was primarily considered by the marina to deal with toxic residues but could be broadened to tackle invasive species fouling hulls. This opportunity could in fact provide an excellent opportunity for Wales to demonstrate to the rest of the EU that they are leading the way in considering these issues.

Initial discussions with both the RYA and Holyhead Marina have indicated that both parties are willing to work towards addressing hull fouling issues.

6 WHAT MEASURES COULD BE USED TO PREVENT SPREAD IF ERADICATION IS NOT CONSIDERED?

If eradication is not considered, the following options are considered:

1. Do nothing
2. Least control - Learn to live with the problems caused by the species and/or leave any control attempts to affected stakeholders
3. Limited control - Monitoring the event and providing stakeholders with information and support with limited eradication efforts in the event of new occurrences
4. Containment of Dv, by controlling movement of vectors (e.g. vessels and moveable equipment) from affected areas within the Marina, to prevent incursion into other areas that are presently Dv-free.
5. Monitor spread beyond stage 1-3 and triggering of containment of Dv by controlling movement of vectors (e.g. vessels and moveable equipment) from affected areas within the Marina, to prevent incursion into other areas that are presently Dv-free

6.1 Option 1: Do Nothing

This option provides a counter-factual as a baseline to compare the benefits and costs of other options. For this analysis, the following assumptions and calculations were made regarding the likely outcomes if no measures were taken to control the infestation of Dv:

- There is a very high likelihood that the pest will spread within Holyhead Harbour and develop more extensive and pendulous growth forms (infestation stage 4-8) within the next 5 years;
- If the pest achieves this there is an increased chance, above that of the likelihood that infestation arrives from Ireland, that the organism will spread to other marinas throughout much of the Welsh coastline if no measures are taken, reaching maximum extent within 10 years;
- If this spread occurs, there is:
 - A high chance that reef areas in at least one SAC would be infected;
 - A high chance that mussel culture sites would be affected (up to 50% coverage in subtidal beds with a potential impact on 25% of total production) and impacted to the point of requiring treatment and/or control.
- It is considered that a monetary value cannot be applied to describe the impacts on conservation areas. Infection of SACs would likely result in monitoring efforts to satisfy conditions laid out in the WFD. Options for eradication would no longer be feasible or achievable. This would also be seen as the cost of a missed opportunity.
- As a simplified estimate with regard to mussel culture, a loss in production of 25% would equate to a loss in production value of £1,375,125 per year. A loss in production of 10% would equate to a loss in production value of £550,050 per year, while a loss in production of 5% would equate to a loss in production value of £275,025 per year. Costs incurred through potential loss to seed sourcing areas and loss of export markets would be expected to be severe.
- If the opportunity to eradicate is missed, and Dv continues to spread, Mussel farmers will perceive the costs to prevent infection on their farms as unfair and may seek compensation. It is advised that the government seek legal advice regarding the potential for litigation from industry if mussel culture is severely impacted and it can be

demonstrated that efforts were not made to control the pest when at a very early stage of infestation in Holyhead Marina.

6.2 Option 2: Least control. Learn to live with the problems caused by the species and/or leave any control attempt to affected stakeholders.

This option entails undertaking no action if a stakeholder survey reveals that stakeholders have little to no concerns. The survey would involve providing stakeholder information on the affects of Dv if it were to spread as well as provide education and advice on identification and control to marine users.

- Marinas manage their own pest problems
- Boat owners develop their own code of practice
- Industry manage their own farms to prevent infection

The same assumptions are maintained as those outlined in Option 1 with the addition that there is:

- On going costs in stakeholder communication – allowing £20,000 for the cost of undertaking 2 years stakeholder communication.

This option is considered highly undesirable given the current extent of infestation and the timely opportunity to react while the pest is at very early stages of infestation. Also given that no one group is considered accountable, government action is required.

Again, it must be emphasised that opportunity will be lost. Invasions are more likely to be addressed successfully in their early stages. Populations that are still localized and have low abundance are more likely to be contained and eradicated than well established populations.

The likelihood of success of this option is low with regard to preventing the spread of Dv, and moderate for uptake of action by affected parties. It is uncertain whether marina or boat users will voluntarily cooperate in hull fouling conditions, however discussions with both the RYA and Holyhead Marina indicate a genuine willingness to address these issues. However, with regard to uptake by individual boat owners, there is a risk that it is perceived that the problem is not their fault (given the lack of data on hull fouling risks in the UK). Furthermore, impacts to boat owners are currently very low. It would also be deemed unfair if marinas and boat users UK wide do not undertake similar programmes. The balance of effort will henceforth fall on industry, being the most likely impacted. While the mussel industry are committed to developing good codes of practice, it may be considered that their efforts in developing preparedness, while the government avoids similar actions, would likely be deemed unreasonable.

6.3 Option 3: Monitoring the event and providing stakeholders with information

To monitor the distribution, impacts and spread of Dv and to provide education and advice on identification and control to marine users. This option involves monitoring the spread of Dv both within and outside Holyhead Harbour and allowing for the option to reassess actions depending on results.

The same assumptions are maintained as in Option 2, with the addition that the process:

- Requires development of an state-wide monitoring manual and plan. £45,000 in the first year (Holyhead + state-wide). £30,000 in subsequent years.
- Involves ongoing costs to undertake monitoring and re-evaluation of the situation

Again, this option is not attractive as it presents an unacceptably high risk that the organism will spread further and control options will become more expensive and less effective. The benefits become difficult to determine as the situation becomes complicated by too many uncertainties.

6.4 Option 4: Containment of Dv, by controlling movement of vectors (e.g. vessels and moveable equipment) from affected areas within the Marina, to prevent incursion into other areas that are presently Dv-free

This option involves the containment of Dv within Holyhead Marina by treating recreational vessels and other vectors that move out of the harbour. This option may or may not involve monitoring efforts to continue.

The objective of this approach would be to contain Dv to existing locations, accepting the negative consequences there and aiming to prevent incursion into other areas that are presently Dv-free.

However at present, practices requiring boats to prove “clean hulls” can be implemented but not enforced. This approach would require regulation of some form. A voluntary Code of Practice is unlikely to be effective, given that the pest is not controlled from other sources. Hence it would require significant resources for managing, communicating and enforcing regulations, and some compliance costs on recreational boat owners and other users.

A critical judgment about this approach is its effectiveness. The major weakness with this approach is that it does not prevent the spread of Dv in mainland UK unless implemented EU-wide (i.e. unless Ireland adopt similar practices). It is considered inevitable that controls will be required on recreational vessels within the EU in the future. This may involve ranking marinas/areas as high risk locations which would thus incur more stringent conditions on vessel owners and possible restrictions on their movements to Dv free areas.

The main action would be controls on movement of vectors from affected areas, for example requiring vessels and moveable equipment to be cleaned before they could be relocated. Because of the practical difficulties in controlling movement and requiring cleaning of recreational vessels, affected areas may have to be closed to those vessels for all or part of the year.

In addition, concerns over effectiveness extend to the fact that it essentially provides for control over the movement of non-natural vectors only. Dv would continue to reproduce within infected areas (subject to any voluntary eradication efforts by the marina), and could potentially spread beyond those areas through natural means such as tidal flow. Coutts and Skinner (2004) suggested that Dv appears less likely to spread naturally in the Marlborough Sounds, because of the low competency period of the larvae and relatively weak water currents, compared to the risk of artificial spread (i.e. through movement of vessels and man-made objects), however it is considered that if left unchecked, infection levels could bloom, thus increasing the risk of natural dispersal by fragmentation as well as reducing any further chances of “nipping the infection in the bud”.

It is considered impractical to assess the cost of this option. The likelihood of success is Low.

The costs under this option would be also ongoing and will continue indefinitely.

6.5 Option 5. Monitor spread beyond stage 1-3 and triggering containment of Dv by controlling movement of vectors (e.g. vessels and moveable equipment) from affected areas within the Marina, to prevent incursion into other areas that are presently Dv-free

It has been determined that the risk of Dv spreading from Holyhead Harbour increases significantly if infestation progresses to stages of development where pendulous growth types form. It was considered from the risk assessment survey that there is a high likelihood that Dv will spread beyond stage 3 within the next 5 years.

Option 5 allows for the monitoring of infestation levels over 2009. If infestation spreads within the harbour and blooms to form pendulous growths, particularly on boat hulls, then strategies could be developed to contain the infestation via vector control (see Option 4). For example, the

marina may be closed to vessels from June to January, or until measures were undertaken to clean vessel hulls. All dredging in the harbour would also cease.

However, this does not prevent the possibility of fragmentation and natural dispersal by currents once pendulous growth forms develop.

Again, it is impractical to consider the costs of this option for vector control. Additional costs for monitoring in Holyhead Harbour are estimated to be £25,000 in the first year (Holyhead only) and £20,000 in subsequent years. Communications programmes are estimated at £10,000 per year and would be required for a minimum of two years. The costs under this option would be also ongoing and will continue indefinitely. The likelihood of success is Low.

7 COST BENEFIT ANALYSIS

There are explicit matters in providing recommendations in this paper: we make no comment on how the required activities would be funded, nor what form any regulation would take. For the purposes of this analysis, our objective was primarily to compare the cost of various activities relative to their benefits. However, given the uncertainty of the impacts of Dv in the UK, the difficulty in applying costs to various options, such as those involving vector control, and the inability to apply monetary values to impacts to on conservation areas, we have simplified the analysis by assessing, as an example, the relative cost-benefit to the mussel industry.

Assessments of costs are evaluated for the next 10 year period. Given the considerable uncertainty about the spread of Dv and its impact on mussel production, we have assumed that:

- 90% of subtidal production will be affected within 10 years if eradication is not achieved.
- Within the next 10 years, 5 of these years will show some sort of adverse affect (i.e. loss in production, closure of seed sourcing areas, loss of export markets etc.)
- Where mussel areas are affected, production will fall by 5% and up to 25%.

We have calculated the costs under the simple scenario of potential loss in production. It should be noted that this is likely to be a vast underestimate of the potential impacts: given the information available we have not included loss of export markets, the effects of closure of seed sourcing areas or the potential loss in industry development (e.g. line culture).

In simplistic terms, the cost of an eradication attempt at Holyhead, including development of a monitoring strategy and a communications strategy for kick-starting hull fouling issues, is calculated at £350,000 to £385,000 over 3 years with ongoing monitoring after 3 years estimated to be ~£15,000 per year. The potential impact to the mussel industry over the next 10 years, where just 5% of production is affected, would be around £1,375,125. The pest has the potential to cause up to 25% loss of production. This would equate to a loss in value of £6,875,625 over 10 years. It is important to recognise that we are restricted to forming broad-brush conclusions, with costs specified in very general terms. This reflects the information available, however obvious conclusions can be drawn from the basic values calculated, further to the fact that this is merely a subset of any real potential cost to industry, marinas, boat owners as well as the government, to name but a few.

With regard to considerations on conservation areas, the cost to SACs cannot be equated in monetary terms, however there are potentially severe financial and legal implications if lack of action results in these areas becoming affected. The likelihood that Dv will infest high value areas and the high potential consequences of its presence in these areas, indicate that these values must be protected if it is reasonable to do so:

The body of evidence in conducting this report has indicated that eradication is both feasible and cost effective. It is also considered timely. It could be argued that given that the opportunity to eradicate exists now, but is likely to be lost in the next few years, lack of action would be deemed unreasonable.

Under the Habitats Directive, Member States have an obligation to take steps to avoid the deterioration of natural habitats and the habitats of species (Article 6(2)). Article 22 of the Habitats Directive also places an obligation on Member States to ensure the regulation of the deliberate introduction of non native species. It is unclear under what circumstances any spread of *Didemnum vexillum* by human vectors would be considered 'deliberate', although it seems likely that movement of vessels where the owners had been advised of the presence of the species would be considered a deliberate action.

8 RECOMMENDATIONS

In essence, the current situation presents a unique opportunity to successfully eradicate Dv from Holyhead Marina. If attempts are not made now and further spread occurs to extend into the natural rock structures, control methods will no longer be feasible or cost effective. In this situation, the marina will essentially act as a stepping stone for the spread of the ascidian within North Wales and beyond, and as reservoirs for its incursion to seabed habitats.

Preliminary research in evaluating methods to eradicate indicates that the programme could be conducted in a cost effective manner. Indeed, the price tag of £350-380,000 over 3 years, with a high likelihood of success and thus minimal expenditure thereafter, appears extremely “cheap” when one reflects on the relative funds applied to managing certain terrestrial pests such as Japanese knotweed (in 2003 the estimated cost to remove Japanese knotweed from Britain was £1.56 billion – see POSTnote 303 - <http://www.parliament.uk/documents/upload/postpn303.pdf>) and Rhododendron (in 1995 an estimated £45 million had been spent to control Rhododendron in Snowdonia alone (Gritten, 1995)). Interestingly, Dv, if not eradicated now, could in fact become the marine equivalent of these two plant pests with regard to future management.

While eradication is considered feasible, there are various considerations in the successful implementation of an eradication program within 2009. The lack of capability in the application of eradication tools in the UK means that efforts are being undertaken using locally unproven techniques. In NZ, experience suggested tools required testing and modification to be successful (Sinner and Coutts, 2003). Any attempt would also need to be conducted prior to water temperatures reaching or exceeding 14°C (probably June 2009) to avoid the chance of larvae reinfesting cleaned surfaces. In addition, absence of any efforts to manage high risk vectors leaves open the opportunity for Dv to reinfest the area. It is also advised that communication with the non-scientific community be conducted.

Difficulties in managing these sort of situations in Wales results from the lack of any sort of Marine Biosecurity Strategy. There is no effective preparedness and response to marine invasives, and this is reflected in the current situation and the time taken to reach a decision on “what to do”. However, despite this, the situation in Holyhead Marina remains at a stage where something can be done. This presents an interesting opportunity to develop a “test case” in how to respond to invasive species.

In this regard, it is suggested that an Eradication Plan be implemented immediately that may serve, not only as a chance to eradicate Dv from Holyhead Marina, but also to develop capability in dealing with the next invasive marine pest that arrives in the UK.

The eradication plan may include some or all of the following:

- Instigation of an Eradication Plan or Strategy that can act as a case-study for Marine Biosecurity preparedness and response, as well as development of capability, in Wales.
- Formation of a Dv working group and development of a global network contact list.
- Develop education and awareness programmes targeted to Holyhead Marina boat owners.
- Conduct eradication treatment trials in 2009 and develop an understanding of the costs, resources and time lines required to eliminate Dv.
- Monitor surrounding substrates in July/August 2009 and review implementation plan depending on results.
- Undertake full scale eradication in 2010 and follow up treatment in 2011 where necessary.
- Work with Holyhead Marina and boat owners to develop hull cleaning strategies.

- Develop research strategies to study the effectiveness of control techniques as well as the biology of Dv (dispersal characteristics, growth rates etc).
- Develop monitoring programmes for improved methods for identifying potential areas where Dv is likely to occur.
- Develop a better understanding of principle vector pathways – e.g. recreational vessels.
- Develop communication with other countries for cooperation in hull fouling/vector management.

9 ACKNOWLEDGEMENTS

Many thanks to all the marina operators and staff for their assistance and providing access and advice, in particular Mr Edward Hughes, Managing Director, Holyhead Marina.

Many thanks are also extended to Kate Griffith, Kirsten Ramsay, Kate Smith, Gabrielle Wyn, Catherine Duigan, Stephen Mowat, Rohan Holt, Adam Cole-King and Flora Kent of CCW, for their feedback and assistance.

Thanks also to Eilir Morgan, Bangor University, for translation of the executive summary.

Also thanks to the following people for their responses to various queries as well as the questionnaire, and often at short notice:

Ashley Coutts, Senior Research Scientist - Marine Pests, Aquenal Pty Ltd, Australia.

Capt. Andrew Cummings, Wellfleet, MA, USA.

Chris Denny, Brendan Gould, Lou Hunt, Bex Ansell, Ranuka Robinson and Naomi Parker, MAF Biosecurity New Zealand.

Dan Minchin, Marine Organism Investigations (MOI), Ballina, Killaloe, Ireland.

Francoise Monniot, Museum National d'Histoire Naturelle (MNHN), Paris, France.

Frank Poole, Puget Sound Drivers (PSD), Seattle, Washington.

G rard Breton, Gerard Breton, Le Havre, France.

Gordon King, Taylor Shellfish Farms, Inc, Washington.

Gretchen Lambert, University of Washington Friday Harbor Laboratories.

James Willisroft, EMS Directorate (Effort Management and Statistics), Marine and Fisheries Agency.

Jennifer Dijkstra, University of New Hampshire (UNH), Durham, New Hampshire.

John Stachowicz, University of California, (UCD), Davis, California.

Kyle Edwards, University of California, (UCD), Davis, California.

Larry Harris, University of New Hampshire (UNH), Durham, New Hampshire.

Mary Carman, Woods Hole Oceanographic Institution (WHOI), Woods Hole, Massachusetts.

Page Valentine, U.S. Geological Survey (USGS), Woods Hole, Massachusetts.

Richard Osman, Smithsonian Environmental Research Center (SERC), St. Leonard, Maryland.

Ruth Sharrat, Green-Blue project officer, Wales.

Susie Tomson, RYA Planning and Environmental Advisor.

10 REFERENCES

- Altman, S. and Whitlatch, R.B. (2007). Space invaders: the effect of small-scale disturbance on invasion success in marine communities. *Journal of Experimental Marine Biology and Ecology*, **342**, 40-53.
- Ashton, G., Boos, C., Shucksmith, R. and Cook, E. (2006). Rapid assessment of the distribution of marine non-native species in marinas in Scotland. *Aquatic Invasions*, **1**, 209-213
- Ashton, G., Boos, C., Shucksmith, R. and Cook, E. (2006). Risk assessment of hull fouling as a vector for marine non-natives in Scotland. *Aquatic Invasions*, **1(4)**, 214-218
- Ates, R. (1998). De druipzakpijp, *Didemnum lahillei* Hartmeyer, 1909 in Zeeland [The sea squirt *Didemnum lahillei* Hartmeyer, 1909 in Zeeland] *Het Zeepaard*, **58(4)**, 101-110
- Bernier, R.Y., Locke, A. and Hanson, J.M. (2009). Lobsters and crabs as potential vectors for tunicate dispersal in the southern Gulf of St. Lawrence, Canada. *Aquatic Invasions*, **4(1)**, 105-110.
- Bullard, S.G. and Whitlatch, R.B. (2009). In situ growth of the colonial ascidian *Didemnum vexillum* under different environmental conditions. *Aquatic Invasions*, **4(1)**, 275-278
- Bullard, S.G., Lambert, G., Carman, M.R., Byrnes, J., Whitlatch, R.B., Ruiz, G., Miller, R.J., Harris, L., Valentine, P.C., Collie, J.S., Pederson, J., McNaught, D.C., Cohen, A.N., Asch, R.G., Dijkstra, J., Heinonen, K. (2007a). The colonial ascidian *Didemnum* sp. A: Current distribution, basic biology and potential threat to marine communities of the northeast and west coasts of North America. *Journal of Experimental Marine Biology and Ecology*, **342**, 99-108
- Bullard, S.G., Sedlack, B., Reinhardt, J.F., Littly, C., Gareau, K., Whitlatch, R.B. (2007b). Fragmentation of colonial ascidians: differences in reattachment capability among species. *Journal of Experimental Marine Biology and Ecology*, **342**, 166-168
- Cardoso, A.C. and Free, G. (2009). Incorporating invasive alien species into ecological assessment in the context of the Water Framework Directive. *Aquatic Invasions*, **3(4)**, 361-366
- Carlton, J., Geller, J., (1993). Ecological roulette: the global transport of non-indigenous marine organisms. *Science*, **261**, 78-82.
- Carlton, J.T. and Hodder J. (1995) Biogeography and dispersal of coastal marine organisms: experimental studies on a replica of a 16th Century sailing vessel. *Marine Biology*, **121**, 721-730
- Carman, M. (2008). The viability of fragments of the invasive colonial tunicate *Didemnum vexillum* in suspension and implications for long distance transport of the species. *American Society of Limnology and Oceanography*, *St. John's Newfoundland*, **June** 8-13
- Connell, S. D. (2000) Floating pontoons create novel habitats for subtidal epibiota. *Journal of Experimental Marine Biology and Ecology*, **247**, 183-194
- Coutts, A. D. M. (2002). A biosecurity investigation of a barge in the Marlborough Sounds. *Cawthron Report No. 744*.
- Coutts, A.D.M. and Forrest, B.M. (2005). Evaluation of eradication tools for the clubbed tunicate *Styela clava*. Cawthron Report, vol. 1110. *Cawthron Institute, Nelson, New Zealand*. December 2005, 48 pp.
- Coutts, A. D. M. and Forrest, B. M. (2007). Development and application of tools for incursion response: Lessons learned from the management of the fouling pest *Didemnum vexillum*. *Journal of Experimental Marine Biology and Ecology*, **342**, 154-162
- Coutts, A. D. M. and Dodgshun, T.J. (2007). The nature and extent of organisms in vessel sea-chests: A protected mechanism for marine bioinvasions. *Marine pollution bulletin*, **54(7)**, 875-86

- Daley, B. A. and Scavia, D. (2008). An Integrated Assessment of the Continued Spread and Potential Impacts of the Colonial Ascidian, *Didemnum* sp. A, in U.S. Waters. *NOAA Technical Memorandum NOS NCCOS 78*, 61 pp.
- Darbyson, E., Locke, A., Hanson, J.M., Willison, J.H.M. (2009). Marine boating habits and the spread of invasive species in the Gulf of St. Lawrence. *Aquatic Invasions*, **4**, 87-94
- Dijkstra, J., Harris, L.G., Westerman, E. (2007). Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. *Journal of Experimental Marine Biology and Ecology*, **342**, 61–68
- Floerl, O. and Inglis, G.J. (2003). Boat harbour design can exacerbate hull fouling. *Austral Ecology*, **28**, 116-127
- Gherardi, F. (2007). Biological invasions in inland waters: an overview. In: Gherardi F (ed) *Biological invaders in inland waters: Profiles, distribution, and threats*. Springer, Dordrecht, pp 3-25
- Gittenberger, A. (2007). Recent population expansions of non-native ascidians in The Netherlands. *Journal of Experimental Marine Biology and Ecology*, **342**, 122–126
- 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*.
- Kott, P. (2002). A complex didemnid ascidian from Whangamata, New Zealand. *Journal of the Marine Biological Association UK*, **82**, 625-628
- Kott, P. (2004). A new species of *Didemnum* (Ascidacea, Tunicata) from the Atlantic coast of North America: *Zootaxa*, **732**, 1-10
- Lengyel, N.L., Collie, J.S. and Valentine, P.C. (2009). The invasive colonial ascidian *Didemnum vexillum* on Georges Bank — Ecological effects and genetic identification. *Aquatic Invasions*, **4(1)**, 143-152
- Locke, A., Hanson, J.M., MacNair, M.G. and Smith, A.H. (2009). Rapid response to non-indigenous species. 2. Case studies of invasive tunicates in Prince Edward Island. *Aquatic Invasions*, **4(1)**, 249-258
- Mercer, J.M., Whitlatch, R.B. and Osman, R.W. (2009). Potential effects of the invasive colonial ascidian (*Didemnum vexillum* Kott, 2002) on pebble-cobble bottom habitats in Long Island Sound, USA. *Aquatic Invasions*, **4(1)**, 133-142
- Miller, C., Kettunen, M. & Shine, C. (2006). Scope options for EU action on invasive alien species (IAS) *Final report for the European Commission*. Institute for European Environmental Policy (IEEP), Brussels, Belgium. 109 pp + Annexes.
- Minchin, D., Floerl, O., Occipinti-Ambrogi, A. and Savini, D. (2006). Small craft and the spread of exotic species. In: Davenport J & Davenport JL (eds) *The Ecology of Transportation: Managing Mobility for the Environment*. Kluwer Academic Publishers. Environmental Pollution 10: 99-118
- Minchin, D. and Sides, E. (2006). Appearance of a cryptogenic tunicate, a *Didemnum* sp. fouling marina pontoons and leisure craft in Ireland. *Aquatic Invasions*, **1(3)**, 143-147
- Osman, R.W., Whitlatch, R.B., (1995). The influence of resident adults on larval settlement-experiments with four species of ascidians. *Journal of Experimental Marine Biology and Ecology*, **190**, 199–220.
- Pannell, A. and Coutts, A.D.M. (2007). Treatment methods used to manage *Didemnum vexillum* in New Zealand. *New Zealand Marine Farming Association Report*. 29 pp + Annexes.

- Pantin, H M. (1991). The sea-bed sediments around the United Kingdom: their grain size, mineral composition and associated bedforms. *BGS Research Report SB/90/1*.
- Royal Yachting Association (2005). Identifying Recreational Cruising Routes, Sailing and Racing Areas within the SEA 6 Area. *A Report for the Department of Trade and Industry*, 52pp
- Royal Yachting Association (2009). UK Coastal Atlas of Recreational Boating. Second Edition. 2009. RYA, Hamble.
- Sinner, J., Coutts, A.D.M., (2003). Benefit-cost analysis of management options for *Didemnum vexillum* in Shakespeare Bay. *Cawthron Report*, vol. **924**. August 2003, 24 pp.
- Stachowicz, J.J., Terwin, J.R., Whitlatch, R.B., Osman, R.W., (2002). Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. *Proceedings of the National Academy of Sciences USA*, **99**, 15497–15500.
- Stefaniak, L., Lambert, G., Gittenberger, A., Zhang, H., Lin, S., Whitlatch, R.B. (2009). Genetic conspecificity of the G. Lambert 28 worldwide populations of *Didemnum vexillum* Kott, 2002. *Aquatic Invasions*, **4**, 29-44
- Valentine, P.C., Carman, M.R., Blackwood, D.S., Heffron, E.J. (2007a). Ecological observations on the colonial ascidian *Didemnum* sp. in a New England tide pool habitat. *Journal of Experimental Marine Biology and Ecology*, **342**, 109-121
- Valentine, P.C., Collie, J.S., Reid, R.N., Asch, R.G., Guida, V.G., Blackwood, D.S. (2007b). 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(1)**, 179-181
- Wallentinus, I. and Nyberg, C.D. (2007). Introduced marine organisms as habitat modifiers. *Marine Pollution Bulletin*, **55**, 323-332.

APPENDIX 1: QUESTIONNAIRE TO ASSESS THE RISK OF DIDEMNUM VEXILLUM IN WALES

Q1 – Given the information provided below, how long do you think Dv has been present at Holyhead Marina for:

- Less than one year
- 1-2 years
- 2-5 years
- More than 5 years

Answer:

Supporting information:

Discovery date:

Discovered by a skilled scientist in July 2008 who was actively surveying the area.

State of infestation:

- Restricted to marina pontoons, chains and 2 boat hulls
- Less than 10% coverage in observed range and no pendulous growths.
- Larvae present in Jan 2009 samples.

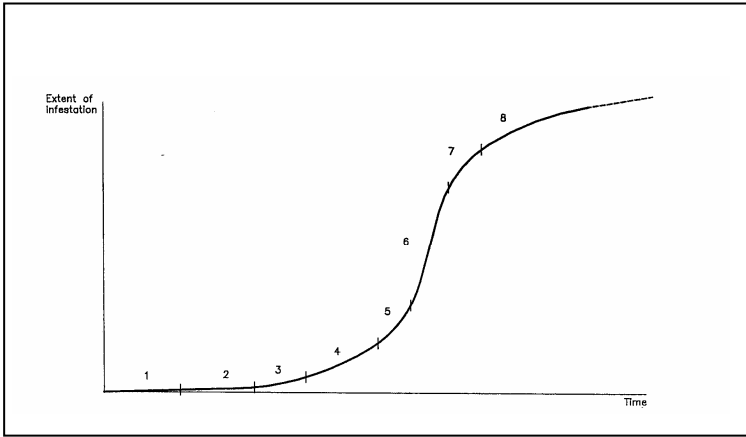


Figure 1 – Holyhead Marina located within Holyhead Harbour

Q2 – Using the infestation curve below, what is the likely extent of infestation at Holyhead Marina?

Answer:

Supporting information:



- the initial lag phase (stages 1-3) when the organism is establishing itself and becoming apparent
- the explosion phase (stages 4-7) when the organism’s population and distribution increases rapidly
- the widespread phase (stage 8) when the organism’s population has stabilised and filled most of the habitat suitable to it.

Number	Shape of Curve	Description
1	Flat	Not yet known in Region but known nearby
2	Flat	One or two known sites
3	Flat	Three to twenty sites
4	Starting upward	Twenty plus sites although still occupying a small proportion of possible sites
5	Steeply upward	Starting to noticeably expand the range and/or intensity of infestation
6	Halfway up	Widespread and continuing to expand the range and/or intensity of infestation
7	Upper Curve	Common through most of the expected habitat in the Region
8	Levelling off	Found in early every expected habitat

Q3 - What is the likelihood that Dv could naturally disappear/die out?

Given your knowledge of Dv, what are the chances that the infestation will naturally die out?

Answer: *Please indicate likelihood using Table 1 below. If possible, provide any supporting comments.*

Table 1. Nomenclature for qualitative likelihoods

Likelihood	Descriptive definition
High	The event would be very likely to occur ¹
Moderate	The event would occur with an even probability
Low	The event would be unlikely to occur
Very low	The event would be very unlikely to occur
Extremely low	The event would be extremely unlikely to occur
Negligible	The event would almost certainly not occur

¹ A probability of 1 means the event will occur and a probability of 0 means the event will never occur. A probability of 10⁻⁶ corresponds to a one in a million chance of an event occurring.

Q4 - Given the information below, what is the likelihood Dv will spread to all available habitats within Holyhead Harbour?

Answer: Please indicate likelihood using Table 1. If possible, provide any supporting comments.

.....

Supporting information:

Holyhead harbour is located on Holy Island. Please view on Google Earth if you have access (search for Holyhead Marina, Holyhead, Isle of Anglesey LL65, UK). Holyhead harbour is protected from the Irish Sea by a 2.4 kilometre breakwater sheltering an area of 260 hectares comprising the Inner, Outer and New Harbours. The approaches to Holyhead are beset by strong tidal currents (up to 6 knots), however Holyhead Bay is relatively quiet with less than 2 knots. Within the harbour it is virtually slack water. The tidal amplitude within the bay is 6m. Depths within the harbour range from 15m at the entrance to 6m approaching the marina. The Marina has 230 berths and is frequented by numerous vessels.

Please refer to Figure 2 – with the exception of the small mooring bouys (red dots numbered 2-9) there is considerable fouling including vigorous growth of native and some non-native species of sea squirts - see Table 2 in attached dive survey (Holt *et al.*, 2009).



Figure 2 – Holyhead Harbour dive survey locations

Q5 - How long will it take for all available habitats to be occupied?

If you consider it likely that Dv will spread to all available habitats and, given your knowledge of the invasiveness of Dv worldwide, over what time period would this be expected to take?

- Less than one year
- 1-2 years
- 2-5 years
- More than 5 years

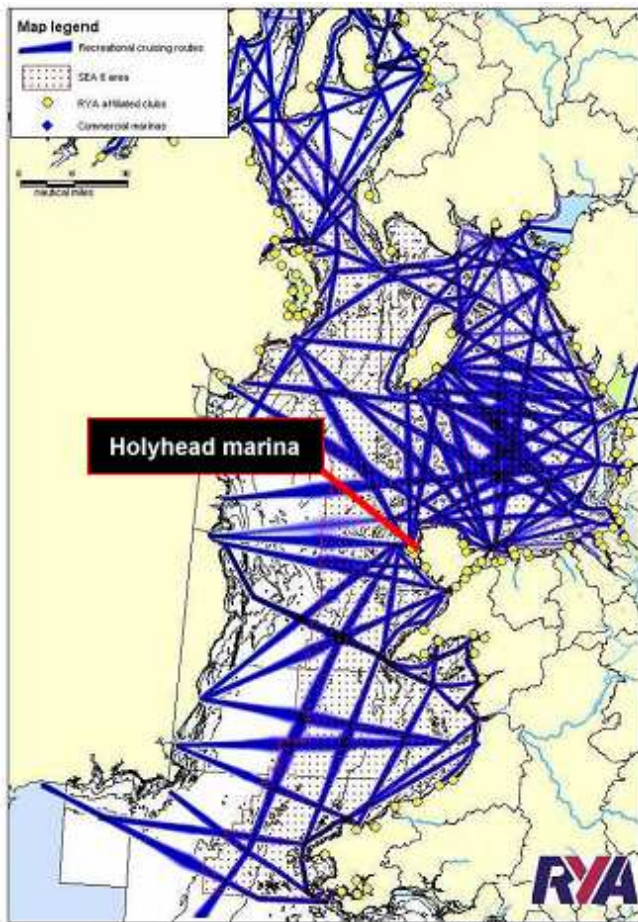
Answer:

Q6 - What is the likelihood Dv will be *transported* by recreational vessels to other Wales locations?

Answer: *Please indicate likelihood using Table 1. If possible, provide any supporting comments.*

.....

.....



Holyhead is one of the UK's busiest ferry ports. There are about 8,000 conventional and fast ferry movements a year and over 500 calls from bulk carriers, cruise liners, coasters and large fishing vessels. Countless numbers of small fishing vessels and leisure craft call at the port. Holyhead harbour, being a harbour of refuge, may be entered in all weather conditions and at all states of the tide. Holyhead marina supports around 230 recreational vessels. In the winter months the marina is usually half full (around 150 vessels). The Irish Sea is a popular recreational boating area with routes to and from the English, Welsh, Scottish and Irish coasts as well as to and from the Isle of Man.

Figure 3 - Recreational cruising routes (Blue pathways) in the Irish Sea – sourced from RYA Report 2005.

Q7 - What is the likelihood DV will establish in other marinas in Wales?

Answer: Please indicate likelihood using Table 1. If possible, provide any supporting comments.

.....

Given our current understanding of the biology of Dv, areas most susceptible to invasion are those with growing season of 14-18°C of 6 months or longer and where mean monthly water temperatures are below 25 °C.

Most coastal areas in Wales have temperatures above 14°C and not exceeding 22°C for at least 6 months of the year (May to November), however some of the Wales mainland coastal areas demonstrate very low temperatures (5-6°C) from November through to April.

There are numerous marinas along the coastline. Data for recreational yachts from 2005 listed around 11,600 club members and 1650 berths within the area displayed in Figure 4 below and including Holyhead Marina.

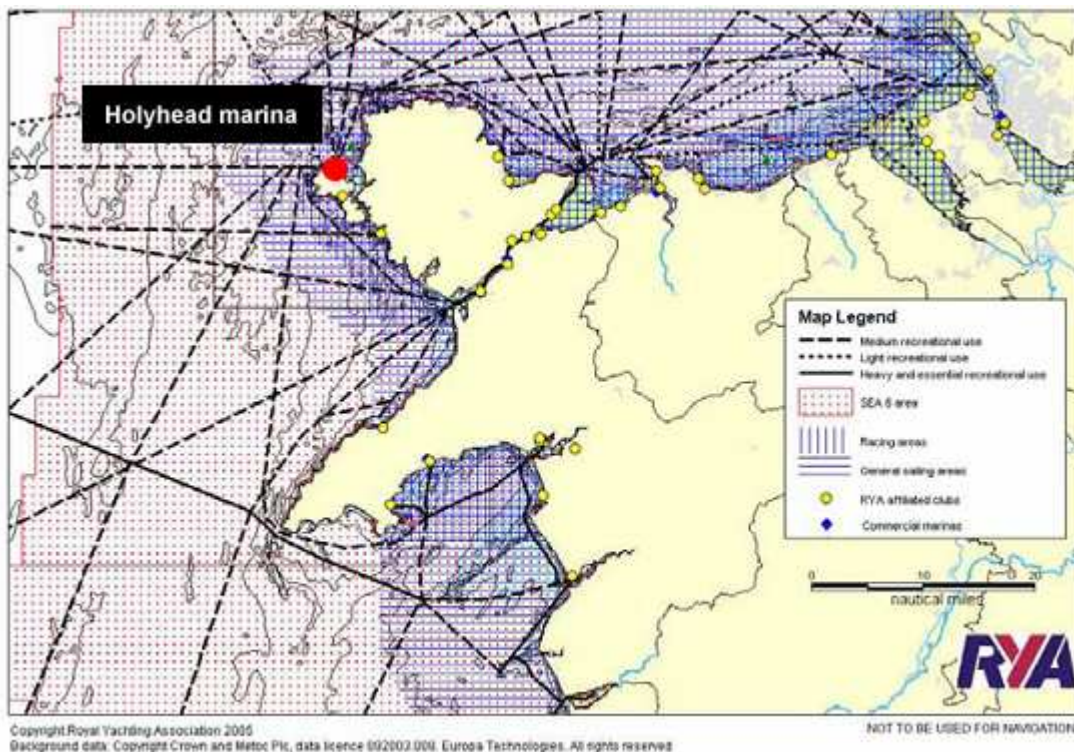


Figure 4. Recreational cruising routes and origins of marinas within the SEA 6 sub-area 2 – Figure extracted from RYA report 2005.

Q8 - What is the likelihood DV will establish in mussel beds in Wales?

Answer: *Please indicate likelihood using Table 1. If possible, provide any supporting comments.*

.....

Supporting information:

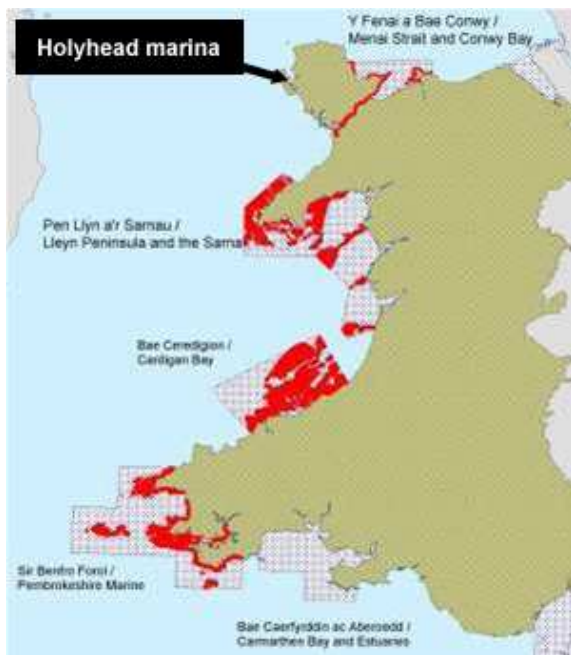
The Menai Strait is the UK’s most important mussel producing area. Mussels are grown to market size on the sea bed and the fishery is dependant on seed mussel, sourced from eroding mussel beds in sites both within the Menai Strait and from around the UK coast. Mussel beds may be subtidal and situated on sand and gravel substratum or intertidal (up to 30m depth) and often concentrated on areas of hard substratum but may occur on sand. There is currently no line culture however there is growing interest in developing this in some offshore locations near Holyhead.

Q9 - What is the likelihood DV will establish in reef areas in Wales?

Answer: *Please indicate likelihood using Table 1. If possible, provide any supporting comments.*

.....

Supporting information:



The following general groups of reef types are:

1. Rocky intertidal reefs
2. Rocky subtidal reefs (bedrock, boulders, cobbles, mixed)
3. Extensive boulder and cobble subtidal reefs – e.g. the Sarnau
4. Biogenic reefs
5. Carbonate reef formed by methane gas leaking from the seabed.

Figure 5 - Marine reef features in Wales (indicated as darker red areas).

Q10 – Consequences

In your experience, what are the likely consequences to:

Marinas

.....

Mussel industry

.....

Conservation sites (reefs, biogenic reefs and intertidal pools)

.....

APPENDIX 2: TIMELINE OF RESPONSE TO AN INCURSION OF THE SLIPPER LIMPET (*CREPIDULA FORNICA*) IN THE MENAI STRAIT IN 2007

Details of a rapid response taken, on advice from the Countryside Council for Wales (CCW), by mussel farmers in the Menai Strait in the event of the accidental introduction of the Slipper Limpet, *Crepidula fornicata*, with mussel seed imports. The action has thus far been successful. Throughout the entire episode, CCW stressed that eradication needed to take place before water temperatures increased and the slipper limpets started releasing larvae in large quantities.

27 February

The presence of slipper limpet amongst mussels in commercial lays in the Menai Strait was confirmed at an evening fisheries liaison meeting.

28 February

CCW contacted the NW&NWSFC, as the body responsible for the management of inshore fisheries and shortly afterwards the mussel company responsible for introducing the slipper limpets. It was requested that immediate action be taken to remove the affected mussels (i.e. all the mussels re-laid from the English Channel needed to be removed immediately from the Menai Strait). Pressure was maintained on a daily basis until agreement reached to take action.

5 March

Operations began to remove the affected load of mussels from the Menai Strait.

Mid March

1200 tonnes of mussels contaminated with slipper limpets and a buffer zone of mussels around the affected area were removed from the Menai Strait.

22 March

CCW undertook a survey of the affected mussel lay area, to assess success of removal. CCW subsequently advised that further dredging was required in affected lays.

End March to early April

Additional dredging in cleared areas undertaken.

18 April

CCW undertook further survey work on cleared lays. CCW subsequently advised that cleared areas should be relaid heavily with 'clean mussels' from Morecambe Bay to smother remaining slipper limpets.

Early May

Cleared areas relaid with 'clean mussels' from Morecambe Bay.

28 September

Commercial mussel lays surveyed by CCW and the NWNWSFC. Two empty shells, but no live slipper limpets were found.

August to September

CCW commissioned a survey of the wider Menai Strait survey to assess whether slipper limpet were present. This work did not find any conclusive evidence for current or historic populations of slipper limpet in the Menai Strait (other than those introduced in the mussel lays themselves).

2009

No live specimens have been found since the accidental introduction in 2007.