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# Potential Eradication and Control Methods for the Management of the Ascidian Didemnum perlucidum in Western Australia

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Cover photograph: Colonies of introduced ascidians *in situ*: *Didemnum perlucidum, Botrylloides violeaceous* and *Botryllus schlosseri*


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# Glossary of tunicate terminology

**Table 1.** Common terms related to ascidian (tunicate) morphology and taxonomy (Rocha 2011).

| Term | Definition |
|---|---|
| Abdomen | Present in colonial ascidians with two or three body regions. It is the part of the animal that includes the digestive tract or oesophagus. In some groups, also includes gonads and the heart. It is posterior to the pharynx. |
| Ampullae | Present in larval and adult colonial ascidians and some larvae of solitary ascidians, grown from the epidermis of the body and are used to attach the animal to the substratum. |
| Adhesive cones | Outer adhesive structures of a larval ascidian used to adhere to a substrate |
| Branchial sac | Perforated sac where respiration and food filtration occurs. It is equivalent to the pharynx in vertebrates. |
| Bud | Undeveloped or embryonic zooid that develops asexually. |
| Budding | Type of vegetative reproductions; may occur in different forms in ascidians |
| Cloacal canal | A channel in the tunic linking the atrial openings of several zooids in a colony. |
| Colonial ascidian | Ascidian with vegetative reproduction, and the individuals originated remain united through the tunic. |
| Colony | Set of several zooids originating from a larva that remains united by the common tunic. |
| Egg | Diploid cell resulting from the fusion of gametes (e.g. ova and sperm) |
| Egg brooding | Retention of eggs in the parent individual until fertilisation. |
| Gonads | Reproductive organs. |
| Larva (pl. larvae) | Stage of the life cycle that is an immature free-swimming form (natant). |
| Larval trunk | Body of the larva, excluding the tail |
| Oesophageal region | Narrow region between the thorax and abdomen, formed by the oesophagus and the rectum, only in colonial ascidians. |
| Ovary | Female gonad |
| Solitary ascidian | An ascidian consisting of a single individual |
| Spicules | Structures made of calcium or aragonite which provides support and strength to the tunic. In the family Didemnidae they are star shaped or spherical |
| Stigmata | Perforations in the branchial sac, with ciliated margin |
| Testis | Male gonad |
| Tunic | Tissue with different concentrations of tunicine fibres (similar to cellulose), blood cells and connective cells surrounding the animal |
| Zooids | Each individual in a colony |

## Other terms used in this text

**Table 2.** List of important terms presented in this review.

| Term | Definition | Reference |
|---|---|---|
| Control | Reduction of a population density. | Genovesi (2011) |
| Containment | To limit the spread of a species by containing its presence within defined geographical boundaries. | Genovesi (2011) |
| DNA barcoding | A tool that uses one or more short gene sequences taken from a standardised portion of the genome and is used to identify species through reference to DNA sequence libraries or databases. | Kress and Erickson (2012) |
| Eradication | Elimination from a site of all individuals of a species (100% mortality). | Genovesi (2011) |
| Polyculture | Polyculture in aquaculture is the association of fish species of different food habits for the effective use of available fish foods in a pond, where wastes produced by one species may be inputs for other species. | Milstein (2005) |

## Executive summary

In Western Australia, the invasive colonial ascidian *Didemnum perlucidum* was first detected in the Swan River Estuary in 2010 and subsequently recorded at multiple locations across the State. Ongoing research by the Department of Fisheries (the Department) indicates that this species exhibits strong seasonal changes in abundance. Although, *D. perlucidum* is able to reproduce sexually (larvae) and asexually (budding) year round, larvae density and settlement, and overall colony size significantly decreases during the colder months (winter). From a management point of view this period would present the best opportunity for an attempt at eradication or control of this species.

Currently there are no eradication or control methods for *D. perlucidum* mainly due to its wide distribution and/or unrecorded negative impacts in introduced locations (e.g. USA). However, this report provides a summary of various methods used to treat artificial and natural substrates affected by introduced colonial and solitary ascidians. Several management methods have been used for the eradication and control of ascidians, which can be classified into three broad categories: chemical, mechanical and biological.

1. Chemical methods involve the use of chemical compounds such as bleach, vinegar, lime, freshwater, sodium hydroxide, and others to kill the target species.
2. Mechanical methods involve the deployment of physical barriers with the aim of promoting unsuitable conditions for the survival of the target species (e.g. low oxygen concentrations) or physical removal (e.g. hand picking) from the fouled surface.
3. Biological methods involve the use of a control agent (i.e. another organism) with the aim of decreasing the abundance of the target species. However, this method of control is poorly understood and tested for colonial ascidians.

Few programs have been previously implemented for the eradication of introduced ascidians. There are many cases where eradication was not feasible due to lack of good understanding of the target species, local conditions, and lack of funds, etc. When implemented, no program was able to achieve eradication of the target species. However, every attempt, irrespective of the outcome, has enhanced the development of new treatments and the identification of critical success factors that dictate the overall outcome of such programs.

The most effective eradication method identified so far is a combination of chemical and mechanical methods. Structures fouled with colonial ascidians can be encapsulated or wrapped and concentrated vinegar (acetic acid 20%) pumped into the encapsulation. This method is cost effective and relatively easy to use if applied correctly and if anoxic conditions are achieved under the wrap. However, the encapsulation method (with or without chemical addition) is non-target specific and cannot be used to control ascidian fouling on cultured organisms (i.e. mussels) or in high value areas (i.e. where there are protected species) without adversely affecting other species. In these situations, alternative methods can be used such as dipping or spraying mussel lines with a chemical or physically removing the target species. Results from previous attempts to manage ascidian species other than *D. perlucidum* have indicated that eradication is unlikely to be achieved and that these control methods often result in high mortality of farmed species. As a last resort or if the level of infestation is very high, fouled structures, vessels or aquaculture gear can be removed from the water and air dried. However, this process has been shown to be extremely expensive and time consuming, and stakeholders were often reluctant to take this action.

It is clear that key factors need to be met prior to any attempt at eradicating a colonial ascidian such as *D. perlucidum* in WA. These key factors are as follows:

1. Good knowledge of the distribution, biology and ecology of the target species.
2. Good knowledge of the geographic and temporal variations of the area to be treated.
3. The commitment of funds for the entire control program and ongoing monitoring surveys.
4. The establishment of efficient lines for decision making and effective communication with stakeholders, agencies and general public.

## Current statement on the incursion of *Didemnum perlucidum* in WA

On 16/12/2013 02:46 PM, Victoria Aitken wrote:

The position currently on *Didemnum perlucidum* is this:

Until recently the Department treated *D. perlucidum* like any pest listed on the Western Australian Prevention List for Introduced Marine Pests (2013). Inclusion on this list means that certain management responses should be followed by vessel operators when a pest is found - including reporting all suspected and confirmed detections to the Department as soon as possible.

Unfortunately, *D. perlucidum* is now confirmed in several locations around the coast of WA. As a consequence, the Department has revised the policy and the management response for this species is now to manage this pest only for high value asset areas. These areas are considered to be State marine parks, lands and waters adjacent to A class reserves, pearling and aquaculture facilities, and ports.

Thus, the Department proposes to provide the following advice for all stakeholders:

- *D. perlucidum* remains listed as a marine pest and suspected and confirmed detections should still be reported so its distribution can be tracked.
- If moving vessels or immersible equipment into, or adjacent to, high value assets areas as mentioned above, stakeholders are requested to comply with any specific Departmental management advice regarding *D. perlucidum*. Actions may include ensuring vessels, or immersible equipment, are clean before entering these areas.

Furthermore, proponents with marine pest conditions within a Ministerial Statement under the Environmental Protection Act 1986 will still be required to undertake actions as per their relevant Statement.

The Department recommends to the OEPA the following specific response actions for *D. perlucidum* found within project areas:

- Establish the extent of the species within the project (i.e. through a delimiting survey); and
- Report any suspected or confirmed detections of the species.

Further action is likely only to be required if infested vessels and equipment are planning to be moved into or near high value asset areas, as mentioned above. If clarity is required, stakeholders will be able to seek guidance from the Department.

Regards,

Victoria Aitken

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# 1.0 Introduction

The introduction of ascidians to natural and artificial substrates in tropical and temperate environments is now commonplace (Lambert 2002, Minchin and Sides 2006, Valentine *et al.* 2007, Lengyel *et al.* 2009). Once introduced, ascidians have the potential to negatively affect the biodiversity and ecological functions or economy of the recipient location (Lengyel *et al.* 2009, Morris and Carman 2012). For example ascidian fouling in Brazil and New Hampshire has been linked to a reduction of growth and increased mussel mortality, resulting in reduced farm productivity and economic losses for the shellfish aquaculture industry (Rocha *et al.* 2009, Auker 2010).

Colonial species, from the family Didemnidae provide many examples of negative impacts. For example, *Didemnum vexillum* has had a detrimental impact on pre-existing sessile hard surfaces communities where it was introduced. Negative impacts include the decline of the brittle star *Ophiothrix fragilis* and the sea urchin *Psammechinus miliaris* (Minchin and Sides 2006), while *Diplosoma listerianum,* another member of the this family, was able to change the community structure in the North Sea (Vance *et al.* 2009).

The origin of *D. perlucidum* remains uncertain. It was first recorded from Guadeloupe Island in the Caribbean but apparently is of Atlantic origin (Monniot 1983). Furthermore, the data available in the literature is often contradictory. For example, Perry (2012) suggests that *D. perlucidum* is native to Guam and Palau, while Golbuu *et al.* (2005) and Lambert (2002, 2003) indicate that this species was introduced to these locations. This confusion is most likely due to the difficulty in identifying this species and lack of historical records.

In 2010, *D. perlucidum* was recorded for the first time in the Swan River Estuary, Perth, Western Australia (WA) fouling settlement plates (Smale and Childs 2011). Since then this species has been found widely distributed across the State, growing on artificial and natural substrates. However, no control or eradication has been attempted for this ascidian. This report provides a review of current management measures used for the eradication and control of introduced colonial and solitary ascidians (Refer to Appendix 1 for a list of species presented in this review), focusing on strategies previously used for the management of the colonial ascidian *D. vexillum*.

# 2.0 *Didemnum perlucidum* characteristics

## 2.1 Common names

A white crust ascidian, white sea squirt, colonial ascidian, compound sea squirt, leather squirt.

## 2.2 Similar species

*D. perlucidum* have been previously misidentified or often confused with other Didemnid species (many native to WA) including: *D. perplexum, D. granulatum, D. cineraceum, D. candidum* and *D. psamathodes* (Monniot 1983, Rocha and Monniot 1995, Kott 2001).

## 2.3 Taxonomic classification

Classification as per Gittenberger *et al.* (2012).

| | |
|---|---|
| Kingdom | Animalia |
| Phylum | Chordata |
| Subphylum | Tunicata |
| Class | Ascidiacea |
| Order | Aplausobranchia |
| Family | Didemnidae |
| Genus | *Didemnum* |
| Species | *Didemnum perlucidum* |

## 2.4 Distribution

*D. perlucidum* was first described from Guadeloupe Island in the Caribbean (Monniot 1983) and subsequently recorded in Brazil (Rocha and Monniot 1995, Rocha *et al.* 2005), West Africa (Monniot and Monniot 1997), the Gulf of Mexico (USA) (Felder and Camp 2009) and the Indo-Pacific (Monniot *et al.* 1991), including Hawaii, Guam, New Caledonia and the Pacific entrance to the Panama Canal (Perry 2012).

*D. perlucidum* is classified as a tropical colonial ascidian which grows on multiple substrates (Rocha and Monniot 1995). In the Caribbean, where this ascidian is considered to be a native species, it grows in low densities and is associated to coral reefs and mangroves (Rocha *et al.* 2009, Kremer *et al.* 2010). In introduced locations, *D. perlucidum* is commonly associated with disturbed habitats (e.g. marinas and harbours and aquaculture facilities) (Kremer *et al.* 2010) where it can heavily foul artificial substrates including buoys, ropes, pylons and vessels (HARC 2012). In these environments, this ascidian is usually observed growing on other organisms such as polychaete tubes, algae, corals, barnacles and solitary ascidians and is frequently associated with other introduced ascidians (e.g. *Styela plicata*) (Kremer *et al.* 2010, 2011).

*D. perlucidum* has also been described as a common fouler in shellfish farms, where it can heavily grow over mussels such as *Perna perna* (Rocha *et al.* 2009), *Mytilus edulis* (Glen Dibbin 2012 pers. comm.) and *Pinctada* oysters (Baptista *et al.* 2007). Interestingly, in Brazil,

*D. perlucidum* was reported as one of the most common fouler on oysters (Baptista *et al.* 2007) but not for cultivated mussels (Rocha *et al.* 2009). The ability of *D. perlucidum* to heavily foul certain cultured bivalves and the actual effect on farm productivity requires further investigation.

*D. perlucidum* has been recorded in natural and artificial marine environments in WA from Busselton to Broome (Department of Fisheries WA 2013) and the Northern Territory in Darwin and surrounding coastal waters (Robert Rose 2012, pers. comm.). In WA this ascidian can survive temperatures between 15 and 30 ºC (CSIRO 2003) and has been recorded at depths of up to 8 m (B. Tilley 2013, pers. comm.). However, it is commonly found in the upper 1–3 m of the water column (Muñoz *et al.* 2013, unpublished data).

## 2.5 Description

Descriptions in the literature demonstrate the plasticity of *D. perlucidum's* morphological and anatomical characteristics. Although diagnostic characteristics (necessary for taxonomic identification) are well defined for this species it is often difficult to find all of them in the same individual. Hence, taxonomic identification of *D. perlucidum* requires extensive observation and in many cases needs to be supported by molecular identification tools (e.g. DNA barcoding).

***External appearance***

The external colouration of ascidians, especially of colonial ascidians, should not be used as an indicator of species identity. For example in WA, the colouration of *D. perlucidum* colonies varies from white to grey, brown, pink or reddish coloration, which indicates that these features are not reliable characteristics for the identification of this species in the field.

The shape of *D. perlucidum* colonies is also variable; it is due to the number of spicules present in its tunic (Rocha and Monniot 1995) and the shape of the fouled substrate (e.g. tube worms) (Mike Page 2013, pers. comm.).

Colonies of *D. perlucidum* form very thin sheets of no more than 2 mm thickness (Monniot *et al.* 1991) and can extend several centimetres in diameter (10 cm) (Monniot 1983, Perry 2012). It has been suggested that the thickness of the sheet is also related to the reproductive state of the colony with thicker colonies occurring when the colony is in its most reproductive state (Perry 2012). This characteristic has been observed for *D. perlucidum* populations in WA with reproductive colonies being thicker at higher larvae densities (Muñoz *et al.* 2013, unpublished data).

***Internal appearance***

Zooids are variable in length measuring up to 1.8 mm (Monniot 1983) and are characterised by the presence of 6–7 stigmata in the first and second row of the branchial sac. The testis is undivided and the sperm duct coiled in 7–8 turns (Monniot 1983, Kott 2001). For an extensive anatomical description of *Didemnum perlucidum* refer to Monniot (1983).

Larvae of *D. perlucidum* are relatively small compared to other *Didemnum* species. They are distinguished by the presence of four large ampullae and three adhesive papillae measuring up to 500 µm in diameter (Figure 1A). Larvae are cylindrical but occasionally flattened with a larval trunk 0.45 mm long. Usually the tail winds only two thirds of the way round (Kott 2001) but sometimes it can be completely wound around the larva (Monniot 1983). These characteristics are only visible with the aid of a microscope using correctly preserved and sexually reproductive specimens, highlighting the need for adherence to specific sample collection and preservation procedures.

*D. perlucidum*'s spicules are always present and commonly found in the superficial layers of the tunic (Rocha and Monniot 1995). They are star shaped with 12 short rounded conical rays (Figure 1B) evident in a visible plane (Monniot 1983); they are relatively small and can vary from 20–40 µm diameter (Monniot 1983, Rocha and Monniot 1995).

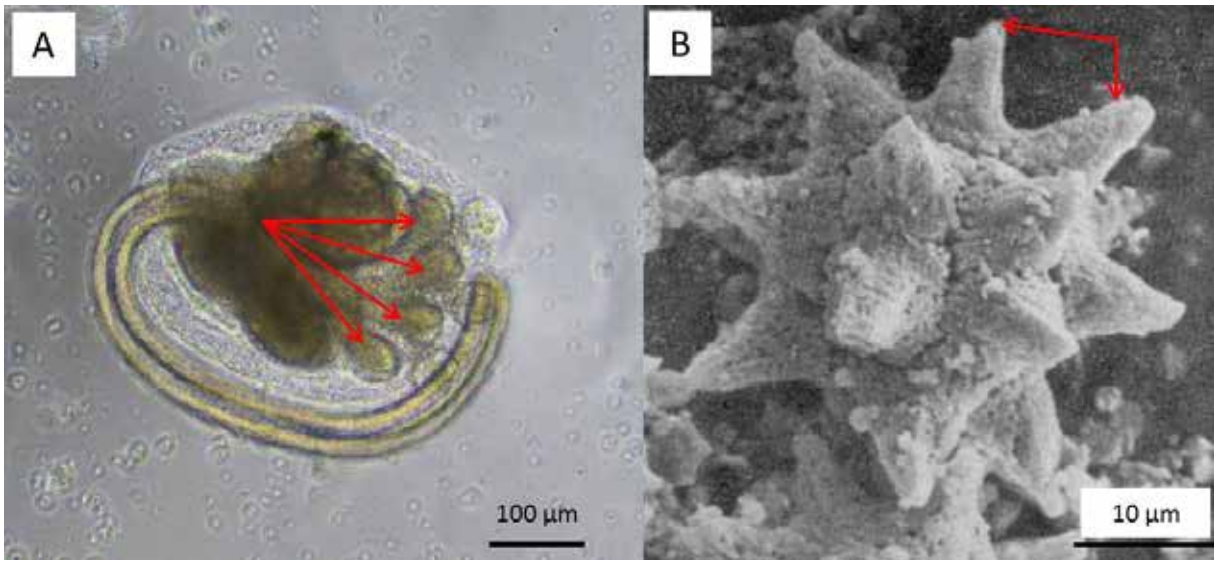


**Figure 1.** **A.** A fully developed *Didemnum perlucidum* larva with four ampullae (red arrows), coiled tail and three adhesive papillae. **B.** Photograph of a spicule taken with a scanning electron microscope (SEM) showing 12 rounded conical rays (red arrows) (photograph by Monniot 1983).

## 2.6 Reproduction

Despite continual reproduction throughout the year, the growth and reproductive efforts of *D. perlucidum* are greater in warmer months with a decrease in colony size or retraction during colder months (Kremer 2008, Kremer *et al.* 2010, Muñoz *et al.* 2013 unpublished results).

An ascidian colony consists of individual elements called zooids. Each zooid is hermaphroditic, presenting male and female gonads in the same individual, and can reproduce both sexually and asexually (Monniot *et al.* 1991).

Literature suggests that *D. perlucidum* is sexually mature within a few weeks of larval settlement (HARC 2012). *D. perlucidum* is highly fecund and has been shown to breed throughout the year releasing large numbers of mature larvae daily. In Santa Catarina, Brazil peak larval release was in March (summer season) when all colonies were reproductive and produced up to 42.7 larvae $cm^{-2}$. In contrast larval production was lowest during October (winter) when only 30 % of colonies were reproductive and produced only 8.9 larvae $cm^{-2}$ (Kremer *et al.* 2010).

### *Sexual*

In *Didemnum* species fertilisation is internal with embryos brooded within the colony (Monniot *et al.* 1991). Larvae are then released into the water column as free swimming larvae (in a process called spawning) as a response to light stimulation (Fletcher and Forrest 2011). However, larvae have only a very short-range active dispersal capacity, commonly settling only a few metres from the parent colony. For *D. perlucidum* it is unknown how long larvae swim prior to settlement, however, for *D. vexillum* larvae swim for approximately 48 hr before settling and

undergoing metamorphosis (Fletcher and Forrest 2011). This process continues until a colony is fully established (Figure 2). These reproductive characteristics haven't been described for *D. perlucidum* in Australia or elsewhere.

### *Asexual*

Clonal propagation of colonies via budding regeneration (asexual) is common in the Didemnidae family (Kott 2001). It is known that a few weeks post-settlement, *D. vexillum* recruits can undergo asexual budding and divide into a two zooid colony. It has been suggested that this method of reproduction plays a major role expanding the size of a *D. vexillum* colony under favourable conditions (e.g. food, space availability) (Dias *et al*. 2008).

Budding has also been described for *D. perlucidum* (Dias *et al.* 2008). However, it is not known how the mechanism of budding aids in the spreading of *D. perlucidum*. Ongoing research on *D. perlucidum* in WA has confirmed that budding regeneration occurs in the oesophageal region, and that both types of reproduction (sexual and asexual) can occur at the same time (Muñoz *et al.* 2013, unpublished data)(Figure 2). This alternation between sexual and asexual reproduction appears to be a significant colonising strategy for this ascidian. It has also been determined that in temperate waters of WA (Hillarys), *D. perlucidum* is reproductive throughout the whole year with the egg/larva density being greatest during the warmest months in summer and autumn. Whether this is the case for tropical populations in WA (Dampier to Broome) or lower latitudes (Busselton) is yet to be investigated.

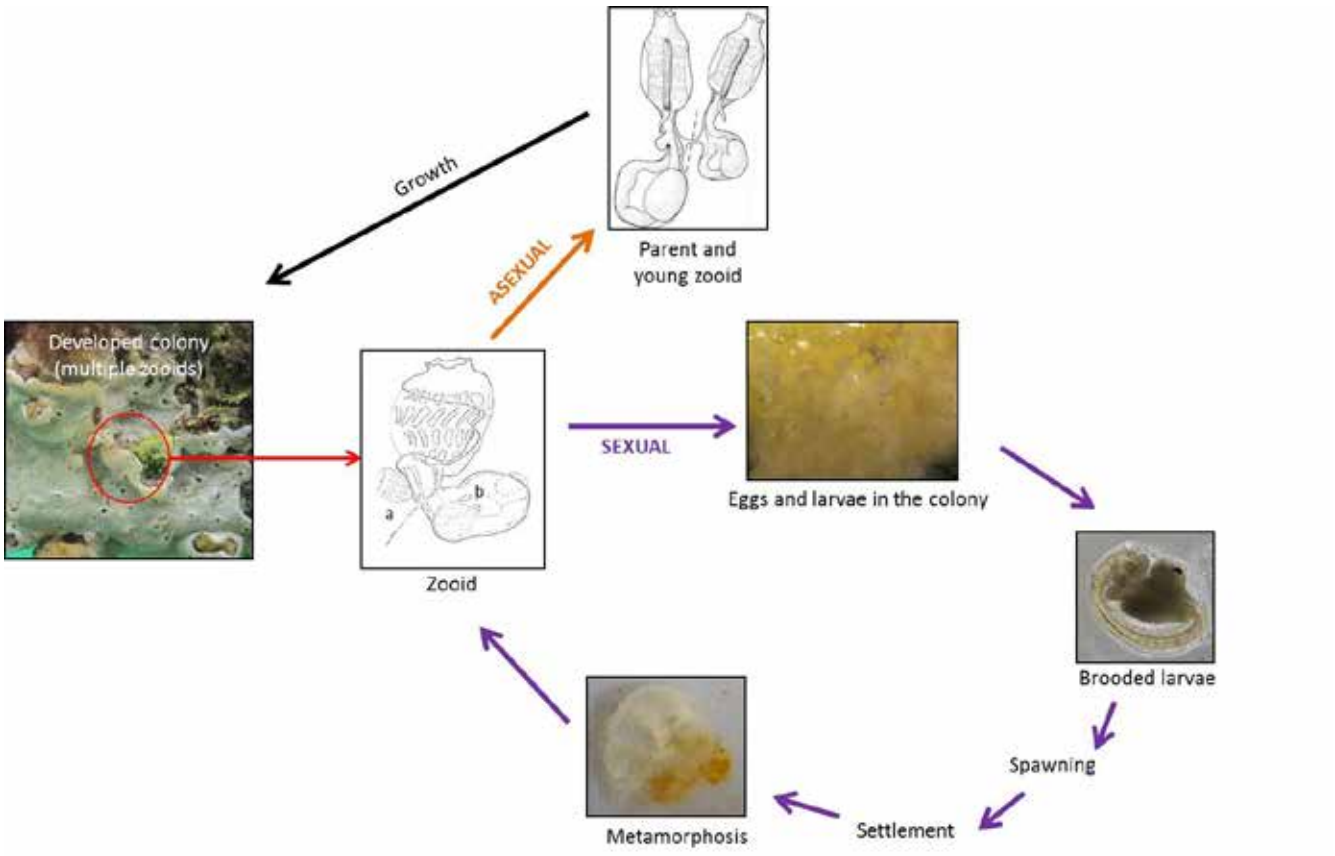


**Figure 2.** *Didemnum perlucidum* life cycle. A reproductive zooid (black and white diagram) shows a bud arising from the oesophageal region (a) and gonads (b) present in the abdomen. (Black and white diagrams by Kott 2001).

## 2.7 Impacts

*D. perlucidum* is considered a species with the potential to be highly invasive due to its high reproductive output, fast growth rate, and ability to occupy disturbed environments (Kremer 2008). This high capacity for colonisation has been observed in WA (Smale and Childs 2011) and USA (Lambert 2009). In 1997, an oil rig was cut in half and sunk in the Gulf of Mexico. The rig was surveyed 14 months later and it was observed that *D. perlucidum* covered most of the rig superstructure that had previously been out of water (Lambert 2009).

*D. perlucidum* has also been recorded as a heavy fouler at a local mussel farm in south-west WA and is anecdotally suspected to increase the mortality of farmed mussels spat by up to 80% (Glenn Dibbin 2012, pers. comm.). These fouling characteristics suggest that this species could outcompete local fouling populations.

## 3.0 Management of colonial and solitary ascidians

Before attempting an eradication or control program within a localised area, the first step should be to undertake a delimiting survey to understand the distribution of the species and the scale of impact. From there decisions can be made on the aims (eradication or control), whether the impacts of the species and the removal techniques are such that any management should be attempted, and the identification of specific and realistic spatial boundaries to be treated. For example, eradication of a 2 $m^2$ area in a national park at the time of first detection followed by ongoing controls and surveys to reduce the likelihood of reinfection. In New Zealand (Pannell and Coutts 2007) and Great Britain (Cook 2010), eradication programs for *D. vexillum* followed an order of management prioritisation from 1 to 4 where 4 resulted in no action:

1. Eradication in high-value areas (e.g. aquaculture areas).
2. Eradication in areas with only low levels of infestation that can be easily treated but has the potential of developing into large infestations.
3. Eradication of large infestations of artificial and natural areas.
4. Large infestations where eradication and control is not feasible, thus no action can be taken.

If eradication and/or control of *D. perlucidum* are to be attempted in WA then clearly established objectives and aims should be agreed prior to the implementation of any program including agreement on funding and ongoing monitoring (refer to Pannell and Coutts 2007 and Coutts and Forrest 2007 for specific details on this approach).

# 4.0 Control and eradication methods

Control methods for invasive species are classified into three broad categories: chemical, mechanical and biological, with combinations of methods (e.g. chemical and mechanical) also possible.

## 4.1 Chemical methods

Chemical control refers to the use of specially formulated chemical compounds to kill or control invasive species intended for the prevention, destruction, repulsion, or mitigation of any pest. Common chemicals aimed at reducing the biomass and cover of colonial and solitary ascidians on aquaculture species/infrastructure are outlined in Table 3. Additional treatments used for the management of the solitary ascidian *S. clava* include citric acid, formalin and detergents (Le Blanc *et al.* 2007) but no information on the treatment time and concentration was provided. These methods consist of dipping and/or spraying mussel lines or oyster cages with the chemical. So far no chemical treatment tested for the management of ascidian fouling has been proven to be successful in eradicating (100% mortality) the target species (i.e. *D. vexillum, Botryllus schlosseri, Botrylloides leachi*). One of the major constraints with this method is the high mortality observed in the farmed species (mussels and oysters). However, the two treatments that best managed to control *D. vexillum* biomass were dipping the lines in a solution of bleach (0.5%) for 2 min (farmed species, *P. canalicula*. Denny (2008) and for oysters, dipping the lines in a solution of hydrated lime (1–5%) for 5 min (farmed species, C*rassostrea gigas,* Roldheiser *et al.* 2010). The latter treatment resulted in the greatest reduction of *D. vexillum* biomass (>90%) and maximum survival rate (>80%) of the farmed species (oysters in this case) (Roldheiser *et al.* 2010).

Chemical methods can be also used in combination with mechanical methods to accelerate the process and increase the likelihood of eradication (e.g. addition of acetic acid to a wrapped pylon) (Pleus *et al.* 2008). For example structures such as pylons and floating pontoons can be wrapped with an impervious material and simultaneously treated with acetic acid in concentrations from 5–20%. For *D. vexillum* the best results to date have been achieved using high concentrations of acetic acid (20%) (Pannell and Coutts 2007). Acetic acid is cheap and presents a low environmental risk (highly soluble in water) compared to other chemicals. A permit to utilise any chemical for eradication purposes might be required, especially for high value assets, as its use might cause negative impacts on the environment.

**Table 3.** Chemical treatments used to control colonial and solitary ascidian fouling in aquaculture facilities.

| Target species | Farmed species | Method | Treatment concentration | Dipping times | Rinse | Effectiveness (best result) | Location tested | Season | Study type | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| *Didemnum vexillum* | *Perna canalicula* | Freshwater | NA | 2, 5, 10 min | No | High mussel mortality | New Zealand | Unknown | Field | Denny (2008) |
| *D. vexillum* | *P. canalicula* | Acetic acid ($C_2H_2O_2$) | 0.1, 0.5, 1, 2, 4, 10% | 1, 2, 3, 5, 10 min | Yes | High mussel mortality | New Zealand | Unknown | Lab/field | Denny (2008) |
| *D. vexillum* | *P. canalicula* | Bleach (NaClO) | 0.1, 0.25, 0.5, 1% | 30 s and 2 min | | 0.5% bleach @ 2 min with max. survival of farmed species, eradication not achieved | New Zealand | Unknown | Lab/field | Denny (2008) |
| *D. vexillum* | *P. canalicula* | Calcium oxide (CaO) | 5, 10% | 20s and 2 min | Yes | High farmed species mortality | New Zealand | Unknown | Lab/field | Denny (2008) |
| *D. vexillum* | *P. canalicula* | Sodium metasilicate ($Na_2O_3Si$) | 3, 6% | 21 s and 2 min | Yes | High farmed species mortality | New Zealand | Unknown | Lab/field | Denny (2008) |
| *D. vexillum* | *P. canalicula* | Sodium hydroxide (NaOH) | 3, 6% | 22 s and 2 min | Yes | High farmed species mortality | New Zealand | Unknown | Lab/field | Denny (2008) |
| *D. vexillum* | *Crassostrea gigas* | Hydrated lime ($CaOH_2$) | 4% | NA | NA | Reduction in cover of target species but subsequent increase of other foulers' biomass due to space availability/ reduction in oyster survival | Vancouver, Canada | Jul–Nov | Field | Switzer *et al.* (2011) |
| *D. vexillum* | *C. gigas* | Brine | 40, 50, 70 ppt | 0.5, 1, 5, 10 min | Unknown | Reduction of *D. vexillum* cover | Tofino, Canada | Winter | Lab/field | Roldheiser *et al.* (2012) |
| *D. vexillum* | *C. gigas* | Freshwater | 0, 5, 20 ppt | 0.5, 1, 5, 10 min | Unknown | Reduction of *D. vexillum* cover | Tofino, Canada | Winter | Lab/field | Roldheiser *et al.* (2012) |

| Target species | Farmed species | Method | Treatment concentration | Dipping times | Rinse | Effectiveness (best result) | Location tested | Season | Study type | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| *D. vexillum* | *C. gigas* | Hydrated lime ($CaOH_2$) | 1, 2, 4% | 0.5, 1, 5, 10 min | Unknown | Best method with best survival of farmed species (>80%) 1-5%/10 min and 4%/5 min | Tofino, Canada | Winter | Lab/field | Roldheiser *et al.* (2012) |
| *D. vexillum* | *C. gigas* | Acetic acid ($C_2H_2O_2$) | 0.25, 1.25, 5% | 0.5, 1, 5, 10 min | Unknown | Reduction of *D. vexillum* cover | Tofino, Canada | Winter | Lab/field | Roldheiser *et al.* (2012) |
| *D. vexillum* | NA | Sodium hydrochloride (HClNa) | concentrated | NA | NA | Potential high mussel mortality | Ireland | NA | Unknown | Kelly and Mcguire (2008) |
| *Ciona intestinalis* | mussels | Virkon® Aquatic | 0.5 – 5% | 30 s | NA | 89% of *C. intestinalis* biomass reduced | Canada | Unknown | Unknown | Paetzold and Davison (2011) |
| *Ciona savignyi* | NA | Ubiquinone-10 Spermidine Muscimol Radicicol Polygodial | Multiple concentrations | NA | NA | Highest mortality of target species achieved with radicicol and polygodial | New Zealand | NA | Lab | Cahill *et al.* (2012) |
| *C. savignyi* | *P. canalicula* | Ubiquinone-10 Radicicol Polygodial | Doses calculated at $IC_{99}$, mussels exposed for 42 days | NA | NA | Ubiquinone 10 and polygodial did not affect *P. canalicula* and inhibit settlement and metamorphosis of *C. savignyi* | New Zealand | NA | Lab | Cahill *et al.* (2013) |

## 4.2 Biological methods

Biological control agents are used to decrease a pest's competitive advantage over native organisms and to weaken the invading population by increasing pest mortality, and/or limiting population expansion, size and reproduction (Pleus *et al.* 2008).

Ascidians have few known predators with most predation occurring during the larval stage or very shortly after settlement and metamorphosis (Pleus *et al.* 2008). Few publications refer to the use of biological control agents for the control or eradication of ascidians and those that do have limited value due to uncontrollable effects encountered during the experimental phase. Switzer *et al.* (2011) tested the effect of the sea urchin *Strongylocentrotus droebachiensis* on oysters (*C. gigas*) heavily fouled with *D. vexillum*. The experiment consisted of placing 20 urchins in oyster trays covered with 5 cm mesh to stop urchins escaping. The results obtained suggested that it's difficult to monitor the control agent (urchins) and there are many biological and environmental factors that can't be controlled. Hence the real effect of the treatment (urchins) on *D. vexillum* remains unknown (Switzer *et al.* 2011). Other publications report the use of the sea star *Asterias vulgaris*, the crabs *Cancer irroratus* and *Carcinus maenas* (Carver *et al.* 2003) and the shrimp *Rhyncocynetes typus* (Dumont *et al.* 2009) to control the solitary ascidian *Ciona intestinalis*. Results showed that these predators were selective feeders, had low feed rates and had a tendency to avoid larger *C. intestinalis* specimens.

Reports of successful eradications using biological control agents against introduced species are few and there are many well-known case studies that illustrate potentially disastrous consequences to the environment, industry, and human health following the introduction of foreign predators or pathogens (Pleus *et al.* 2008).

Biological control is not currently recommended as a viable option for the control of *D. perlucidum* in WA.

## 4.3 Physical/mechanical methods

Physical control refers to the use of physical manipulation of a pest with or without the aid of machinery to cut, shear, shred, crush, press, lift, convey, transport, and/or remove an invasive pest and associated organic material from water bodies (Pleus *et al.* 2008). This method also includes the establishment of physical barriers (e.g. fences, wrapping) to prevent the dispersal of a pest or to create specific environmental conditions unsuitable for the survival of a pest (e.g. reduction of oxygen in the water) (Pleus *et al.* 2008).

The mechanical methods used to control ascidians can be classed into two major groups: 1) physical removal of target species or fouled structures and 2) the encapsulation of submerged structures *in situ*. These are outlined in Table 4.

The first method is the manual collection of the target species (Pannell and Coutts 2007) or removal of the submerged structures fouled by the target species, such as aquaculture structures (e.g. nets, lines, cages), moorings and vessels (Kelly and Maguire 2008). Caution must be exercised during all attempts involving the physical removal of target ascidian species as there is potential for the species to be spread by fragmentation. It has been observed that dislodged fragments (in the water) of *Botryllus schlosseri* (colonial ascidian) are viable for a period of 18 days (Paetzold and Davison 2010).

*In situ* removal of solitary ascidians such as *S. clava* has been proven to be an effective control

method. However, this method is costly in terms of time and effort as each individual needs to be handpicked (NIWA 2013). Furthermore, the stress induced by the physical removal of the individuals could also promote the release of larvae before they are removed from the water (Mike Page 2013, pers. comm.).

Submerged structures that have been removed should be air dried (Carman *et al.* 2010) and/or treated to facilitate the cleaning of structures (e.g. manual scrubbing) (Switzer *et al.* 2011). Alternative methods for cleaning docked structures/vessels is the use of pressurised seawater above water to remove colonial or solitary ascidians from vessel hulls (Arens *et al.* 2010) or the use of direct pressure blasting to remove fouling from mussel lines (Paetzon and Davison 2010). However attempts must be made to capture the removed fouling and dispose of it on land.

The encapsulation method consists of placing a physical barrier over fouled surfaces such as pylons, wharfs/pontoons, vessels, moorings and chains, aquaculture cages, and certain areas of the seabed. Encapsulation can be achieved by wrapping the fouled structure with plastic bags; high-grade geotextile fabric, plastic silage covers and plastic wrap (Pannell and Coutts 2007). The purpose of "wrapping" fouled structures *in situ* is to establish a physical barrier to prevent water exchange and create anoxic (low oxygen) conditions around the target species and ultimately kill it.

Encapsulation of structures has been proven to be the most effective method for the eradication of *D. vexillum,* if anoxic conditions are achieved. The time required to achieve full eradication depends on the type of affected structure (e.g. size, surface characteristics). For example, for pylons and vessels, eradication can be achieved in 7 days, while for infected seabed it might take up to 14 days. This period can be reduced during the summer when the water temperature is higher (C. Wellington 2013, pers. comm.). However, this method like many other control methods, is indiscriminate (non-target specific) and is likely to kill all the organisms attached to the structure. A summary of mechanical and physical methods tested for the control of *D. vexillum* and other ascidians is provided in Table 4.

## 5.0 Combining methods for control/eradication

There are various methods that have been trialled or proposed for the eradication of *D. vexillum* in introduced locations such as New Zealand (Coutts and Forrest 2007; Pannell and Coutts 2007), Scotland (Nimmo *et al.* 2011), Wales (Kleeman 2001, Holt and Cordingley 2011), England (Cook 2010), Ireland (Kelly and Maguire 2008), and Canada (Switzer *et al.* 2011). These programs included a combination of physical and chemical methods such as: wrapping fouled infrastructure with plastic and adding a chemical accelerant to kill it, or removal of infected infrastructure from the water to allow drying, and adding biocides to infected areas. For a comparison of the effectiveness of the different treatments refer to Table 5. Theoretically, using a combination of these methods would provide the best opportunity for eradicating target populations of *D. vexillum* or any other introduced ascidian.

**Table 4.** Mechanical and physical methods used for the control and eradication of *Didemnum vexillum* and other introduced ascidians.

| Fouler | Substrate | Description | Treatment | Effectiveness against target organism (eradication) | Location | Study type | Reference |
|---|---|---|---|---|---|---|---|
| *Didemnum vexillum* | Submerged structures | Remove moorings, vessels and aquaculture installations, by slipping/ dry docking, high pressure water cleaning, then desiccation for 48 hrs | Not fully tested | Not tested | Ireland | Field | Kelly and Maguire (2008) |
| *D. vexillum* | Oysters | Turn oyster bags regularly | | Reduces colonisation of *D. vexillum* on the bags | Ireland | Field | Kelly and Maguire (2008) |
| *D. vexillum* | Seabed | Use of high grade geotextile fabric (smallest pore size) | | Effective only if anoxic conditions are achieved under the fabric | Ireland | Field | Kelly and Maguire (2008) |
| *D. vexillum* | Seabed (beneath jetties/ pontoons, moorings, vessels and mussel farms) | Larger infestations were covered with plastic silage covers attached with rocks or pegs, water under the cover pumped out | 14 days | Effective only if anoxic conditions are achieved under the silage cover | New Zealand | Field | Pannell and Coutts (2007) |
| *D. vexillum* | Pontoons | Plastic encapsulation using silage covers with or without accelerant | | Easy to apply as don't require complex equipment<br>Effective only if anoxic conditions are achieved under the silage cover | Holyhead Marina, Wales | NA | Kleeman (2009); Laing *et al.* (2010) |
| *D. vexillum* | Pontoons | "Set-n-forget" plastic encapsulation method using silage covers | 7 days, most effective if 20% acetic acid is added | 100% effective if applied correctly | New Zealand | Field | Pannell and Coutts (2007) |
| *D. vexillum* | Anchor chains | Plastic wrapping | | As above | Holyhead Marina, Wales | NA | Kleeman (2009); Laing *et al.* (2010) |
| *D. vexillum* | Moorings | In situ plastic "Set-n-forget" encapsulation technique | 4–7 days treatment | 100% effective if applied correctly, *D. vexillum* was killed in 89% of moorings treated | New Zealand | Field | Pannell and Coutts (2007) |

| Fouler | Substrate | Description | Treatment | Effectiveness against target organism (eradication) | Location | Study type | Reference |
|---|---|---|---|---|---|---|---|
| *D. vexillum* | Pylons | Wrapping by divers, Donagheys plastic balage wrap (0.75 x 1500 x 25 m) | | 100% effective if applied correctly, acts in 7 days in optimal conditions | New Zealand | Field | Pannell and Coutts (2007) |
| *D. vexillum* | Boat hulls | Plastic encapsulation using silage covers with or without chemical accelerant | | As above | Holyhead Marina, Wales | NA | Kleeman (2009); Laing *et al*. (2010) |
| *D. vexillum* | Boat hulls | *In situ* plastic "Set-n-forget" encapsulation technique | 7 days treatment with addition of 20% acetic acid | 100% effective if applied correctly | New Zealand | Field | Pannell and Coutts (2007) |
| *D. vexillum* | C. gigas | Manual scrubbing | | Reduction of cover but increase of other foulers (*B. schlosseri*) biomass due to space availability | Vancouver, Canada | Field | Switzer *et al*. (2011) |
| *D. vexillum* | Mussels | Air drying of fouled mussels (nets, cages) | | Not effective, mussels died at 5 hr exposure time while *D. vexillum* survived >6 hr exposure time | USA (east coast) | Field | Carman *et al.* (2010) |
| *D. vexillum* | Floating pontoons/ wharfs | Air drying of structures | Left for 2 weeks to dry | Effective if applied correctly | New Zealand | Field | Pannell and Coutts (2007) |
| *D. vexillum* | Seaweed beds (Carpophyllum sp.) | Manual removal | Divers used knives to remove all affected seaweed, placed in a plastic bag and disposed of | The success of this treatment is very dependent upon good underwater visibility and ongoing monitoring | New Zealand | Field | Pannell and Coutts (2007) |
| *D. vexillum* | Shellfish aquaculture gear | Combination | Manual removal on site, removal of ascidians with pressurised water and contain runoff water, dry off gear | Controls *D. vexillum* | British Columbia, Canada | Commercial Field | BC Shellfish Growers Association (2013) |
| *Botryllus schlosseri* | Mussels | Spray mussel socks with hand held high pressure seawater | 700 psi | Not effective, dislodged fragments viable for >18 days | St Peters Bay, Canada | Field | Paetzold and Davison (2010) |

| Fouler | Substrate | Description | Treatment | Effectiveness against target organism (eradication) | Location | Study type | Reference |
|---|---|---|---|---|---|---|---|
| *B. schlosseri Botryllodes violaceous* | Mussels | Out-of-water pressurized seawater spray | 700 and 40 psi, spray every 3 weeks | High pressure spray of mussel close to harvest season (Nov) showed greatest reduction of target species biomass (85%) | St Peter Bay & Savage Harbour, Canada | Field | Arens *et al.* (2010) |
| *Ciona savignyi* | Submerged structures | In water exposure of structures to UV radiation from sunlight | Sunlight radiation | Adults seem to be affected, however, early life history phases (larvae) are resistant to high exposure levels | Puget Sound, Washington | Field | Olah (2001) |
| *Eudistoma elongatum* | Oysters | Heat treatment | Burning of fouled structure using a LPG burning torch | Limited to areas with full accessibility | New Zealand | Field | Morrisey *et al.* (2009) |
| Multiple species | Not specified | Underwater hydraulic pressure washing | Not tested on *D. vexillum* | Highly effective at removing all living organisms, it can be used only on hard surfaces (e.g. concrete, metal) | Washington | NA | Pleus *et al.* (2008) |
| Multiple species | Seabed or underwater structures | Suction | Not tested on *D. vexillum* | Not tested on ascidians, used in Hawaii to remove pest algae | Hawaii, USA | Field | Pleus *et al.* (2008) |

## 6.0 Novel methods in aquaculture

In areas where fouling problems are mainly caused by ascidians, standard practice by the aquaculture industry has been to use nets treated with antifouling containing copper-oxide (Crab Project 2013). These nets can be periodically removed from the cages and washed on shore in industrial net washers (Crab Project 2013). However, the use of copper-based coatings can negatively affect a farm's productivity by increasing mortality of the farmed species due to increased levels of dissolved copper in the water. In finfish aquaculture a higher risk of amoebic gill disease has been documented when copper treated nets were used (Douglas-Helders *et al.* 2003).

Recently developed methods such as the use of Aquagrid® and metal mesh nettings are being introduced to reduce the predation effect rather than the biofouling effect. The Aquagrid® doesn't require antifouling as it is cleaned with an underwater disc cleaner on a 3-4 week cycle. This new netting type is more expensive than traditional nylon nets and labour costs can be significantly higher due to the greater weight of the nets/cages (Crab Project 2013). The finfish aquaculture industry is investigating the possibility of placing mussels around the fish cages to provide "new" available substrate for ascidians to settle on and so prevent the settlement of other fouling organisms on the cages (Dürr and Watson 2009). This is because cages with low levels of fouling are easier and cheaper to clean. However, there is no indication of what type of mussel would be used as a "substrate" nor how the ongoing management of the "fouled" shellfish will be managed.

The use of special shellfish coatings (which are applied directly to shells), and epoxy based and slow release antifouling coatings (applied to cages and gear) have been also assessed by the aquaculture industry (De Nys and Ison, 2004; Svane *et al.* 2006). Although the use of antifouling coatings has potential, and applications and some commercial products have been developed along the way (e.g. PearlSafe® in Australia), several problems have been observed during testing. These include reduced efficiency due to unsuccessful application of the coating to nets, cracking of net samples after low water pressure washes; the coating peeling off soon after immersion and increased weight of coated gear (Dürr and Watson 2009). It has been also observed that the efficacy of these coatings varies between geographical locations and is seasonally dependant (De Nys and Ison 2004). Thus these technologies are still under development and require additional research to increase their efficacy before they can be utilised at a commercial scale.

Other methods for the control of biofouling in aquaculture currently being investigated include new materials for the construction of aquaculture cages (fouling-release coatings that are silicone based), use of electrical methods to generate biocides (i.e. chlorine radicals) or pH changes, adoption of polyculture techniques and development of new protocols for shellfish handling and immersion techniques (Crab Project 2013). These methods are still far from being applicable at commercial level and require further testing.

New methodologies are also being explored to control early stages of the ascidian life cycle with the aim of decreasing population growth (Bellas 2006). For example, the compound medetomidine can reduce *S. clava* larval motility (Willis and Woods 2011), while zinc pyrithione and potassium monopersulphonate triple salt based disinfectant used in the aquaculture industry (Virkon® Aquatic) have shown to decrease *C. intestinalis* larval settlement and overall biomass (Bellas 2005, Paetzold and Davison 2011). Recent experiments have focused on the use of natural compounds such as radicol and polygodial which can inhibit larval metamorphosis and increase mortality of the solitary ascidian *C. savignyi* (Cahill *et al.* 2012). Overall, there is still a need to develop highly-targeted compounds/antifouling agents, efficient methods of application (e.g. slow release underwater coatings and pellets) and better understanding of the treatments' effects and modes of action.

# 7.0 Case studies of ascidian management programs

## 7.1 Holyhead Marina, Wales

In October 2009, a rapid response eradication program for *D. vexillum* began in Holyhead Harbour (Wales), led by the Countryside Council for Wales (CCW). The marina supports 520 pontoons ranging from 1.5 to 80 $m^2$ (Kleeman 2009). Initially plastic wrappings were used to isolate, smother and kill *D. vexillum* growing on the pontoons. At the end of 2009, permission was granted to use a chemical accelerant, calcium hypochlorite ($Ca(ClO)_2$). This process was reported as an extremely labour intensive exercise, however, by May 2010 the entire marina was surveyed and it was determined to be clear of *D. vexillum* colonies. In August 2010, a monitoring survey revealed that small colonies of *D. vexillum* were present in the marina. Re-settlement of larvae released during the treatment procedure and difficulties experienced in sealing the bags around the numerous chains and ropes securing the pontoons to the seabed were considered the most likely reason for this re-colonisation in the marina. After this detection a second eradication program was planned, but in October 2010 a further survey revealed that an even larger area was re-infected. A new eradication program was proposed including the following improvements:

1. Increased labour to ensure that at least 60% of the submerged structures (i.e. pontoon and chains) in the marina and swinging moorings in the vicinity of the marina were "wrapped" and treated rather than treating a smaller "block" of pontoons at one time.
2. Treatment to occur during the coldest water temperatures to avoid larval dispersal.
3. Removal of as many "structures" as possible for air drying or bleach spraying.
4. Establishment of a detailed surveillance program for at least 2 years following treatment to detect any re-infection of the marina.

In January 2011, due to insufficient funds and time the improved eradication program was not implemented (Holt and Cordingley 2011). To date, *D. vexillum* at this location has not been eradicated. The total cost of this program was estimated to be around AUS $679,423 (~£400,000) (Nimmo 2011).

## 7.2 New Zealand

In October 2001, an unidentified Didemnid ascidian was recorded for the first time in New Zealand smothering wharf piles and moorings in the northern harbour of Shakespeare Bay. 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 (Coutts and Forrest 2007). The species was subsequently identified as *D. vexillum*, but its status as indigenous or non-indigenous was disputed. Nevertheless, its presence was regarded as a significant threat to the mussel industry because of its demonstrated invasiveness on artificial structures, and its ability to over-grow mussels. After initial detection, several delimiting surveys were required to determine the distribution of the species around the detection area. By July 2003, *D. vexillum* was well established on surrounding pylons, new pontoons, artificial seabed (artificial rip rap rock), other barges, recreational vessels and a salmon farm in the area. Despite the farm being relocated 35 km from the infestation point, *D. vexillum* was able to re-colonise the cages. The dispersal of this ascidian was considered to be mainly due to:

1. The effect of anthropogenic activities and vessel movement.
2. The assumption that  had limited capacity to establish in natural environments.

In 2003, a benefit cost analysis was developed to determine control options for this ascidian (Sinner and Coutts 2003). A decision was taken to attempt eradication of the species from New Zealand as soon as possible. For this, several treatment methods were tested to eradicate *D. vexillum* from the seabed underneath infected barges and submerged structures including removal and air drying of structures and encapsulation of pylons, pontoons and vessel hulls (Pannell and Coutts 2007). Several methods to eradicate *D. vexillum* from the seabed were tested including the application of lime, concrete powder, hot water blasting and burning (i.e. torch) (Coutts and Forrest 2007). While some managed to kill *D. vexillum*, they were not considered economically feasible given the size of the infected area (10 000 $m^2$). The use of the encapsulation method on the infected seabed with geotextile fabric was unsuccessful due to the gaps present underneath the fabric and lack of an adequate seal, allowing for water exchange and survival of *D. vexillum* (refer to Table 1 and 2 for details).

Many methods used were effective in eradicating *D. vexillum* from particular substrata while others were less effective. The overall combination of methods failed to completely eradicate this ascidian. Hence, many structures and treated areas gradually became re-infected.

The total cost for the labour and material used during the eradication effort at Shakespeare Bay was estimated to be AUS $588,381 (NZ $346,400) (Coutts and Forrest 2007).

# 8.0 Considerations for future responses

Irrespective of the method used, an eradication or control program relies on many factors which in many occasions cannot be controlled or are unknown. Careful analysis of the "problem" and consultation with stakeholders should be undertaken before implementation of any eradication and/or control program.

## 8.1 Target area

The size of the area to be treated plays a major role in the final outcome of an eradication program. So far, there have been no successful eradication programs documented for *D. vexillum* or any other introduced ascidian. In Holyhead Marina, Wales, an eradication program was feasible mainly due to the size of the treated area (smaller as compared to other locations) being a localised infestation (6,138.88 $m^2$) and the first detection of *D. vexillum* for Wales's waters. In contrast, eradication of *D. vexillum* in locations such as England and New Zealand has been more complex due to its wider distribution in those locations (Kleeman 2009). Similarly, in the Gulf of Mexico, USA, *D. perlucidum* is considered so widely distributed and well established that no eradication or control has been considered for this species (Gretchen Lambert 2013, pers. comm.).

## 8.2 Habitat

So far there is no method available which targets only the species to be eradicated (in this case *D. perlucidum*). In New Zealand, the physical removal of *D. vexillum* from natural substrates also reduced the biomass of other native organisms (seaweeds) (Pannell and Coutts 2007). The use of physical barriers or mechanical devices might also affect antifouling coatings or increase physical damage to pylons or pontoons.

The use of chemicals, such as acetic acid, lime and bleach, especially in high concentrations, can negatively affect native organisms (including protected species) and the environment and their use must be considered in line with appropriate environmental and health legislation. The impact of chemicals in natural or highly-valued habitats needs to be carefully analysed and their use justified prior to use.

## 8.3 Environmental conditions

In New Zealand, the success of most methods tested on *D. vexillum* was highly dependent on environmental conditions. For those methods where diving was required (e.g. physical removal) good underwater visibility was a major constraint. Additionally, in the case of methods involving isolation/encapsulation of structures (pylons, wharfs, pontoon, chains and moorings, seabed, etc.) factors such as tidal movement, currents and winds played a major role in keeping the wrap or cover in place, affecting whether anoxic conditions were achieved (Pannell and Coutts 2007).

## 8.4 Temporal and geographic variations

Temporal and geographic variations in local environmental conditions are known to occur in the marine environment. These variations might impact on the expected success of an eradication program. It was determined that production of larvae by *D. vexillum* at Holyhead Marina decreased significantly below 8 °C. Thus the best chance of successful eradication was when

larvae production was minimal. This stresses the importance of regular monitoring to determine suitable times to conduct an eradication/control program for *D. perlucidum*.

Geographic variations in environmental conditions may also occur, even between locations separated by relatively short distances (e.g. hundreds of metres). These variations might influence the results of an eradication program despite use of the same methodologies and/or target species.

## 8.5 Substratum characteristics

Studies have shown that the morphology or characteristics of a structure's surface and presence of certain organisms (e.g. polychaete tubes and oysters) can puncture 25 µm thick isolation covers (plastic bags) allowing water exchange and preventing the desired anoxic conditions. Uneven surface characteristics of pylons can also decrease the effectiveness of wrapped structures (Pannell and Coutts 2007).

## 8.6 Target species characteristics

As previously observed for *D. vexillum* and other introduced ascidians, in order to achieve a successful eradication program, information on the target species is mandatory. This information should consider seasonal changes in the target species (e.g. biomass, cover), mechanisms of propagation (e.g. sexual and asexual), reproductive characteristics (seasonal production of larvae and eggs, larvae survival) and substrate preferences of the larvae among others.

## 8.7 Decision making and response protocol

One of the most important lessons learned from control or eradication programs elsewhere is that for a program to be successful an incursion response protocol needs to be in place, as well as clear lines of communication and decision making. In New Zealand, when *D. vexillum* was first detected, there was reluctance by regulatory agencies, port companies and the aquaculture industry to take responsibility for managing this ascidian (Coutts and Forrest 2007). Furthermore, the local government and ports' funding to continue an eradication program was not secured, reflecting the uncertainty as to the likely success of the program. Finally, stakeholders like the mussel industry, were not prepared to take any responsibility due to the fear of setting a precedent for other biosecurity threats, especially because at the beginning there were not clear impacts of *D. vexillum* on the aquaculture industry (Coutts and Forrest 2007). Hence, it took more than six months to finalise the formulation of a management plan for this ascidian, by that time *D. vexillum* had spread within Shakespeare Bay beyond its confined point of initial incursion.

The absence of proven cost-effective incursion response tools available to the New Zealand authorities was another factor that contributed to the failure of the eradication attempt. Several methods were developed as a result of the invasion response which varied in effectiveness. Valuable time was also lost in trialling other methods allowing for re-infestation of treated areas and infestation of new areas. These problems were mainly the result of a lack of effective quality assurance (systems), insufficient funds and a lack of overall program management (Coutts and Forrest 2007).

# 9.0 Conclusions and recommendations

This review identifies that key requirements for the management of the ascidian *D. perlucidum* are similar to previous observations on eradication and/or control attempts for other invasive ascidians (Myres *et al.* 2000, Coutts and Forrest 2007). These requirements include:

1. Baseline knowledge of the target species and "infected" environments
2. An effective monitoring regime and quarantine measures
3. Clear lines of authority and decision making
4. Availability and commitment of funds to achieve the program's goals including ongoing monitoring
5. Effective collaboration with stakeholders and public in general

Despite several attempts at eradicating introduced ascidians around the world, to date none has been successful. However, each attempt has significantly enhanced the development of treatments and the identification of key factors that affect the overall outcomes of any management program. The encapsulation method (with or without accelerant) is perhaps one of the most efficient methods and can be applied to a wide variety of substrates. So far, no chemical and mechanical method has been successful in eradicating *D. vexillum* from mussel and oyster farms and treatment has often resulted in high mortality of the farmed species. A comparison of cost, effectiveness, advantages and disadvantages for the methods to manage *D. perlucidum* is presented in Table 5.

Based on husbandry practices and farm environmental conditions a control program using a combination of methods (e.g. chemical spray + air dry) could be used to control *D. perlucidum's* fouling effects. However, eradication of *D. perlucidum* from natural environments is likely to affect native populations (e.g. seagrasses beds). This approach would have to be carefully considered, developed and evaluated by a multidisciplinary panel prior to any eradication attempt. Figure 3 presents an overall view of potential eradication methods considering infestation of *D. perlucidum* in high and low value assets.

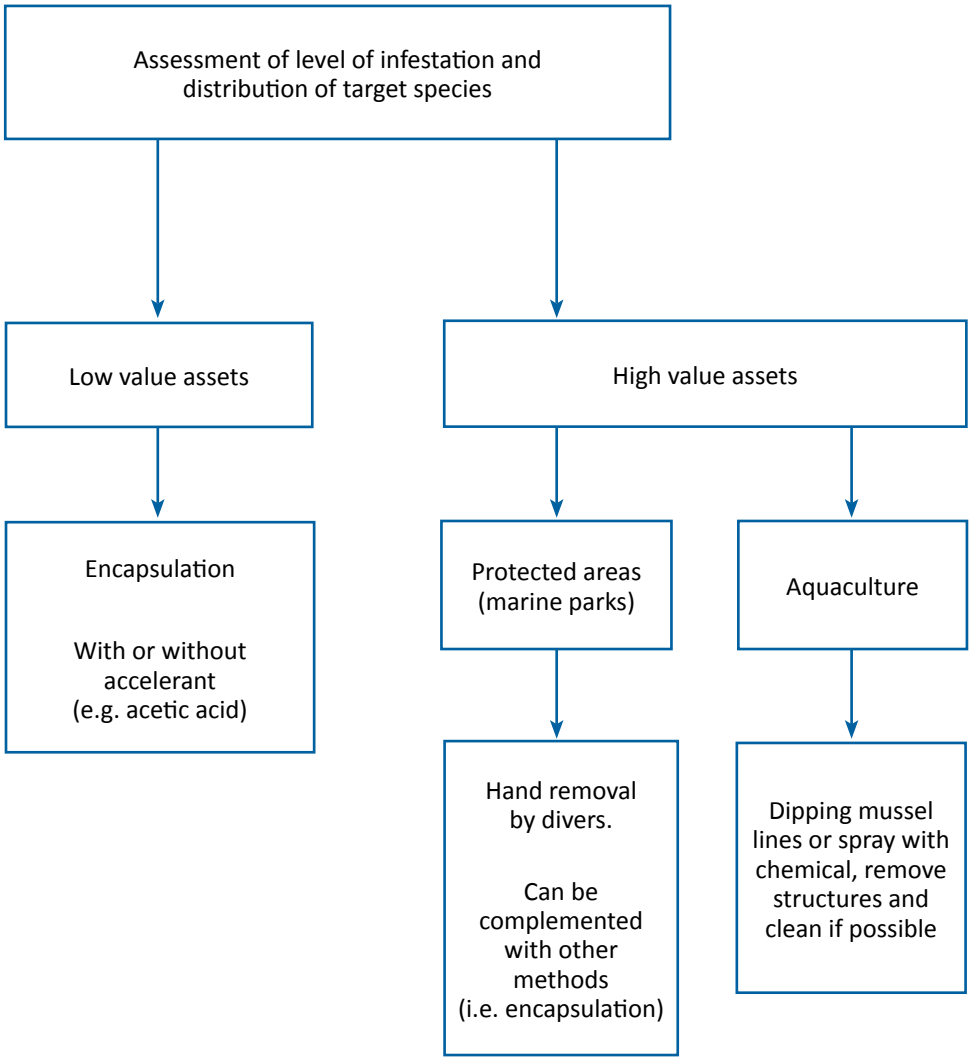


**Figure 3.** General view of a plan to manage the *Didemnum perlucidum* in WA environments.

Based on previous management results for *D. vexillum* around the world and in light of the wide distribution of *D. perlucidum* in WA, a program to locally eradicate *D. perlucidum* could be attempted for specific areas of the State (e.g. diversity hot spots, aquaculture sites)following careful review and testing of eradication methods to be used. However, if other management measures are not put in place, such as strict quarantine and biofouling control, and ongoing monitoring programs, it is likely that re-colonisation would occur. If a local eradication program for *D. perlucidum* is to be attempted, the target area needs to be well delimited and relatively small for the program to have the highest probability of success. Winter-spring is when *D. perlucidum* colonies suffer a significant reduction in the area covered by colony and there is a decrease in colonies' larvae density, which may offer the best opportunity to attempt an eradication program.

**Table 5.** Summary of main treatments: efficacy, cost and relative advantages and/or disadvantages. The feasibility of each treatment, considering previous factors, is indicated with the colours green (feasible), yellow (could be considered) and red (not feasible). Note that a permit to use chemicals (*) or remove fouled animals or plants in the target area might be required.

| Traffic light | Treatment | Effectiveness against target organism (eradication) | Cost | Advantages | Disadvantages |
|---|---|---|---|---|---|
| **Chemical** | | | | | |
|  | Freshwater | Reduces cover/biomass but not 100% effective against target *D. vexillum* | Low | Cheap<br>Easily available | High mortality of farmed and non-farmed organisms<br>No permit is required |
|  | Acetic acid | Not effective against *D. vexillum* | Low | Accelerates the process when applied with the wrapping method<br>Proven to be one of the most effective chemical treatments | High mortality of farmed and non-farmed organisms<br>Concentrated acetic acid is hazardous to work with* |
|  | Bleach/Calcium oxide | Reduces cover/biomass but not 100% effective against target *D. vexillum* | Low | Effective in high concentrations<br>Easy to apply | High mortality of farmed (mussels and oysters) and non-farmed organisms<br>Hazardous to work with, can affect surrounding environment* |
|  | Hydrated lime | Reduces cover/biomass but not 100% effective against target *D. vexillum* | Low | Effective to control *D. vexillum* on *C. gigas* oysters<br>Easy to apply | Hazardous to work with<br>Can be toxic to other marine organisms* |
| **Physical/mechanical** | | | | | |
|  | Removal of submerged structures, air drying under direct sunlight (UV) | Not effective in NZ against target *D. vexillum* | Low | No equipment required – except to remove from water<br>No training required | Not target specific – treatment causes mortality of target and non-target organisms<br>Expensive to lift structures, collateral damage might occur<br>Labour intensive<br>High probability of fragment dislodgment, *D. vexillum* survives (>6 days) |

| Traffic light | Treatment | Effectiveness against target organism (eradication) | Cost | Advantages | Disadvantages |
|---|---|---|---|---|---|
|  | Handpicked (fouled seaweed) | Not effective against *D. vexillum* | Medium | Target specific | Diving & training required<br>Visibility dependant<br>Large quantities of biomass may need to be disposed of<br>Fragment dislodgment might occur |
|  | Manual scrubbing of mussel lines | Not effective in NZ against *D. vexillum* | Low | Cheap | Not target specific – treatment causes mortality of target and non-target organisms<br>Labour intensive<br>Fragment dislodgment might occur |
|  | Wrapping (encapsulation) | Not effective if the surface is not adequately wrapped | Medium-high (depends on materials and labour) | Easy and fast to apply<br>No complex equipment is required<br>Can be left *in situ* for long periods<br>Silage covers are reusable | Not target specific – treatment causes mortality of target and non-target organisms<br>Oysters and tubeworms can puncture a 25 µm plastic<br>High probability of fragment dislodgment<br>Large quantity of plastic needs to be removed and disposed off<br>Mobile fouled organisms (crabs) might escape from the wrapping<br>Not applicable to aquaculture<br>Certain training of divers and frequent inspections are required<br>Large quantities of detached material might be released into the water |
|  | Seabed cover | Not effective against *D. vexillum* | Medium-high (depends on materials and labour) | Applicable to specific and highly infected area | Requires trained staff and diving<br>Highly dependent on currents and tidal changes<br>Not target specific – treatment causes mortality of target and non-target organisms |
|  | Pressurised seawater to clean mussel lines or vessel hulls (above water) | Not effective against *D. vexillum* or *B. schlosseri* | Low | No negative effects to farm's productivity (mussels) | Pressurised seawater to clean mussel lines or vessel hulls (above water) |

| Traffic light | Treatment | Effectiveness against target organism (eradication) | Cost | Advantages | Disadvantages |
|---|---|---|---|---|---|
|  | Burning | Not effective against *Eudistoma elongatum* | Low | Applicable to specific areas<br>Cheap<br>Easy to apply | Non target specific<br>Applicable only to accessible areas (trucks)<br>Applicable only to intertidal populations |
|  | Suction | Not tested against ascidians | Medium - High | Applicable to submerged structures<br>Easy to apply<br>Can be used in combination with other methods | Not target specific – treatment causes mortality of target and non-target organisms<br>Fragments can be left behind<br>Requires divers and well-engineered equipment<br>Large containers/tanks are required to store suctioned water with fragments |
| **Biological** | | | | | |
|  | Control agent | Not effective against *D. vexillum* or *C. intestinalis*<br>Not enough information available | Unknown | Use of available resources (native species) | The increase abundance of the control agent in an area might negatively affect other native species |

It is recommended that the following actions are undertaken before attempting an eradication program for *D. perlucidum* in WA:

1. Develop a well-established network of experts and trained personnel to ensure that identification of *D. perlucidum* is fast and accurate.
2. Carry out additional surveys to determine the distribution of *D. perlucidum* in the State and evaluate, based on the target area, if eradication would be feasible.
3. Clearly identify which sectors would be negatively impacted by *D. perlucidum* and how.
4. Evaluate if sufficient knowledge on the biology of *D. perlucidum* in WA is available. Lack of understanding of the target species and assumptions on its behaviour could lead to an unsuccessful outcome for the program.
5. Collect as much information as possible (e.g. environmental conditions) about the area where eradication is planned.
6. Notify and establish efficient communication channels with stakeholders, government agencies and public prior to undertaking an eradication program.
7. Identify potential routes and mechanisms for the spread of *D. perlucidum* inter and intrastate. This would consider commercial and recreational vessels, and aquaculture traffic.
8. Identify if sufficient funds and/or resources will be available to complete the program and support necessary ongoing monitoring.
9. Identify disposal options for treated water and *D. perlucidum*/biofouling biomass.
10. Based on characteristics of the target area, identify and trial potential control/eradication methods.
11. Develop a site specific management and monitoring plan.

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