APPENDIX No. 9

Management Options for *Didemnum vexillum*

Management options for the invasive ascidian *Didemnum vexillum*, Mweeloon Bay, Co. Galway

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Cover Photo: Mweeloon oyster farm (Photo credit: Martina O'Brien; 19 January, 2018).

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1. Summary

The report provides details on the background, biology, ecology and responses to tested control treatments of the invasive ascidian *Didemnum vexillum (*hereafter *Didemnum)*. Findings from a site visit to Mweeloon (oyster farm) are also outlined. Relevant details on the principles of containment and control are included to provide scientific reasoning and support for selected management options and monitoring protocols outlined in the final section of the report. The general strategy recommended in this report involves substantially reducing *Didemnum* abundance within the first year of the programme. Recommended management activities for subsequent years (i.e. year 2 and onwards) are suggested in order to maintain the reduced abundance levels achieved in the first year of the control programme. Overall reductions in abundance will be achieved through the application of simple but effective chemical (i.e. vinegar sprays) and physical/mechanical (e.g. desiccation, hand removal) control techniques which have been shown to substantially reduce *Didemnum's* abundance in similar field settings. It is intended that the control and monitoring programmes are implemented over a long-term period (i.e. years). The report is divided into two distinct sections (i) scientific reasoning and support for proposed control programme (sections 2 – 7) and (ii) practical details (protocols and methodologies) of control and monitoring programmes (section 8).

2. Background

Didemnum, a colonial ascidian (sea-squirt), native to Japan has become widely invasive in coastal marine ecosystems over the past two decades (Stefaniak et al. 2009; Lambert 2009). It has established in over 100 locations throughout the U.S.A., Canada, Europe and New Zealand (Stefaniak et al. 2009). The main mechanism of this species' impact is it's capacity to successfully outcompete with native marine invertebrates for space (Gittenberger 2007; Bullard and Whitlatch 2009; Carman and Grunden 2010). It can rapidly overgrow natural and man-made substrates (Minchin and Sides 2006). Bullard and Whitlatch, (2009) recorded a 60% increase in *Didemnum*'s mean biomass over a 2 week period (Bullard and Whitlatch 2009). Where *Didemnum* becomes established it can decrease the complexity of habitats and bring about shifts in the structure and diversity of communities (Dijkstra et al. 2007; Lengyel et al. 2009; Mercer et al. 2009). In Georges Bank in the U.S., colonies of *Didemnum* have formed extensive mats over hundreds of square kilometres of pebble-gravel habitats which support diverse benthic invertebrate communities and a range of juvenile fish species (Lengyel et al. 2009; Valentine et al. 2007b). Within aquaculture *Didemnum* can extensively foul equipment and stock resulting in increased operational and labour costs (Fitridge et al. 2012). Overgrowth can also reduce the growth and survival of commercially important species (e.g. *Magallana gigas, Ostrea edulis, Mytilus edulis* etc.) (Piola et al. 2010; Switzer et al. 2011; Rolheiser et al. 2012; Auker 2010; Fletcher, Forrest and Bell 2013a).

Didemnum was first documented in Ireland in June 2005 where it was discovered fouling *emersed* man-made structures within Malahide marina (Minchin & Sides, 2006), North Co. Dublin. The hulls of boats, pontoons, chains, ropes and buoys were extensively fouled and assemblages of native biota were overgrown by colonies of this invader (Minchin & Sides, 2006). Subsequent to this initial discovery it has been documented at a number of other sites throughout the country including; Clew Bay, Carlingford Lough, Galway Bay, Strangford Lough and most recently in Dunmanus Bay, Co. Cork (pers. obsv.), (Minchin and Sides 2006; Kleeman 2009; Miley 2014; Kelly and Maguire 2008; Minchin 2007; Nunn and Minchin 2009). First recorded sightings of this species in Galway Bay were made in July 2007 at Parknallagh. While no formal survey has been conducted in the area, reports of the species found at sites to the north and south of its initial recorded location have been made, suggesting that the species has spread from an initial introduced source population or that multiple independent introductions have occurred within Galway Bay. However, the distribution of this species throughout Galway Bay has not been precisely documented.

3. Site visit: Mweeloon Bay

Visit purpose: Establish presence/absence of *Didemnum vexillum* (Capet sea squirt) and extent of invasion	
Date: 19/01/2018	**Site type/location:** Intertidal trestle system oyster farm, Mweeloon Bay, Co. Galway.
Cultured species: *Magallana gigas* (previously, *Crassostrea gigas*)	

Report: A licensed intertidal trestle system oyster farm in Mweeloon Bay was visited on January 19th 2018 (see map, figure 1). At the site the invasive ascidian *Didemnum vexillum* was found growing over a range of artificial and natural substrates. Biofouled substrates included; oyster trestles, bags, rubber ties, algae and other biofouling marine invertebrates. Cultured oysters did not appear to be directly fouled by *Didemnum*. Unused stacks of oyster trestles present at the site were heavily fouled with algae (predominantly *Fucus serratus*). *Didemnum* fouling occurred on a small portion of fucoid fronds attached to these unused trestles.

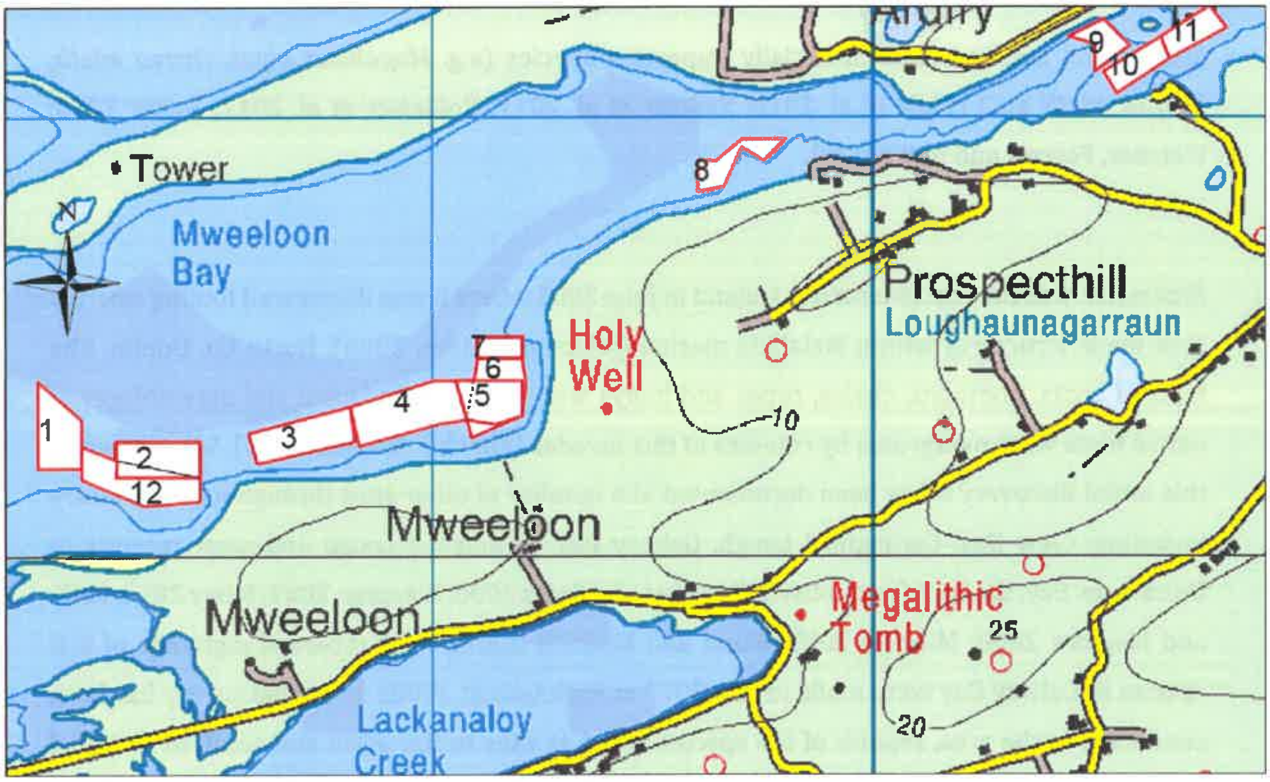

Figure 1: Map showing location of oyster farms, Mweeloon, Co. Galway, Ireland.

A large portion of the site appeared to be free of visible colonies of *Didemnum* (and other) fouling species. Both bags and trestles on the northern end of the site were 'clean', suggesting that bags may have been in the water for a relatively short period of time. Deeper portions of the site did not appear to harbour greater abundances of *Didemnum* as has been noted at other oyster farm sites (pers. obsv.). Overall *Didemnum*'s distribution was patchy and its abundance appeared to be low. However, where it was found colonies covered large portions of oyster bags, encapsulated rubber ties and covered algal fronds. High abundance levels at the time of year the site was visited would not be expected, as *Didemnum* die back takes place from late August to late February/March. Colonies ranged in size and morphology from encrusting two dimensional mats to characteristic lobed growth forms (see Figure 2). A small number of colonies were found growing over algae (*Ascophyllum nodosum*) in the upper intertidal portion of the site. The predominant seabed substrate at the site was sand. Other substrates included rocks, boulders, and discarded oyster trestles. *Didemnum* thrives on hard substrates, however soft moving substrates like sand would not be conducive to natural settlement by *Didemnum* colonies (i.e. it is unlikely to become established on the seabed at the site or within its immediate vicinity). However, *Didemnum* has been found to establish on mussel druses growing on the seabed and on soft substrates in environments with low current velocities. Seabed substrate would likely influence the spread of *Didemnum* to subtidal habitats within the vicinity. It is not known how the species arrived on the site.

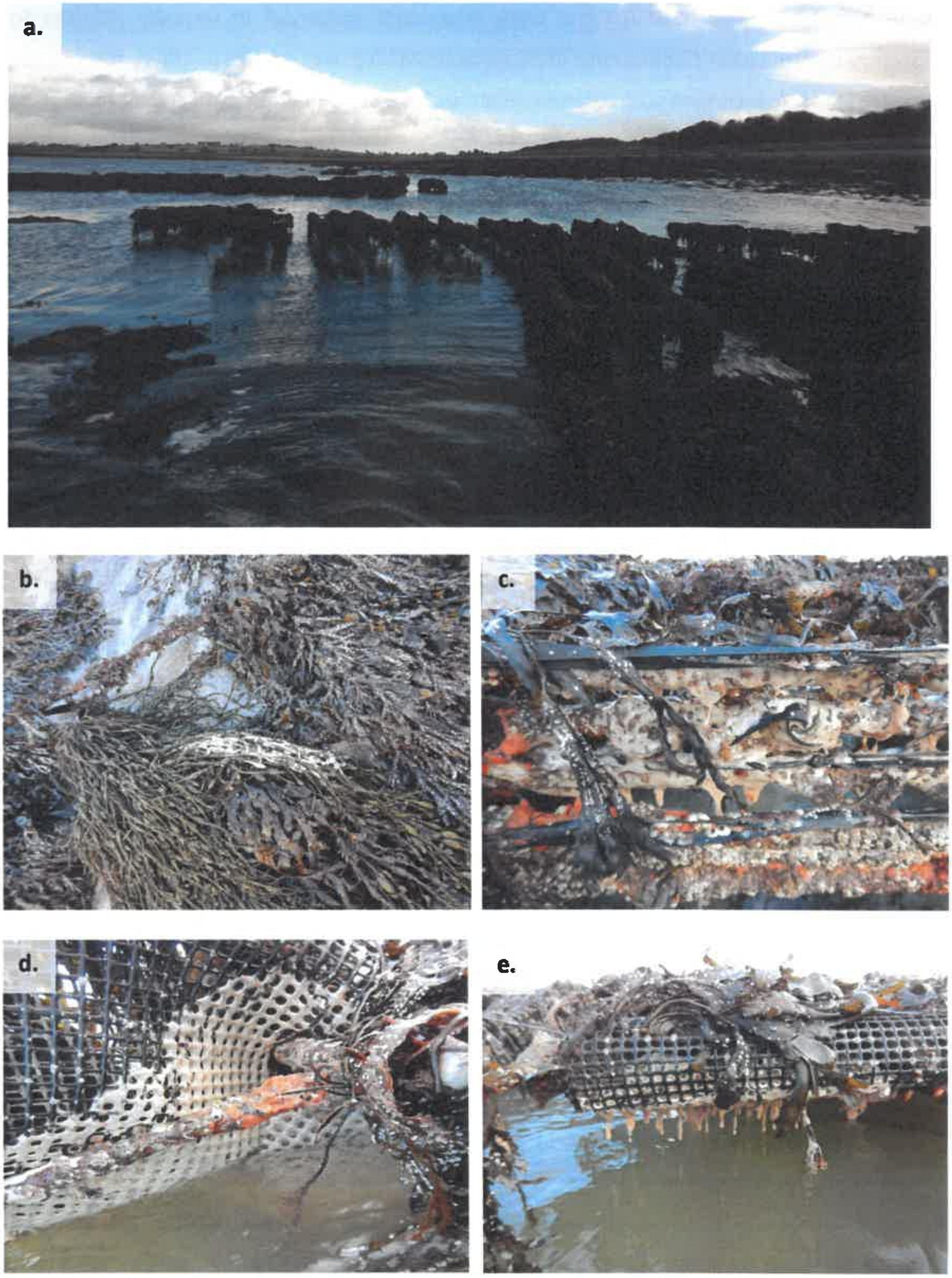

Figure 2: (a) Mweeloon bay oyster farm (b) *Didemnum* fouling *A. nodosum* (c) *Didemnum* fouling trestle frame and rubber ties (d) Encrusting 2-D mats of *Didemnum* growing over mesh oyster bags (e) 3-D lobed growth form of *Didemnum*

Action taken: Samples of *Didemnum* were taken and preserved in formalin solution for laboratory identification. Photographs of *Didemnum* growing over a range of different substrates were also taken. A document with recommendations for containment, control and mitigation was compiled (see section 4 - 8).

Summary of potential risks: The main threat that this species poses is to the management of the farm site. Fouling will likely result in increased labour costs over time. Fouling on bags can also restrict water flow, disrupting the flow of food and water to oysters contained inside. Other potential impacts include the local spread of *Didemnum* to (i) other aquaculture sites in the vicinity and (ii) the natural environment through natural dispersal (i.e. larval and fragment dispersal). Free floating fragments (as small as 2cm) act as viable propagules of *Didemnum*, which can be transported in the water column by tidal movement/hydrodynamics to other sites and areas. *Didemnum* could also be spread regionally to other aquaculture sites through equipment and stock transfer. To determine and fully understand the risk of impact and spread of *Didemnum* at this site, a formal risk assessment would be required.

4. Management options

Didemnum has been present in Galway bay since 2007 and has established at multiple local and regional locations since. It is widely accepted that once non-native populations have successfully established, eradication is often unfeasible and impacts on native biodiversity and ecosystems can occur (Gherardi and Angiolini 2004; Myers et al. 2000; Clout and Williams 2009). Given that *Didemnum* has been established in Galway since 2007 and has established in numerous other locations on both a local and national scale, eradication no longer represents a feasible management option (Grice 2009). A comprehensive control and monitoring programme represent the most appropriate course of action (Grice 2009; Clout and Williams 2009). Unlike eradication, control programmes have no definitive 'end point' and therefore generally require an indefinite commitment of resources (Grice 2009). Finding cost effective and time efficient control strategies for invasive species is, therefore, crucial to ensuring their long term and sustainable management (Atalah et al. 2013; Atalah et al. 2015; Taylor and Hastings 2004; Hewitt et al. 2005; Bax et al. 2001; Grice 2009).

The global concern surrounding the introduction of *Didemnum* and its potential impacts on marine ecosystems and industries (e.g. shellfish aquaculture) (Carman and Grunden 2010; Fletcher et al. 2013b; Rolheiser et al. 2012; Switzer et al. 2011) has resulted in the development and evaluation of an extensive range of biological, mechanical and chemical control techniques (Carman et al. 2009; Epelbaum et al. 2009; Rolheiser et al. 2012; Switzer et al. 2011; Piola et al. 2010; Coutts and Forrest 2007; Holt and Cordingley 2011; Denny 2008). Details of these control options, their level of success, treatment context and location are summarised in Table 1. Mechanical controls tested include; water blasting, covering with biodegradable geotextiles, air drying, PVC wrapping, hand removal and wire brushing. Some of these treatments, while effective, are labour intensive and impractical for large scale, long term control of *Didemnum* (Coutts and Forrest 2007). Chemical treatments such as vinegar, bleach, sodium meta-silicate, lime, copper and freshwater have been tested at a range of concentrations and exposure time using dip or spray applications. Some of these chemical treatments were up to 100% effective at removing *Didemnum* (Denny 2008; Rolheiser et al. 2012; Piola et al. 2010). Chemical treatments have also often been used in combination with other treatment types (i.e. plastic encapsulation) to accelerate treatment effects. A diverse range of biological control agents were tested, organisms evaluated included crabs, nudibranchs, sea stars, sea urchins, periwinkles and sea slugs. While a number of the species tested consumed living or decaying *Didemnum* colonies, none were deemed effective control agents with the capacity to substantially reduce *Didemnum*'s abundance (Carman et al. 2009; Epelbaum et al. 2009). The success and suitability of these mechanical, chemical and biological control treatments varied greatly: many were found to be entirely ineffective or unsuitable for the specific context required. Details of these studies and their outcomes are summarised in the table below (Table 1.

Table 1: Table summarising trailed *Didemnum vexillum* controls within and outside of aquaculture (O'Brien 2018).

Type	Treatment	Context	Location	Outcome	Reference
Mechanical /Physical	Underwater vacuum system with 50µm mesh filter (direct removal of biomass)	Barge & seabed within marina	Field	Reduced biomass	(Coutts and Forrest 2007)
	'Made to order' heavy duty reinforced PVC bags (anoxic conditions) *combined with chemical accelerant	Pontoons, mooring chains, buoys, wharfs & floating docks	Field	Varied: Successful where bag integrity maintained. Failed where bags were damaged by boats and waves.	(Holt and Cordingley 2011)
	Beaching of large barges (desiccation stress)	Large barges (~ 80 m x 25 m)	Field	Unsuccessful	(Coutts and Forrest 2007)
	Polythene wrapping (anoxic conditions) *combined with chemical accelerant	Wharf piles, structurally unsound barges	Field	Varied: Successful where wrap integrity maintained. Failed where wraps became loose or damaged.	(Coutts and Forrest 2007)
	Uncontaminated dredge spoil ~ 70 % Silt/clay 20% sand and 10% small cobble (anoxic conditions i.e. to smoother colonies)	Seabed beneath wharfs in marina (covering total area of 80 x 40 m)	Field	Successful	(Coutts and Forrest 2007)
	Bidum A24 grade geotextile fabric (anoxic conditions)	Rip rap in marina	Field	Unsuccessful gaps between joins of sheets of fabric allowed water exchange	(Coutts and Forrest 2007)

	Removal from water, water blasted in quarantined area returned after 48 hours (biomass removal)	Moorings within marina	Field	Successful	(Coutts and Forrest 2007)
	Manual cleaning with soft wire brushes (biomass removal)	Shellfish aquaculture	Field	Reduced biomass	(Switzer et al. 2011)
Chemical	<u>Calcium oxide (lime)</u> **Trialled application methods:** dips and sprays **Trialled concentrations (%):** 1, 2, 3, 4, 5, 10, 20 (prepared using freshwater or seawater) **Trialled exposure times (mins):** 0.2, 0.5, 1, 2, 3, 4, 5, 10	Aquaculture transfer, shellfish aquaculture, biofouling assemblages on artificial and natural substrates	Laboratory/Field	~ 30 -100% *Didemnum* mortality	(Denny 2008; Switzer et al. 2011; Rolheiser et al. 2012)
	<u>Sodium hypochlorite (bleach)</u> **Trialled application methods:** dips and sprays **Tested concentrations (%):** 0.1, 0.25, 0.5, 1, 2, 5, 10, 20 (prepared using seawater) **Tested exposure times:** 3 s, 20 s, 30 s, 2 mins, 30 mins, 3 hrs, 6 hrs, 10 hrs.	Aquaculture transfer, biofouling assemblages on artificial and natural substrates	Laboratory/Field	60 – 100% *Didemnum* mortality	(Denny 2008)
	<u>Acetic acid (vinegar)</u> **Trialled application methods:** dips and sprays **Tested concentrations (%):** 0.1, 0.5, 1, 2, 4, 5, 10, 20 (prepared using freshwater or seawater) **Tested exposure times:** 3 s, 5 s, 20 s, 1 min, 2 mins, 3 mins, 5 mins, 10 mins, 30 mins, 3 hrs, 6 hrs & 10 hrs.	Aquaculture transfer, shellfish aquaculture, biofouling assemblages on artificial and natural substrates	Field	~20-100% *Didemnum* mortality	(Denny 2008; Rolheiser et al. 2012; Piola et al. 2010)

<u>Freshwater</u> **Trialled application method:** dips **Tested concentrations (ppt):** 0, 5, 20 (prepared using instant ocean and freshwater) **Tested exposure times (mins):** 0.5, 1, 2, 5, 10	Aquaculture transfer, shellfish aquaculture	Field	~ 0 – 87 % *Didemnum* mortality	(Denny 2008; Rolheiser et al. 2012)
<u>Brine</u> **Trialled application method:** dips **Tested concentration (ppt):** 40, 50, 70 (prepared using instant ocean and freshwater) **Tested exposure times (mins):** 0.5, 1, 5, 10	Shellfish aquaculture	Field	Unsuccessful	(Rolheiser et al. 2012)
<u>Sodium metasilicate</u> **Trialled application method(s):** dips with seawater rinse **Tested concentrations (%):** 3, 6 (prepared using seawater) **Tested exposure times:** 20 secs, 2 min	Aquaculture transfer	Field	~ 15 – 100% *Didemnum* mortality	(Denny 2008)
<u>Sodium hydroxide (caustic soda)</u> **Trialled application method(s):** dips with seawater rinse **Tested concentrations (%):** 3, 6 (prepared using seawater) **Tested exposure times:** 20 secs, 2 min	Aquaculture transfer	Filed	~ 80 – 100% *Didemnum* mortality	(Denny 2008)

	Pre-mixed granulised chlorine (200g / m^3 added to polyethylene wrapped structures)	Structurally unsound barges within marina	Field	Successful where wrap integrity maintained	(Coutts and Forrest 2007)
	Granulised calcium hypochlorite (added to structures covered in PVC bags)	Wharf, pontoons, buoys & mooring chains	Field	Successful where bag integrity maintained	(Holt and Cordingley 2011)
	Anti-fouling paint application after cleaning in quarantine	Recreational vessels within marina	Field	Successful	(Coutts and Forrest 2007)
Biological	*Strongylocentrotus droebachiensis* (Green sea urchin)	Shellfish aquaculture	Laboratory/ Field	Consumed *Didemnum* but limited value as control agent	(Switzer et al. 2011; Epelbaum et al. 2009)
	Strongylocentrotus franciscanus (Red sea urchin)	Shellfish aquaculture	Laboratory	Consumed *Didemnum* but limited value as control agent	(Epelbaum et al. 2009)
	Hermissenda crassicornis (Opalescent sea slug)	Shellfish aquaculture	Laboratory	Unsuccessful	(Epelbaum et al. 2009)
	Dermasterias imbricate (Leather sea star)	Shellfish aquaculture	Laboratory	Unsuccessful	(Epelbaum et al. 2009)
	Evasterias troschelii (Mottled sea star)	Shellfish aquaculture	Laboratory	Unsuccessful	(Epelbaum et al. 2009)
	Pisaster ochraceus (Purple sea star)	Shellfish aquaculture	Laboratory	Unsuccessful	(Epelbaum et al. 2009)
	Cancer productus (Red rock crab)	Shellfish aquaculture	Laboratory	Unsuccessful	(Epelbaum et al. 2009)
	Carnicus maenas (Dungeness crab)	Shellfish aquaculture	Laboratory	Unsuccessful	(Epelbaum et al. 2009)
	Cancer gracilis (Graceful crab)	Shellfish aquaculture	Laboratory	Unsuccessful	(Epelbaum et al. 2009)

Carcinus maenas (European green crab)	Shellfish aquaculture	Laboratory	Unsuccessful	(Epelbaum et al. 2009)
Caprella spp. (Skeleton shrimp)	Shellfish aquaculture	Laboratory	Unsuccessful	(Epelbaum et al. 2009)
Littorina littorea (Common periwinkle)	Shellfish aquaculture	Laboratory/ Field	Consumed *Didemnum* but limited value as control agent	(Carman et al. 2009)
Fusitriton oregonensis (Oregon hairy triton)	Shellfish aquaculture	Laboratory	Unsuccessful	(Epelbaum et al. 2009)

5. Selected controls treatments & management activities

Controlling invasive species in aquaculture is a challenging task, as treatments need to be powerful enough to remove target species while also ensuring that the health and survival of aquaculture stock (non-target species) is maintained (Switzer et al. 2011). Often substrates on farm sites (e.g. trestles) cannot be removed and so must be treated in situ (Coutts and Forrest 2007). Given that there is limited time available where substrates are exposed, treatments that can be applied rapidly and act immediately on target organisms with minimal impacts on non-target species are required. Control treatment types selected for Mweeloon oyster farm represent those that best fulfil these selection criteria from the suite of management options available for *Didemnum* (Table 1).

Bag turning

This method is routinely used by farmers to reduce biofouling on mesh oyster bags in intertidal trestle system oyster farms. In these systems oysters are grown in mesh bags suspended above the sea bed by metal frames (trestles) which can be accessed during periods of low tide (Figure 3). This growing method allows water to flow through to oysters enabling them to feed and survive. However over time biofouling organisms accumulate, water flow is reduced and impacts on cultured species may occur (Fitridge et al. 2012). Where oyster bags are regularly turned, invertebrate fouling species (which predominantly accumulate on the shaded underside of bags) are exposed to direct sunlight (i.e. UV, heat and desiccation stress, (Bingham & Reyns, 1999)) during periods of low tide, when aquaculture equipment and stock are fully or partially *emersed*. Additionally, as bags are turned the exposure of algal fouling species (which typically attach to the upward sun-exposed side of oyster bags) to sunlight is obstructed and the abundance of these species is simultaneously reduced.

Vinegar spraying

Chemical controls (e.g. acetic acid, hydrated lime, bleach etc.) are also often used to reduce fouling and can be a cost effective and time efficient alternative for limiting invasive fouling in shellfish aquaculture (Rolheiser et al. 2012; Switzer et al. 2011; Denny 2008; Locke et al. 2009; Coutts and Forrest 2007; Carman et al. 2010; Carman et al. 2015). Acetic acid is commonly used on oyster and mussel farms where equipment and stock are dipped (for specified durations) in or sprayed with ~ 4 – 5% acetic acid solutions (Locke et al. 2009). Treatments act on targeted organism by damaging cell membranes ultimately causing organisms to die, consequently reducing biofouling cover and biomass (Ferguson et al. 2016; Locke et al. 2009). Piola *et al.* (2010) showed that acetic acid spray treatments applied to *Didemnum* reduced its cover by more than 80%. However *Didemnum* was able to regenerate and repeated applications were required (Locke et al. 2009; Piola et al. 2010). At a concentration of < 5% these treatments are chemically equivalent to household vinegar and are thus perceived as "eco-friendly", posing little or no threat to the environment, particularly at sites where strong tidal cycles regularly circulate large volumes of water (Locke et al. 2009). Control treatments such as acetic acid are often used in combination with other control methods (e.g. added to plastic wrapping of pontoons) to act as a chemical accelerant, to slower acting physical or mechanical methods (Atalah et al. 2016; Coutts and Forrest 2007; Holt and Cordingley 2011). Piola *et al.* (2010) found that where spray treatments were applied, exposure times of even 10 minutes substantially increased the effectiveness of treatments. Similar results were found by O'Brien (2018) where fouling was significantly reduced when repeated spray treatments were used in combination with repeated air exposure. This involved administering treatments three times over a six week period (i.e. treatments were applied once every two weeks during the corresponding Spring tides).

Additional treatment details:

While vinegar is an effective control treatment that can substantially reduce the abundance of *Didemnum*, repeat treatments are required as *Didemnum* has the capacity to recover from individual sprays treatments.

6. Factors influencing control success

While treatment selection is a key step in devising successful management programmes other factors can also influence control success (Coutts and Forrest 2007; Grice 2009; Holt and Cordingley 2011; Taylor and Hastings 2004; Dumbauld et al. 2006). Factors such as the frequency, timing and duration between treatment applications can strongly influence the overall success of control programmes (Mehta et al. 2007; Raghu et al. 2006; Wong et al. 2012; Taylor and Hastings 2004). A recent study found that these types of factors significantly influenced the effectiveness of combined 'bag turning' and 'vinegar spray' *Didemnum* control treatments (O'Brien, 2018). By assessing these factors this study was able to identify a range of treatment regimes capable of significantly reducing *Didemnum*'s coverage in an aquaculture setting and uncover why some treatment regimes were more effective than others (O'Brien, 2018). Results from this study showed that while many treatments took immediate effect, others manifested more gradually over time and the effects of some treatments were sustained over the entire season, while the suppressive effects of others wore off over time allowing *Didemnum* to recover and re-establish high coverage levels on oyster bags.

In the context of this management programme, a 'turn and spray' combined treatment was selected with applications times spread out over *Didemnum*'s active growth phase. This treatment regime offers a low cost, sustainable, long term and effective management option. This control strategy involved turning and spraying oyster bags with vinegar at the beginning, middle and end of *Didemnum*'s active growth season (see Section 8 for further details and management methodology). Spreading treatments out in this way allowed treatments to immediately reduced *Didemnum*'s abundance and then subsequently maintain consistently low coverage levels throughout its active growth phase. This was achieved by removing remnant colonies (colonies which have survived over winter), and subsequently targeting newly established/recovering colonies over the course of *Didemnum*'s growth season. The capacity of this treatment regime to limit coverage levels at the end of *Didemnum*'s active growth phase would likely confer control benefits in subsequent years as the removal of overwintering colonies inhibits reestablishment and expansion during *Didemnum*'s early growth phase. While the study did not assess whether achieving low levels of abundance at this point of transition in *Didemnum*'s life cycle, a study by Stachowicz et al. (2002) found that the extent of colony "die-back" (due to reductions in winter water temperatures) amongst invasive ascidians determined their abundance in subsequent years (Stachowicz et al. 2002). Therefore it follows that where abundance of *Didemnum* can be reduced during this phase of regression abundance in subsequent years will also be reduced.

Where resources are limited or control work cannot be initiated early in *Didemnum*'s active growth cycle targeting colonies at the end of the growth season and focusing management efforts during *Didemnum*'s die back phase offer the greatest potential for control success. Turning and spraying fouled oyster bags in late July and throughout August (see Section 8 for treatment details and methodology) significantly reducing coverage levels, leaving less than 5% coverage on bags prior to the initiation of colony die back. This 'clustered' treatment regime involved administering treatments three times over a six week period late in *Didemnum*'s active growth phase (i.e. treatments were applied once every two weeks when trestles and bags were exposed during spring tides).

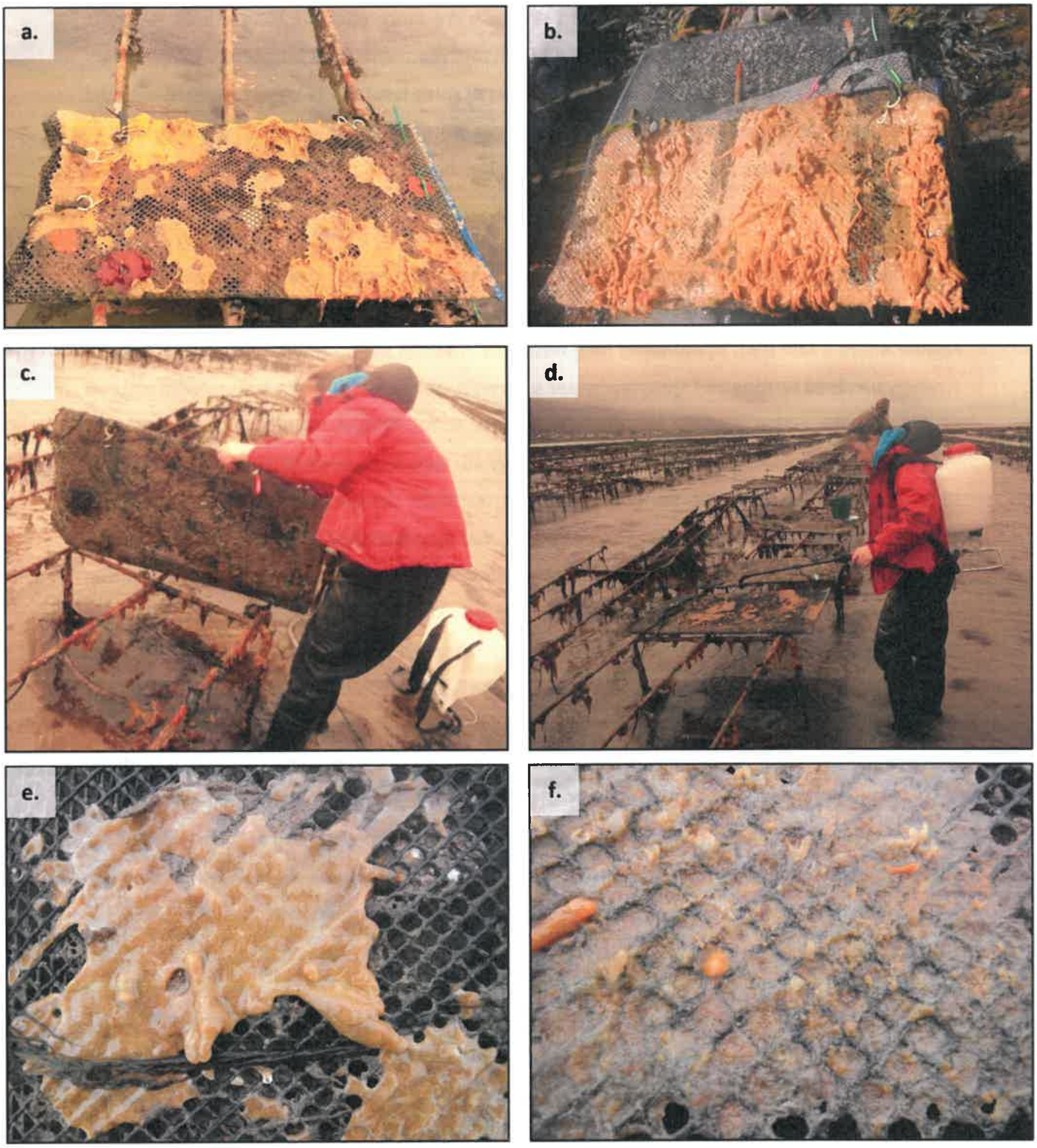

Figure 3 (a) Typiacl *Didemnum* fouling in control plots (May) (b) Extensive fouling in control plots (June) (c) bag turning treatment (d) vinegar spray treatments (e) *Didemnum* colony immediately after vinegar spray treatment (f) effects of vinegar spray treatment on *Didemnum* tunic (2 days after treatment application) (O'Brien 2018).

Physical/manual removal

Removing unwanted invasive species by hand or using scrapers or blades is a well established, basic and effective method for removing small amounts of biofouling material (Clout and Williams 2009; Coutts and Forrest 2007). Physical removal carried out during reproductively inactive phases of a species life cycle can be an effective method of reducing species abundance (Switzer et al. 2011). This is generally not attempted at a large scale as invasive species often establish high levels of biomass and removal can be impractical (Piola et al. 2009). Hand removal may also promote fragmentation and/or larval release (Reinhardt et al. 2012; Morris and Carman 2012). In the context of this management plan, this treatment is intended for removing algal fouling from oyster trestles during *Didemnum*'s dormant life cycle stage. Algae fouling trestles provide ample colonisable surface area for colony establishment and spread. This action will require a considerable investment of time and resources, however it will facilitate the long-term success of this management programme. Where possible trestles should be cleared of algal fouling on a regular basis to inhibit a build-up of epiphytic fouling organisms.

Plastic wrapping

Plastic wrapping/encapsulation is becoming a more frequently used control tool for managing invasive species in aquatic environments (see Figure 4) (Atalah et al. 2016; Piola et al. 2009; Coutts and Forrest 2007; Holt and Cordingley 2011). This method involves covering structures (e.g. pontoon, boat hulls, mooring chains, ropes) or areas of seabed (e.g. pebble-gravel habitats, boulders) in plastic or other barrier fabrics. These barriers inhibit water flow and light penetration (Atalah et al. 2007; Piola et al. 2009). As encapsulated organisms continue to respire, anoxic conditions develop within wrap structures/areas. The combined effects of wrapping (i.e. lack of food, water, light, oxygen and consequent toxic conditions) cause encapsulated organisms to die off (Atalah et al. 2016). This method has been employed effectively as part of *Didemnum* eradication programmes carried out in the U.K. and New Zealand where the integrity of wrapped structures was maintained (Coutts and Forrest 2007; Holt and Cordingley 2011). This method has also been used in combination with chemical controls (e.g. bleach or acetic acid). Chemicals used act as accelerants, rapidly compounding the effects of wrapping techniques (Coutts and Forrest 2007; Holt and Cordingley 2011; Atalah et al. 2016). This combined approach is often necessary where the integrity of wrapping material is likely to be jeopardised due to external conditions (e.g. in aquatic environments exposed to strong wave forces).

Additional treatment details:

This method could be used in place of spraying and hand removal of biofouling from trestles. To our knowledge this has not been attempted in aquaculture as trestles. However it has been attempted on mooring chains, pontoons, jetty piling and a range of substrates that may be considered difficult to navigate. While this method may not be feasible due to limited resources it is an option that can be considered and piloted if total removal of fouling from trestles is required.

Figure 4: Geotextile fabric used to encapsulate pontoon fouling (image Clout & Williams 2010)

7. Principles of containment and control

The identification of effective control methods is an important step in designing management strategies for invasive species: however, evaluating other fundamental aspects associated with their practical implementation should also be assessed. Invasive species can be managed, controlled and monitored more effectively where knowledge of the target species' biology, ecology, management options/responses to various management strategies, distribution (at both local and regional scales) can be obtained (Grice 2009).

7.1 Assemble knowledge of species' biology, ecology and responses to management

Environmental tolerances & habitat preferences

Relevance to control/monitoring:

This information can assist control and monitoring by providing specific details about the types of conditions and habitats that *Didemnum* is likely to be found in and those that support its survival and spread.

Didemnum has been recorded in waters where temperatures range from - 2 and > 25 °C and water salinities range from 26 – 30 ppt (Gittenberger 2010; Daniel and Therriault 2007; Valentine, Carman, et al. 2007; Bullard et al. 2007). Like many other invasive species *Didemnum* can tolerate considerable fluctuations in both water temperature and salinity (Gittenberger 2010; Valentine, Carman, et al. 2007; Bullard and Whitlatch 2009; Herborg et al. 2009; Cohen et al. 2011). Valentine et al. (2007) observed colonies of *Didemnum* growing in New England tidepool habitats where daily water temperatures fluctuated by 11°C (Valentine, Carman, et al. 2007). Gröner et al. (2011) found that *Didemnum* could tolerate fluctuations in salinity, (i.e. declines in salinity of > 4 units). Similarly, Gittenberger (2010) found that established colonies of *Didemnum* could withstand considerable changes in water temperatures and salinities in the Netherlands (Gröner et al. 2011; Gittenberger 2010). Furthermore Lenz et al. (2011) demonstrated that this was a capacity possessed by *Didemnum* that taxonomically related native ascidians lacked (Lenz et al. 2011). Invasive colonies of *Didemnum* have been found growing in water depths ranging from just below the waterline on marina and harbour walls to depths of > 80 m (Georges Bank in the US) (Gittenberger 2010; Valentine, Collie, et al. 2007). *Didemnum* prefers shaded conditions, not directly exposed to sunlight and will often settle on shaded underside of artificial and natural substrates (Monniot et al. 1991; Lambert 2005).

Like many other invasive species, *Didemnum* can tolerate a wide range of environmental conditions; a trait which is believed to facilitate both the initial introduction of this species to new environments (i.e. areas beyond their native range) and its subsequent expansion and spread within those environments (i.e. local and regional spread) (Gittenberger 2010). *Didemnum* has been found in ecosystems where water quality has been significantly diminished (e.g. harbours, marinas) but it also thrives in environments associated with high water quality (i.e. shellfish growing waters) (Carman et al. 2007; Carman et al. 2010). Similar to other invasive ascidian species *Didemnum* can tolerate environments with high levels of suspended particulate matter (Lambert 2002; Minchin and Sides 2006). However persistent inputs of sediment can cause siphons to close and colonies to die over time, as respiratory and feeding processes cease (Monniot et al. 1991).

While *Didemnum* can grow over sand on the seabed (Gittenberger 2010) shifting sandy substrates do not appear to be conducive to the establishment and continued survival of this species (Hopkins et al. 2011). *Didemnum* is closely associated with artificial substrates which are present in abundance in marinas, harbours (e.g. mooring ropes and chains, boat hulls, breakwater walls, pontoons etc.) and shellfish aquaculture (e.g. trestles, oyster bags, mussel long lines and floats, hooks, rubber ties, mooring floats and buoys) (Minchin and Sides 2006; Gittenberger 2007; Holt and Cordingley 2011; Coutts and Forrest 2007). However it is not restricted to these artificial habitats and can overgrow natural substrates (i.e. boulders, rocks, pebble gravel habitats) (Lengyel et al. 2009; Valentine, Collie, et al. 2007; Valentine, Carman, et al. 2007; Miley 2014). In Georges Bank in the US it grows over large expanses of the sea floor (Lengyel et al. 2009; Valentine, Collie, et al. 2007). More recently in Kent, U.K. colonies of *Didemnum* have established a patchy but expansive distribution on the sea bed (Graham et al. 2015). *Didemnum* can also overgrow and encapsulate other marine invertebrates (e.g. barnacles, sea grass, mussels, oysters, tube worms, algae, bryozoans etc.)(Minchin and Sides 2006; Carman and Grunden 2010; Gittenberger 2007). Notable exceptions include anemones (e.g. *Metridium senile*) and other ascidian species (e.g. *Apilidium glabrum*) (pres. obsv.). While no survey on the species distribution has been conducted in Ireland, sightings of *Didemnum* vexillum growth on the seabed have been made (Clew Bay, Malahide Marina pers. obsv.). It is unclear whether populations of the species that become established on the seabed can survive for long periods as high sediment build up can cause the species inhalant siphons to close.

Didemnum's growth cycle

Relevance to control/monitoring:

This section provides key information about *Didemnum*'s life cycle and its changing morphological appearance throughout different phases of its growth. Knowing the period of peak and low abundance allow control work to be planned in advance so that excessive movement is avoided during these times to limit fragmentation/larval release. It also identifies the period where *Didemnum*'s is at its most vulnerable point and consequently most susceptible to treatment effects (i.e during periods of die back).

Didemnum follows a seasonal growth cycle, with early growth of remnant 'overwintered' colonies initiating in May and continuing through to July (Valentine, Carman, et al. 2007). At this stage colonies expand through asexual budding, forming thin two dimensional encrusting mats over substrates (see Figure 4) with small lobed protrusions (< 2cm) gradually developing at cloacal apertures dispersed across the surface of colony tunics (Valentine, Carman, et al. 2007). Colonies continue to expand and thicken and lobes continue to grow and extend outward (i.e. 3-dimensionally) from encrusting colonies in June and July (Valentine, Carman, et al. 2007). In late July and through to early September colonies grow more rapidly covering large surface areas and further increasing the width and length of lobes. Where two independently growing colonies meet, depending on genetic compatibility (i.e. similarity) they may fuse or conflict (Smith et al. 2012). During this 'late growth phase', lobes can extend to more than 1 metre in length (Figure 5) (Coutts and Forrest 2007). The width of lobes can vary greatly from their base to their growing end with narrowing occurring at irregular points along the length of each lobe (Valentine, Carman, et al. 2007; Reinhardt et al. 2012). This form of growth contributes to the 'pinched-off' candle wax morphology of colonies and may also increase the probability of fragmentation, particularly where strong wave forces or other forms of disturbance occur (Reinhardt et al. 2012). Where lobes fragment they represent viable propagules of *Didemnum* as they can survive and continue to grow (sexually reproduce) in suspension for up to three weeks. Fragments as small as 3 cm that have detached from colonies can reattach to substrates and continue to grow (Bullard and Whitlatch 2009).

As temperatures begin to drop in late September and early October colonies begin to "degenerate" leading to their disintegration (Valentine, Carman, et al. 2007). This process is marked by a number of morphological changes including the thickening of colony margins, prolonged opening of zooids and brown spots over colony surface (representing an accumulation of fecal pellets at cloacal canals): colonies reduce back to thin encrusting 2-D mats (reduction in lobe abundance and size) some often with the appearance of peeling off substrates(Valentine, Carman, et al. 2007). The extent to which colonies 'die back' is determined by local temperature conditions (Stachowicz et al. 2002). Colonies that survive over winter begin to grow and expand once again when conditions become favourable (Valentine, Carman, et al. 2007). This suggests that the extent to which colonies die-back over the winter period determines their abundance and seasonal reproductive cycles for the following year. Where colony die-back is minimal, growth and reproduction initiate early the following year, extending the growing and reproductive season and progressively increasing the abundance of *Didemnum* in invaded habitats. Therefore where remnant colonies can be removed, the abundance of *Didemnum* will be substantially reduced.

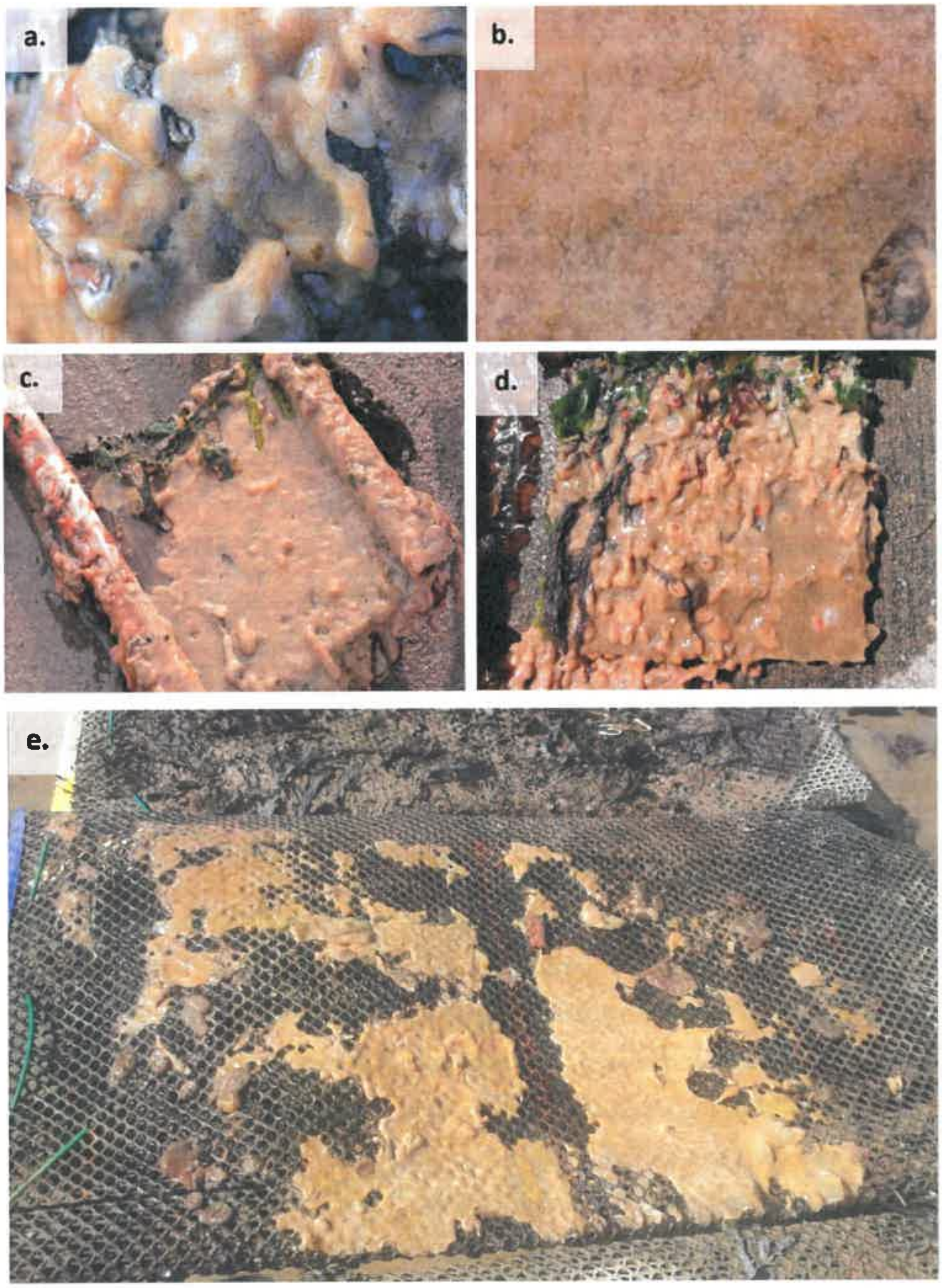

Figure 5 (a – e) Encrusting 2-D mats of *Didemnum* fouling a range of artificial substrates (characteristic white spicules visual in (a). (O'Brien, 2018)

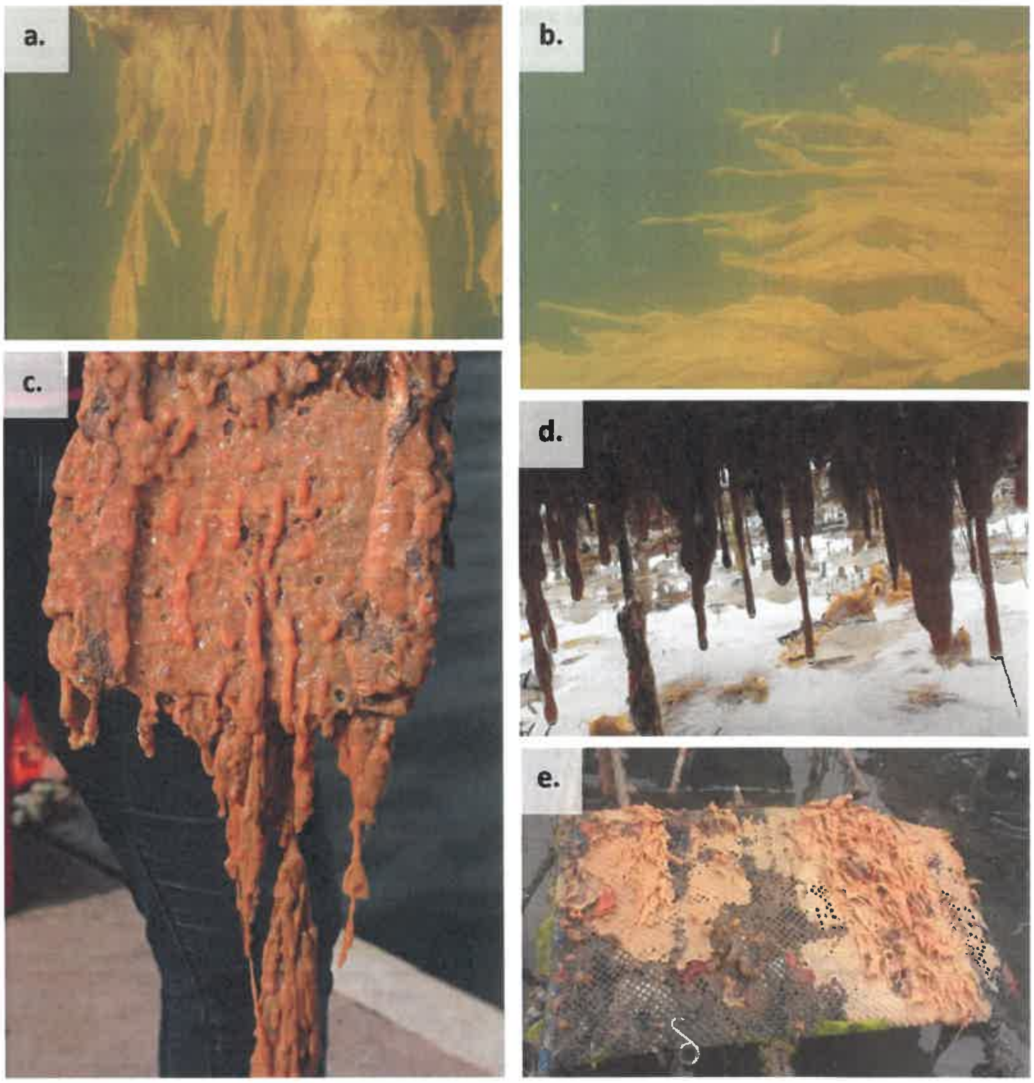

Figure 6: (a – e) Characteristic long tendril/lobed growth form of ***Didemnum*** covering a range of artificial substrates (O'Brien, 2018)

Seasonal reproductive patterns and natural dispersal capacity of Didemnum vexillum larvae

Relevance to control/monitoring: During periods of active reproduction, control work may encourage the release of larvae. Knowledge of the duration and point in time when *Didemnum* is most reproductively active allows managers to select more appropriate times for applying control treatments. Furthermore knowledge of the natural dispersal capacity of Didemnid larvae provides critical information about this species capacity to spread beyond initial source populations, or from external populations to sites where control work is being carried out.

In the U.S. larval recruitment occurred over a 3.5 – 5 month period; however in New Zealand recruitment periods were substantially longer; active recruitment occurred for 9 months and mature larvae were found in colony tissue samples all year round (i.e. including the 3 months where active recruitment ceased and water temperatures dipped below 12 °C, suggesting *Didemnum* has the capacity to recruit year round) (Valentine et al. 2009; Fletcher, Forrest, Atalah, et al. 2013). The initiation and end of recruitment periods are strongly influence by seasonal temperature cycles (for both locations). However critical temperature thresholds for both the onset and cessation of recruitment vary on a local and global scale (Valentine et al. 2009; Fletcher, Forrest, Atalah, et al. 2013). In the U.S. recruitment began between temperatures of 14 – 20°C and ceased where temperatures dropped below 9.8 – 9.3°C at different regional sites. While in New Zealand recruitment onset temperatures were ~13°C and the lowest recorded temperature for recruitment was 11.1°C (Fletcher, Forrest, Atalah, et al. 2013). Upon release tadpole larvae are positively phototactic, swimming towards the light but they then subsequently turn away from the light seeking shaded substrates for settlement (Monniot et al. 1991). Fletcher et al. 2013 found that *Didemnum* "free-swimming larval" stage was considerably longer than initially thought (i.e. substantially longer than the assumed <2 hour period) (Fletcher and Forrest 2011). Larval settlement trials, found that ~56% of larvae had successfully settled and metamorphosed into juvenile recruits after 24 hours (Fletcher and Forrest 2011). A field-based study conducted by Fletcher and Forrest (2011) found that recruitment occurred on settlement plates at 250 m from source populations. Where this information was used alongside exponential decay models results indicated that recruitment could occur beyond 250 m from source populations and possibly even > 1 km where local conditions allow (Fletcher, Forrest and Bell 2013b).

7.2 Map distribution and abundance

No formal survey of *Didemnum*'s distribution has been carried out in Galway bay. Current information on its distribution throughout the bay remains anecdotal. Improving knowledge of the distribution of *Didemnum* would assists with predictions of its spread and would allow the strategic targeting of management actions for its control. Determining the local distribution of *Didemnum* would also allow managers to assess the connectivity between sites and the likelihood of propagule exchange. Where *Didemnum* is locally absent control programmes could refocus on containment efforts (to inhibit and slow the spread of this species).

7.3 Exploit times when invasive species populations are low

To maximise control success treatments can be scheduled so that they corressponds with times where species growth has slowed or ceased (i.e. where overall abundance is limited), during reproductively inactive phases of a species life cycle (Ramula et al. 2008). Treatment application can also be scheduled to coincide with vulnerable life stages of pests, where treatments may have a greater impact on invasive populations (Van Driesche and Bellows 1996; Dauer *et al.* 2012; Wong *et al.* 2012). Early life stages are often more vulnerable to stress than later stages of species development (Qiu and Qian 1998; Gosselin and Qian 1997). This can be due to the physiological strain associated with fast growth during juvenile stages, or other vulnerabilities linked with early life stages (e.g. smaller size, underdeveloped protective structures, behaviours, mechanisms or properties) (Cassis et al. 2011). However for these same reasons, the growth and life stage of non-target species (e.g. native and cultured species) must also be considered when selecting the time at which to apply control treatments (Simmons et al. 2007).

7.4 Monitor the consequences

Monitoring programmes are an essential component of control programmes as they provide feedback so that the programme can be modified or abandoned if it is found to be ineffective. Monitoring could also attempt documenting changes in the distribution and or abundance of an invasive species (Grice 2009). Well-designed monitoring programmes can contribute to knowledge of invader response to treatment effects (Holt and Cordingley 2011). A range of options for monitoring *Didemnum*'s response to treatments are outlined in Section 8 of this report. Monitoring can involve rapid presence/absence surveys to immediately determine if and where *Didemnum* colonies have re-established so that rapid response can be carried out where necessary. Survey methods for determining the effectiveness of treatments on *Didemnum* are also outlined in Section 8.

7.5 Co-ordinated invasive species management

The rate of spread and establishment of marine invasive species is believed to be increasing and aquaculture is a key pathway for its arrival and spread (Carlton and Geller 1993; Ruiz et al. 1999; Shenkar and Swalla 2011; Lambert 2007). As the site will be intensively monitored over the duration of the control programme, this presents an opportunity for early detection of new invasive species introductions. Where possible, if time and resources allow, the control team could familiarise themselves with high impact 'most unwanted' marine invasive species (other than those targeted). Ascidians are a particularly prolific group of invasive species that have demonstrated a high propensity for spread and establishment in new environments (Lambert 2007; Carman et al. 2010; Marins et al. 2010). Should new introductions occur the same methods of monitoring and control could be applied to these potentially functionally similar invasive species.

8. Management plan

Didemnum vexillum management plan

Recommended actions – methodologies & protocols

8.1 Monitoring, baseline survey

1. **Baseline survey – assessing and monitoring the abundance of *Didemnum***

Note: Prior to undertaking survey control work all team members should familiarise themselves with *Didemnum* and its key characteristics. Morphologically similar/frequently confused species include ascidians *Didemnum maculosum* and *Aplidium glabrum*. Identification aids for *Didemnum* are included in the supplementary documents section of this report.

Note: VIRCON tablets should be used to disinfect boots and sampling/control equipment before visiting other oyster sites/marine environments.

8.1.1 Oyster farm

Oyster farm

Note: The site can be divided into smaller more manageable sections/subsections. Different sections (e.g. Section A, Section B) can be based on natural separation of trestle areas (e.g. north/south, see figure 8 below). Trestle rows within each section can be numbered (plastic key rings with waterproof paper labels attached to trestles legs have been previously used). Trestles rows can be further divided into 'start' 'middle' and 'end' sections. Dividing the site into these sections will enable a systematic and efficient method for surveying/ control treatment application.

Figure 7: Mweeloon oyster farm break down of site into distinct sections. E.g. Section A(left) and section (B) right (image obtained from Aquafact Ltd.).

Type: Presence/absence
Note: This survey type should be conducted when (i) assessing the effectiveness of treatments at the end of year 1 (Determine whether treated colonies require further control treatment applications) (ii) for monitoring colony re-establishment in Year 2. (iii) this survey can also be used on other oyster farms to rapidly establish whether *Didemnum* is present or absent.

Timing and frequency
The time and frequency at which surveys are conducted depend on the overall aim of survey.

1. Where the aim is to assess whether any colonies of *Didemnum* are present after control work has been carried out. The survey should be conducted in late January after control work has been completed. It is important to monitor *Didemnum* recovery for at least three weeks, as seemingly destroyed colonies can rebound within this timeframe.

2. Where the aim is to monitor and rapidly detect the establishment of emerging colonies during *Didemnum*'s active growth phase, multiple surveys should be conducted. These should be conducted in early May, late July and late August.

3. Where the aim of the survey is to establish whether *Didemnum* is present on a site (if external sites are surveyed), the survey should be conducted during *Didemnum's* active growth phase (i.e. from April – September) and ideally at peak *Didemnum* growth (i.e. July). During this time *Didemnum* colonies are at their most visually apparent.

Survey methodologies detailed below have been adapted from Kochmann *et al.* (2013) and Miley (2014).

We propose that timed searches are conducted at each of the farm sites. 40 – 45 person minutes should be spent searching for *Didemnum* colonies. This search should include the full suite of artificial and natural substrates present at the aquaculture site and those in its immediate vicinity. Substrates to be inspected include; trestles, oyster bags, rubber bag ties/hooks, other biofouling organisms present on the site (i.e. fouling sea squirts and algae) and any other artificial structures present on the site. Natural hard substrates (e.g. boulders, rocks etc) and sessile organisms (e.g. tube worms) that may have established on the seabed within the farm should also be inspected for the presence of *Didemnum*.

Each line of trestles should be walked along and visually inspected. Where farms are large and time does not allow for the entire farm to be inspected, a random sample of trestles should be selected for inspection. The selection should include trestles on the periphery and at the centre of the farm and in shallow and deeper depths at the site as these represent distinct habitats (i.e. sheltered/exposed, upper/lower intertidal). ~30 – 50 oyster bags should be randomly selected and inspected for fouling. Particular attention should be paid to the underside of oyster bags, as *Didemnum* preferentially settles on shaded surfaces (Monniot et al. 1991).

Location: Oyster farm
Survey type/purpose: Quantification of abundance

Where time allows and greater detail of the abundance at the site is sought/required visual estimates of *Didemnum*'s abundance at site can be made using the SACFOR scale (Connor et al. 2004). Abundance can be determined by assigning sites to one of seven categories (colonies per m^2) to be used are: Superabundant (> 40% / m^2), Abundant (20 – 39% / m^2), Common (10 – 19% / m^2), Frequent (5 – 9% / m^2), Occasional (1 – 5% / m^2), Rare (<1% / m^2) and Absent (0% / m^2).

8.1.2 Oyster bags

Location: Oyster bags
Survey type/purpose: Quantification

Where quantification of *Didemnum* coverage on oyster bags is required a visual estimate, method can also be employed. ~30 – 50 oyster bags should be randomly selected from site (ensuring selection includes various sections and subsections detailed above). Abundance on oyster bags should be collected following methods adapted from O'Brien 2018.

A constructed rectangular quadrat (74 x 52.2 cm, divided with strings into 20 rectangles of 16 x 10.5 cm) matching the shape of the oyster bags (i.e. area available for sampling) can be used to estimate the percentage cover of *Didemnum*. Using a visual estimation method (adapted from Dethier et al. 1993), a score ranging from 1 – 4 should be assigned to *Didemnum* present within each sub-quadrat. A '1', '2', '3' or '4' score should be assigned where *Didemnum* is present in approximately $^1/_4$, $^2/_4$, $^3/_4$ and $^4/_4$ of each sub-quadrat respectively. A '0', score should be assigned where *Didemnum* is absent. Where *Didemnum* occupied less than a quarter of a sub-quadrat (~16 x 10.5), sub-quadrats should be visually divided further into 8ths or 16ths to obtain a more accurate approximation its abundance. Visual estimates should be obtained for each side of oyster bags and combined to get an average percentage cover.

As colonies of *Didemnum* expand, lobes form, growing 3-dimensionally outward from substrates. This is an important aspect of colony expansion and growth and can significantly increase *Didemnum*'s fouling biomass and potentially influence the effects of control treatments. To capture the quantity of this form of growth, the percentage cover of lobed *Didemnum* can also be recorded (using quadrats as described above).

Additional considerations: Given that oyster bags are turned or moved regularly by farmers to limit the accumulation of biofouling organisms, maintain oyster shape or carry out other operational processes (i.e. grading, splitting, harvesting), it will be important to ascertain details of the management practices. Variation in *Didemnum*'s coverage between different farm sites may be due to bag turning schedules or other management practices rather than ecological factors (i.e. invasion stage, local abiotic/biotic factors). As such obtaining information from farmers can supplement data collected and give more complete understanding of the extent of the invasion at different sites.

8.1.3 Natural Environment

Location: Upper intertidal (natural substrates) within the vicinity of oyster farm
Type: Presence/absence

We propose that timed searches are conducted in the upper intertidal area of the farm sites. 40 – 45 person minutes should be spent searching for *Didemnum* colonies in the upper intertidal/ littoral zone directly adjacent to and to the north and south of each aquaculture site. This search should include inspecting algae (*Didemnum* commonly overgrows Fuccoids and *Ascophyllum nodosum*), rocks and boulders and other natural substrates present in the area. Where colonies are found they should be marked so that subsequent control efforts can be targeted, and levels of success monitored. To establish the extent to which *Didemnum* has spread from the oyster farm, timed searches can be carried out at preselected distances (i.e. 50 m, 200 m, and 400 m) from the farm site of interest.

Location: Upper intertidal (natural substrates) within the vicinity of oyster farm
Type: Abundance

Where time allows and greater detail of the abundance is sought/required visual estimates of *Didemnum*'s abundance at site can be made using the SACFOR scale (Connor et al. 2004). Abundance can be determined by assigning sites to one of seven categories (colonies per m^2) based on cover estimated during a timed search (i.e. 5 minutes per 100 x 100 m area): Superabundant (> 40% / m^2), Abundant (20 – 39% / m^2), Common (10 – 19% / m^2), Frequent (5 – 9% / m^2), Occasional (1 – 5% / m^2), Rare (<1% / m^2) and Absent (0% / m^2).

Where greater detail on the abundance of *Didemnum* is required, transects and quadrats can be used to visually estimate abundance. Two 30 x 1 m transects should be placed at each preselected distance (running perpendicularly to the shore line). Along each transect a 1 x 1 m strung quadrat can be placed at two metres intervals, to give 15 quadrats per transect. Within each quadrat, percentage cover of *Didemnum* can be estimated visually using the same methods outlined above (for oyster bags).

8.1.4 Subtidal

Subtidal
Timed searches along transect lines can be conducted in subtidal habitats. Video footage using 'Gopro' or other underwater cameras can aid searches through inspection of footage after field survey. If *Didemnum* is found, its abundance can be visually estimated using SACFOR abundance scale.

Note: Should difficulties/uncertainties with *Didemnum*'s identification arise at any point during survey or monitoring activities high quality images can be taken and sent to taxonomic experts for identification. Where photos are insufficient for confirmation, specimen samples can be taken (following methods outlined in 'preservation protocols' included in supplementary material) and sent to taxonomic experts.

8.2 Control programme year 1

Control programme – management options

Note 1: A survey quantifying baseline levels of *Didemnum* on the oyster farm/oyster bags and natural environment could be conducted prior to commencement of the control programme (ideally during peak growth). This would allow progress to be tracked year on year. Repeat surveys should be conducted at the same time in subsequent years.

Note 2: Recommendations detailed below are separated based on phase of control programme (i.e. year 1 /2), substrate type and point in life cycle (i.e. during *Didemnum's* active growth/dormant phase) where treatments should be applied or other management actions should be taken.

Note 3: Recommendations are made based on the specific life cycle phase that *Didemnum* is in. While particular treatment times are given (see Appendix 1), *Didemnum*'s life cycle is strongly influenced by water temperatures and other environmental factors (i.e. freshwater inputs) (see section 7.1). Therefore 'active life cycle' phases and 'domancy' (i.e. degeneration of colonies) may shift in time in response to the water temperature in any given year (i.e. colony growth may initiate earlier or cease later where seasonal temperatures are higher or lower than average). Where this occurs treatment times may have to be amended to ensure effective control (e.g. if die off is delayed due to warmer autumn/winter temperatures treatments may need to continue until active growth ceases).

8.2.1 Oyster trestles

Recommended Actions – Year 1

Substrate type: Trestles

Life cycle phase: Active growth

General treatment details: Each row of trestles should be visually inspected. Where exposed *Didemnum* colonies are encountered vinegar spray treatments should be applied. A knapsack sprayer (e.g. Solo 473P 10-12 litre or similar) should be used to dispense 5% acetic acid (i.e. household vinegar) treatments. Household vinegar can be directly applied without dilution as it is already a 5% acetic acid solution. Approximately 0.15 ml/cm^2 should be administered to all exposed *Didemnum* colonies on trestles *(see note below). Sprays should be applied as soon after low tide as possible (i.e. once trestles are *emersed*).

***Note:** Where a constant pressure is maintained by continuously pumping the sprayer handle the volume of solution dispense over a specified duration should remain the same. Pilot work can be carried out to determine the time required to dispense the desired volume of solution. In the field timers can be used for treating specific areas of trestle frames.

Timing & frequency: Treatments should be applied at the beginning, middle and end of *Didemnum*'s active growth phase (i.e. three times over the course of its active growth period). This would involve applying treatments approximately once every two months (i.e. once during every fifth spring tide (approx.)). See treatment schedule template in Appendix 1 for further details. (Note: This corresponds to 'Turn and Spray Low Frequency Spread' treatments evaluated in O'Brien (2018), see Section 6 for further details).

Spreading treatments out in this way will allow controls to target and remove remnant (overwintered) colonies, recovering and newly establishing colonies. The capacity of this treatment regime to limit coverage levels at the end of *Didemnum*'s active growth phase will also confer control benefits in subsequent years as the removal of overwintering colonies inhibits reestablishment and expansion in successive years (Stachowicz et al. 2002).

Additional considerations: Difficulties may be encountered when trying to apply spray treatments to trestles. Oyster bags cover large portions of the trestle frames. Removing bags to specifically treat trestles would be labour intensive and time inefficient. Attempts to treat these areas could be co-ordinated with day to day farming operations (i.e. grading, bag splitting or other farming practices where bags are temporarily removed from trestle frames).

At deeper portions of farm sites lower down the shore, trestles may never become fully *emersed* during low spring tides. In this case it is recommended that colonies that lie below the water line at low tide are not treated or removed during *Didemnum's* active growth phase. To do so would likely encourage larval release, colony fragmentation and species spread and consequently undermine control and management efforts overall. Spring tides that bring about extreme low tide conditions may present an opportunity to apply treatments to these hard to access colonies.

Substrate type: Trestles

Life cycle phase: <u>Dormant</u>

General treatment details: At this point vinegar sprays applied during *Didemnum*'s active growth season should have substantially reduced *Didemnum*'s abundance and trestles should be largely free of *Didemnum* fouling. Should treatments applied during *Didemnum*'s active growth phase have been less successful than anticipated there are a number of options that can be pursued during dormancy.

1. If large volumes of *Didemnum* remain on trestles after treatment during active growth season, treatments can continue as above (i.e. application of vinegar sprays during low water, spring tides as outline above (approximately once every two months).

2. Where small patches of *Didemnum* remain targeted spray treatments can be applied to those specific colonies following the treatment schedule outlined. Alternatively, repeat sprays can be clustered (applied once or more during one spring tide, or applied once during consecutive spring tides) until all colonies are fully removed or reduced to a

satisfactory level. Longer periods of trestle exposure will increase the effectiveness of spray treatments.

3. Trestles could be monitored throughout the dormant season and treatments could be applied where colonies remain/become re-established.

All other fouling organisms on trestles (i.e. algae) can be removed by hand/using scrapers (in so far as is practicable/feasible with available resources) during this life cycle period. All material collected from trestles should be placed in sieve buckets with a mesh size that prevents collected organisms from escaping. Material should be brought back to the shore and disposed of appropriately, ensuring that collected organisms do not re-enter the marine environment. Any broken or unused trestles should be removed from site and placed away from the water where they can dry prior to disposal/scrap.

Additional considerations: Difficulties may persist with trestles that are continuously submerged. These trestles should be examined (using a Bathyscope if required) for the presence of *Didemnum* colonies. Spring tides that bring about extreme low tide conditions may present the opportunity to treat these colonies. If no such opportunities occur fouling present that cannot be treated should be removed by hand using scrapers as described above.

Alternative treatments for trestle fouling during *Didemnum*'s dormant phase include PVC wrapping. Plastic wrapping has been used in previous eradication programmes to encapsulate substrates fouled with *Didemnum*. This wrapping creates a barrier effectively cutting off water exchange and consequently inducing anoxic conditions which cause *Didemnum* and other fouling organisms to die. PVC wrapping can be attached to trestle frames with cable ties and PVC tape. Despite the high level of control success applying the wrapping to trestles structures may prove difficult and time consuming. Further details of this control treatment are included in the supplementary material document (details of wrapping methods are outlined in Holt & Cordingly 2011 'Eradication of the non-native carpet ascidian (sea squirt) *Didemnum* vexillum in Holyhead Harbour: Progress, methods and results to spring 2011'.

8.2.2 Oyster bags

Substrate type: Oyster bags

Life cycle phase: <u>Active growth</u>

General treatment details: Oyster bags should be turned and sprayed with vinegar (following the methods outlined above).

Timing and frequency: These treatments should be applied together at the beginning, middle and end of *Didemnum*'s active growth phase (i.e. approximately once every two months; see treatment schedule template in Appendix 1). Spreading treatments out in this way will allow them to act on *Didemnum*'s remnant, recovering and newly establishing colonies, thus maintaining a low level of coverage throughout this active growth phase

Additional considerations: Where possible any bags removed from the site during this life cycle phase (for grading, bag splitting or harvesting processes) should not be returned to the site. A stock of 'clean' replacement bags should be kept and used for oysters after grading/bag splitting activities. Excessive movement of bags for farming practices (i.e. turning, grading etc) during the peak of *Didemnum*'s growth/abundance (i.e. late June/early July) should be limited where possible.

Biofouled bags taken on shore from the intertidal farm can be treated using 5% acetic acid baths (i.e. place bags in large vats of vinegar). Bags should be dipped for ~ 10 minutes and then left away from the shore to desiccate/die off after chemical treatments have been applied. Where possible bags should be left in direct sunlight (causing UV, heat and desiccation stress). Where bags have been pre-treated with vinegar dips, a 2 week air drying period will suffice. Where bags do not receive chemical treatments they should be left to air dry for at least one month (*Didemnum* has been shown to survive for more than 3 weeks on beached barges in New Zealand). These clean bags can then be reused so that a continuous cycle of replacing fouled with clean bags can be maintained.

Vinegar is generally considered to be an environmentally friendly, benign treatment. However this 5% solution has biocidal impacts on both algae and invertebrates. Effects on the wider environment should be given due consideration before applying at large scale (i.e. farm/bay scale). Impacts on oysters should also be monitored closely as studies have reported variable effects on oyster survival after exposure to vinegar spray /dips.

Substrate type: Oyster bags

Life cycle phase: <u>Dormant</u>

General treatment details: During this dormant phase of *Didemnum*'s life cycle fouling levels on bags should be assessed using the visual inspection methods outlined in the monitoring and survey section of this report. Consultation with the farmer will allow the control team to know what bags have been recently turned and where efforts need to be concentrated to remove remnant colonies. A number of options for managing fouling on bags can be considered at this point:

(i) Bag turning and spraying can continue following the same treatment regime used during *Didemnum*'s active growth phase (i.e. once every two months).

(ii) Where treatments have been effective and bags are completely free of fouling or little *Didemnum* fouling remains, vinegar sprays can be targeted at the small volume of remnant colonies and the most heavily fouled side of the bags should remain exposed (i.e. face up). Bags should then be monitored post treatment (following methods outlined in the monitoring section of this report) and retreated with vinegar sprays where necessary.

(iii) Remove & replace; All bags can be collected and removed from the site to allow oysters to be transferred into clean un-fouled replacement bags (if bags have been used on the farm previously ensure that all fouling has died before using). This could be co-ordinated with other farm operations (i.e. grading, bag splitting or harvesting). Fouled bags can be treated in acetic acid baths followed by air drying (as described above).

Additional considerations: (see 'oysters bags – active growth' section above).

8.2.3 Other artificial substrates

Substrate type: Other artificial substrates

Life cycle phase: Active growth

General treatment details: Where other artificial substrates are fouled with *Didemnum*, they can be treated using vinegar sprays and exposure following the same treatment regimes as trestles and oyster bags (i.e. sprays applied to exposed substrates once every two months during periods of spring tide; approximately once during every fifth spring tide).

No attempt should be made to remove other artificial substrates (e.g. hooks, rubber ties, unused/broken trestles) from the farm site during *Didemnum*'s active growth phase. Where possible any additional unused/unnecessary substrates should not be left out on the farm as they provide colonisable substrate area for *Didemnum* and other fouling organisms.

Only loose fragments of *Didemnum* that have detached and are floating/drifting freely should be collected. Collected colonies should be dispose of on land where there is no chance of returning to the sea.

Substrate type: Other artificial substrates

Life cycle phase: Dormant

General treatment details: To limit the availability of colonisable surface area, all unnecessary equipment should be removed from the farm site (i.e. unused rubber ties, hooks, jonks, bags etc.). Where these items are required for reuse they should be placed in acetic acid baths for 10 minutes period and allow to air dry for 2 weeks.

Where substrates cannot be removed, they should be treated in situ with vinegar sprays (following methods outlined above). Repeat spray treatments should be administered if required.

8.2.4 Natural substrates

Substrate type: Natural substrates (upper intertidal)

Life cycle phase: Active growth

General treatment details: Where *Didemnum* has been found fouling natural substrates such as algae, rocks or boulders in the upper intertidal these colonies should be treated with vinegar sprays during *Didemnum*'s active growth phase.

Timing and frequency: Substrates should be treated following application regimes outlined above (i.e. once every two months, approximately once during every fifth spring tide). Again sprays should be applied during spring tides to increase duration of immersion of substrates.

Additional considerations: Plots where colonies have established should be marked in manner which will allow easy relocation for monitoring and retreatment. Notes can also be made for each established colony to assist with locating when required.

Substrate type: Natural substrates (upper intertidal)

Life cycle phase: <u>Dormant</u>

General treatment details: Where acetic acid sprays have been applied to natural substrates and colonies still remain (even minute colonies) retreatment sprays should be applied. Given the complexity of natural substrates vinegar sprays may not be effective on some algal/invertebrate species. *Didemnum* fouled species can be physically removed during inactive growth phase (using blades or scrapers). Any material collected should be disposed of in an appropriate manner ensuring that colonies do not re-enter the marine environment.

Timing and frequency: Where large volumes of *Didemnum* remain on natural substrates treatments can be applied following the same treatment regime used during *Didemnum*'s active growth phase (i.e. once every fifth spring tide). Alternatively spray treatments can be applied and reapplied to target specific colonies fouling natural substrates. Physical removal of material can be carried out during this period.

Additional considerations: Plots where colonies have established should be marked in manner which will allow easy relocation for monitoring and retreatment. Notes can also be made for each established colony to assist with locating when required.

A rapid monitoring survey may be conducted prior to the end of Year 1 to locate and treat any remnant colonies. Depending on the level of success from control activities carried out in Year 1 control activities, colonies of *Didemnum* may not be apparent on the site.

8.3 Control programme year 2 - ongoing

Recommended management actions - Year 2

Year 2 and onwards

1. The overall aim for Year 1 control work was to substantially reduce fouling through the implementation of an intensive control programme. In Year 2 and onwards the aim of control will be to maintain this newly achieved low abundance level by carrying out monitoring and continuing to apply control treatments (using techniques and treatment schedules outlined in the 'year 1' section of this report) and incorporating best practice management methods into the day to day running of the oyster farm as well as implementing other *Didemnum* control methods where necessary.

2. Prior to the commencement of 'Year 2' treatments, monitoring surveys should be conducted to determine whether any colonies remain on any of the various substrate types on and within

the surrounding area of the farm. If colonies are found at relatively low abundances treatments can be targeted and applied immediately to remove discrete populations.

3. Evaluating the level of success of the 'Year 1' control programme should be carried out to determine whether treatments were effective and if the same programme can be implemented again to achieve similar results. Surveys to determine abundance levels should be conducted during periods of peak *Didemnum* abundance (i.e. mid-late July). If data has been collected for this period on the site prior to the commencement of control treatment applications, comparisons can be made and the level of success can gauged directly. However where this information is unavailable, the level of success can be evaluated by assessing whether treatments have achieved the aims and goals of the project. These aims should be made clear when devising the programme (e.g. setting specific thresholds for the acceptable level of *Didemnum* abundance).

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10. Appendices

Appendix 1. Sample treatment schedule details for *Didemnum* control programme, showing number of tides, month, date and total number of days elapsed from initial treatment. Treatment type, associated frequencies, variances and timings are shown. Assessment times and response variables assessed over the course of the experiment are highlighted (filled arrows). Grey shaded boxes indicate the application of treatment. Numbers within those boxes denote the number of applications received (up to and including that point in the control programme) out of the assigned total number of applications for the treatment (i.e. Low frequency total = 3), (ammended from O'Brien, 2018)

							Treatment Time 1 MAY				Treatment Time 2 JULY			Treatment Time 3 AUGUST	Assessment evaluate treatment effects
Treatment type	Frequency	Spring tide #		Spring Tide 1	Spring Tide 2	Spring Tide 3	Spring Tide 4	Spring Tide 5	Spring Tide 6	Spring Tide 7	Spring Tide 8	Spring Tide 9	Spring Tide10	Spring Tide 11	Approx. end of active growth phase (may vary according to water temperatures)
		Month		Mar/Apr	Apr	Apr/May	May	May	June	June	July	July	Aug	Aug	Sept
		Date		29th - 3rd	14th - 18th	26th - 1st	13th - 18th	27th - 30th	12th -17th	27th – 29th	12th - 17th	27th - 30th	9th - 15th	25th - 29th	9th - 15th
		Days		1 – 6	17 - 21	29 - 34	46 - 51	60 – 63	76 - 81	91 - 93	106 - 111	121 - 124	134 - 140	150 - 154	164 -171
		Variance	Timing												
Turn & Spray	Low	Spread	Through-out growth season	1/3					2/3					3/3	