

Rapid Communication

Catch me if you can! The story of a colonial ascidian's takeover bid in Western Australia

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Abstract

In 2010, *Didemnum perlucidum* F. Monniot, 1983, a potentially invasive colonial ascidian, was found for the first time in Western Australia (WA). During routine introduced marine pest monitoring along the WA coast in 2011–2012, the Department of Fisheries confirmed additional disjunct populations in a span of ~ 2,800 km along the coast. Colonies of the ascidian were apparently flourishing in water temperatures from tropical through to temperate. Colour was not a reliable detection method for this colonial ascidian. Colonies in winter showed the expected senescence, becoming smaller, friable and often partially detached from the substrate. While winter monitoring would reduce likelihood of detection, it may present the best opportunity for eradication. Due to the widespread distribution, eradication of *D. perlucidum* is now unlikely. However, implementing local control measures to protect high value areas, such as marine parks or protected areas, from incursions remains feasible. A robust monitoring program is vital to detecting *D. perlucidum* early in its invasion history, and that would provide the best opportunity for local eradication effort.

Key words: sea squirt, non-indigenous species, monitoring, invasive species, temperate

Introduction

The colonial tunicate *Didemnum perlucidum* F. Monniot 1983, originally described from the Caribbean Sea, is known as a tropical/subtropical colonial ascidian and widely distributed in the Atlantic and the Indo-West Pacific, including: Indonesia, Micronesia, Guam, and New Caledonia (Monniot 1983; Coles et al. 2002; Lambert 2002; Witman and Smith 2003; Rocha et al. 2005; Golbuu et al. 2005; Perry 2012). In the Gulf of Mexico and Santa Catarina in Southern Brazil, where the ascidian is non-native, it has demonstrated invasive characteristics including continuous reproduction, high fecundity, ability to colonize both bare and occupied substrates year round, and ability to overgrow a wide range of organisms (Lambert 2002; Kremer et al. 2010). Although there is limited information on the biology and ecology of the species, information regarding management of *D. perlucidum* in its non-native range is non-existent.

In 2010, *D. perlucidum* was detected in the Swan River, Perth, Western Australia (WA) on experimental settlement panels (Smale and Childs 2011). Concurrently, in New South Wales a white colonial ascidian suspected of being the invasive species *Didemnum vexillum* Kott, 2002 was detected in Twofold Bay. There were considerable difficulties in obtaining a correct taxonomic identification for the species; however, using molecular tools the species was confirmed in 2012 as *D. perlucidum* (GenBank JQ731740). Prior to 2010, this species had not been reported in Australian waters (Kott 2001), despite the review of extensive collections of Australian ascidian fauna by Kott (see 1985, 1990a,b, 1992a,b, 1997, 1998, 2001, 2005) and sampling of ascidian fauna in WA by McDonald (2005a, b). In 2011–2012, the Department of Fisheries, Western Australia (DoF) initiated introduced marine pest (IMP) monitoring along the WA coast, targeting high risk locations (i.e. the national monitoring network: NMN) identified by the Australian governments

(Commonwealth, States and Northern Territory). The WA monitoring locations are commercial trading ports and surrounding vector nodes including, amongst others, marinas, anchorages, slipways, dry docks, public jetties, natural areas, and aquaculture facilities. One objective of the program was to detect species displaying potentially invasive characteristics. During this IMP monitoring and other opportunistic sampling by DoF, extensive colonies of a colonial ascidian were detected along the WA coast. The populations were disjunct but spanned 2,800 km (coastline distance). Many of these colonies were overgrowing other species and hence displaying a potentially invasive habit. During this time, there were also reports from members of the aquaculture industry and other stakeholders of extensive white growth on aquaculture infrastructure. Based on descriptions provided to DoF, all colonies were suspected to be the recently detected colonial ascidian *Didemnum perlucidum*.

The aims of this paper were: to report on the widespread distribution of the newly detected tropical/subtropical colonial ascidian *Didemnum perlucidum* along the WA coast; to identify factors influencing the detectability of this species during monitoring programs; and to identify potential opportunities for eradication.

Materials and methods

Western Australia has over 20,781 km of convoluted coastline (including islands) spanning the latitudes between 13°44'36" N and 35°08'10" S. The result is an unusually large range in water temperature from tropical waters (~21–31°C annual range) in the north to temperate waters (~15–20°C annual range) in the south, with a tropical/temperate transitional zone in between (Figure 1). DoF undertakes IMP monitoring along the WA coast in all three zones.

In 2011–2012, colony fragments (n=403) of suspected *D. perlucidum* were collected from natural habitats (seagrass beds) and artificial habitats (pylons, aquaculture equipment and settlement collectors) at locations from WA's north to the south, including NMN locations, other ports, marinas and aquaculture facilities (Figure 1, Table S1). DoF divers, using SCUBA, collected samples from pylons in marine port and marina areas, and estuarine seagrass beds. Samples (handpicked by DoF) were collected from marine aquaculture infrastructure and from settlement collectors located within marine port environments. One sample (Barrow Island, Table S1) was collected

by an external party who undertakes IMP monitoring as a WA state government requirement.

Collected colonies of suspected *D. perlucidum* were relaxed in seawater with menthol crystals. Each colony was split into two portions: one preserved in 95% ethanol and the other in formalin-seawater 4%. Their identification was later confirmed using taxonomic and/or molecular techniques (see Table S1 for details). Taxonomic identifications were confirmed by Gretchen Lambert (University of Washington, USA) and/or Mike Page, (National Institute of Water and Atmospheric Research (NIWA), New Zealand). Molecular identification was performed following the extraction of DNA from 20 mg of tissue using the Fisher Biotech Genomic DNA® extraction kit according to the manufacturer's instructions. The procedure developed by Stefaniak et al. (2009) was followed. The criterion for positive molecular identification was established when sequences had a 100% match with a sequence previously identified as *D. perlucidum* (Genbank accession JQ731735, Swan River, WA, identified by Gretchen Lambert, University of Washington). The molecular analysis showed that *D. perlucidum* colony samples (n=252) collected from across WA had identical sequences over an approximately 500 nucleotide region of the CO1 mitochondrial gene for *D. perlucidum*. Locations where samples were positively identified as *D. perlucidum* are shown in Table S1 and Figure 1.

During the initial stages of collection it was noted the colour and thickness of the *D. perlucidum* colonies differed with the geographical location of the colonies, potentially affecting the detectability of the species. Colour was therefore recorded as a qualitative measure undertaken by the same observer when colonies were *in situ* or directly after collection. Tunic thickness of preserved *D. perlucidum* samples was also measured by taking three replicate measurements from the centre of each individual colony using digital callipers. In addition, fifteen colonies collected from the cooler temperate waters were dissected and examined under the stereo microscope for the presence of eggs and larvae. This was to determine whether they were reproductively active under WA's cooler temperate conditions.

Results and discussion

Didemnum perlucidum was first documented from Western Australia in 2010 in one location: the Swan River during an experiment on fouling responses to simulated climate change using

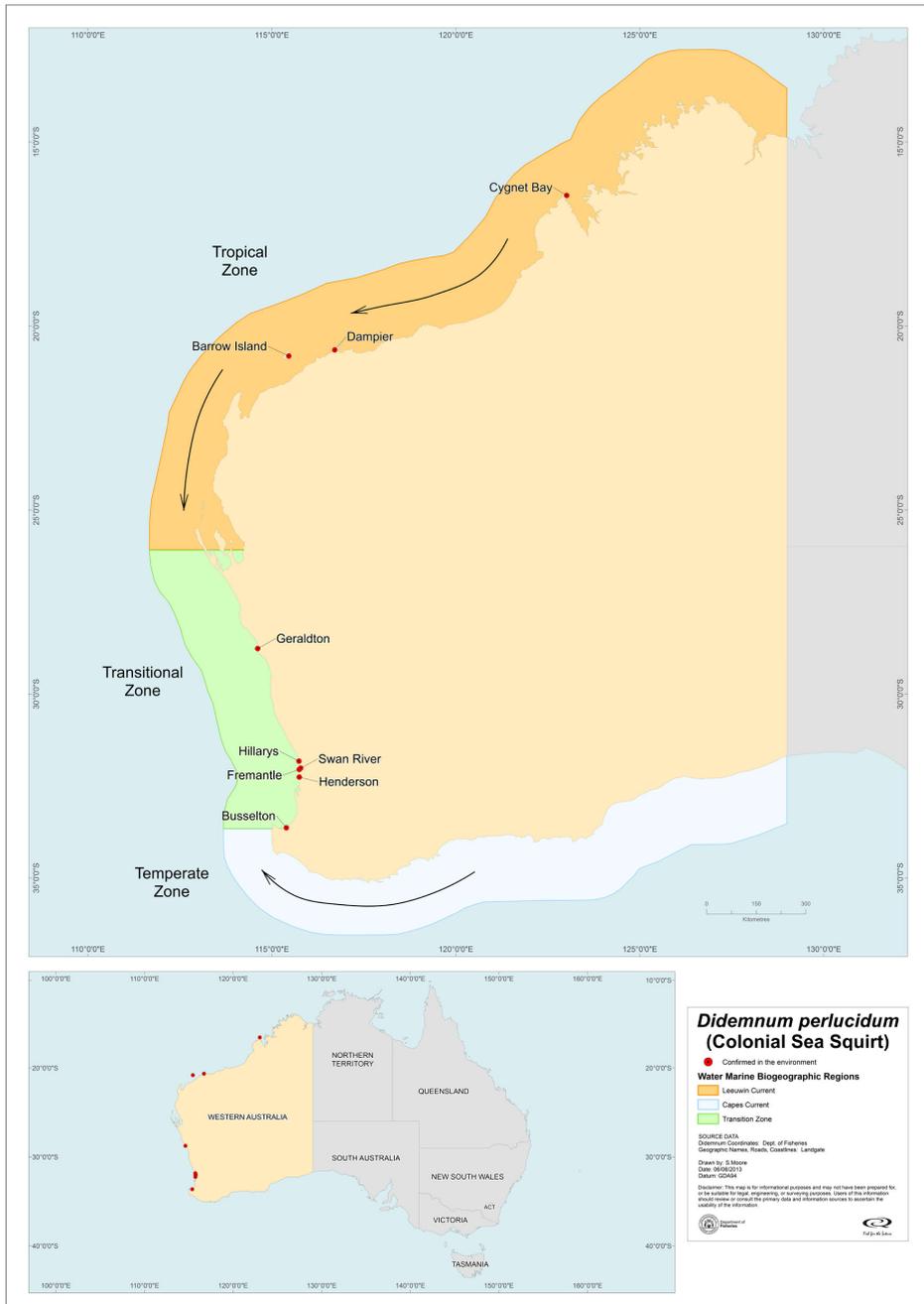


Figure 1. Tropical and temperate distribution of *Didemnum perlicudum* in Western Australia (2012 data). Note: a transitional zone exists between the tropical and temperate coastal waters of Western Australia. For details see supplementary Table S1.

settlement panels (Smale and Childs 2011). Just one year later, DoF has confirmed detections of populations in numerous locations spanning approximately 2,800 km along WA's coastline and encompassing marine water temperatures from temperate to tropical (~15 to 31°C, annual range)

(Table S1, Figure 1). Colonies of *D. perlicudum* were found in natural habitats as well as artificial habitats.

The recent detection (2010) of *D. perlicudum* comes despite the review of extensive collections of ascidian fauna from around Australia (Kott

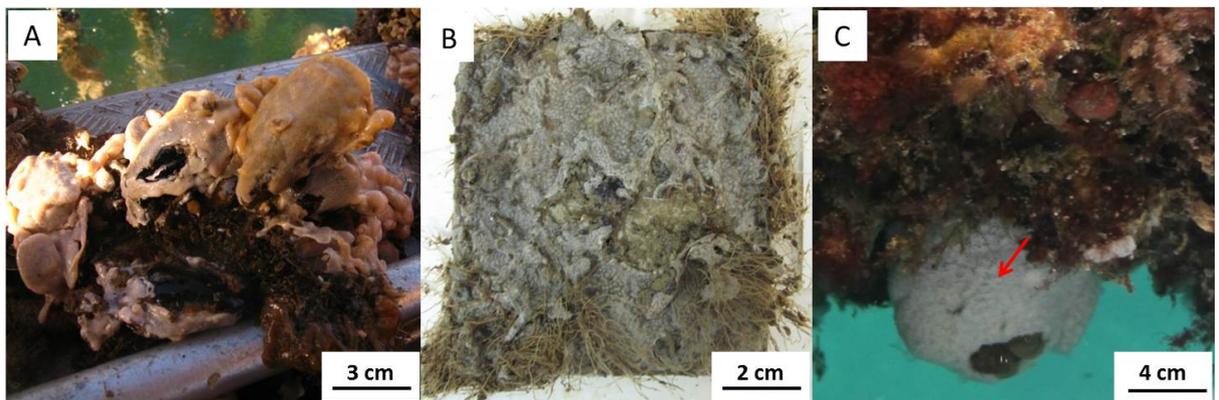


Figure 2. *Didemnum perlucidum* colour variations. A. Pink/orange colonies of growing over cultivated mussel *Mytilus edulis*; B. Grey colonies growing on PVC settlement plates; C. White colonies associated with the solitary ascidian *Styela plicata*. Photographs by the Department of Fisheries.

1985; 1990a,b; 1992a,b; 1997; 1998; 2001; 2005) and sampling of ascidian fauna in WA (McDonald 2005a, b). There are two possible explanations for *D. perlucidum*'s recent detection and rapid spatial expansion into numerous disjunct populations. Firstly, the species could have been present in the Western Australian ascidian fauna earlier but remained undetected because of low abundance or have been overlooked taxonomically. A second possibility is that the species was newly introduced into WA by either multiple inoculation events or a single event with rapid secondary dispersal. *Didemnum* species produce lecithotrophic (non-feeding) larvae that are short-lived and disperse no more than several meters from the parent colony (Davis and Butler 1989; Svane and Young 1989). However, the extended latitudinal distribution (populations spanning 2,800 km) of *D. perlucidum* in WA cannot be explained by natural larval dispersal alone. Colonial ascidians are also recognised as one of the most common invaders primarily translocated as biofouling (Bullard et al. 2007; Lambert and Lambert 2003). As the majority of DoF's detections were associated with ports and marinas (Table S1), we suggest vessel movements either into the state or within the state were the most likely translocation mechanism behind its now widespread distribution.

In the literature, *D. perlucidum* is commonly described as a white encrusting ascidian occurring in tropical waters (Kremer et al. 2010), but it is also possible to observe colonies ranging from yellow to a reddish colour (Perry 2012). When undertaking IMP monitoring, there is a reliance

on visual cues, such as colour, to trigger further investigation of a suspect species. In our study, we observed a range of colours in the *D. perlucidum* colonies. The greatest colour variation occurred in the cooler waters of the WA temperate regions (Figure 2A-C). These colour variants included a pinkish-orange colour in Henderson (Figure 2A), a grey colour in Fremantle (Figure 2B), and the occasional occurrence of yellow and dark brown colonies. In contrast, samples from tropical areas were predominantly white (Figure 2C). As DoF provides scientific and technical advice on IMP monitoring to other stakeholders, we recommend colour not be used as morphological feature for field identification of *D. perlucidum*.

A pattern of growth and recession has been described and linked to seasonal fluctuations in environmental conditions for colonial ascidians (Turon and Becerro 1992). This same relationship was observed in the *D. perlucidum* colonies along the WA coast. Our preliminary observations of colonies in the WA temperate region showed that in summer, colonies formed extensive mats covering ~100 to 900 cm² in area. These were uniform in structure (Figure 3A-B) and occasionally had lobules arising from the tunic of the colony, resulting in a "bumpy" appearance (Figure 3C). In contrast, winter colonies were much smaller in area (~1 to 25 cm²), had no lobules, and displayed a friable, crumbly, and irregular surfaces (Figure 3D). These smaller winter fragments frequently had detached portions of tissue hanging free from the substrate (Figures 3E-F). Thickness of the colonies only varied temporally for samples

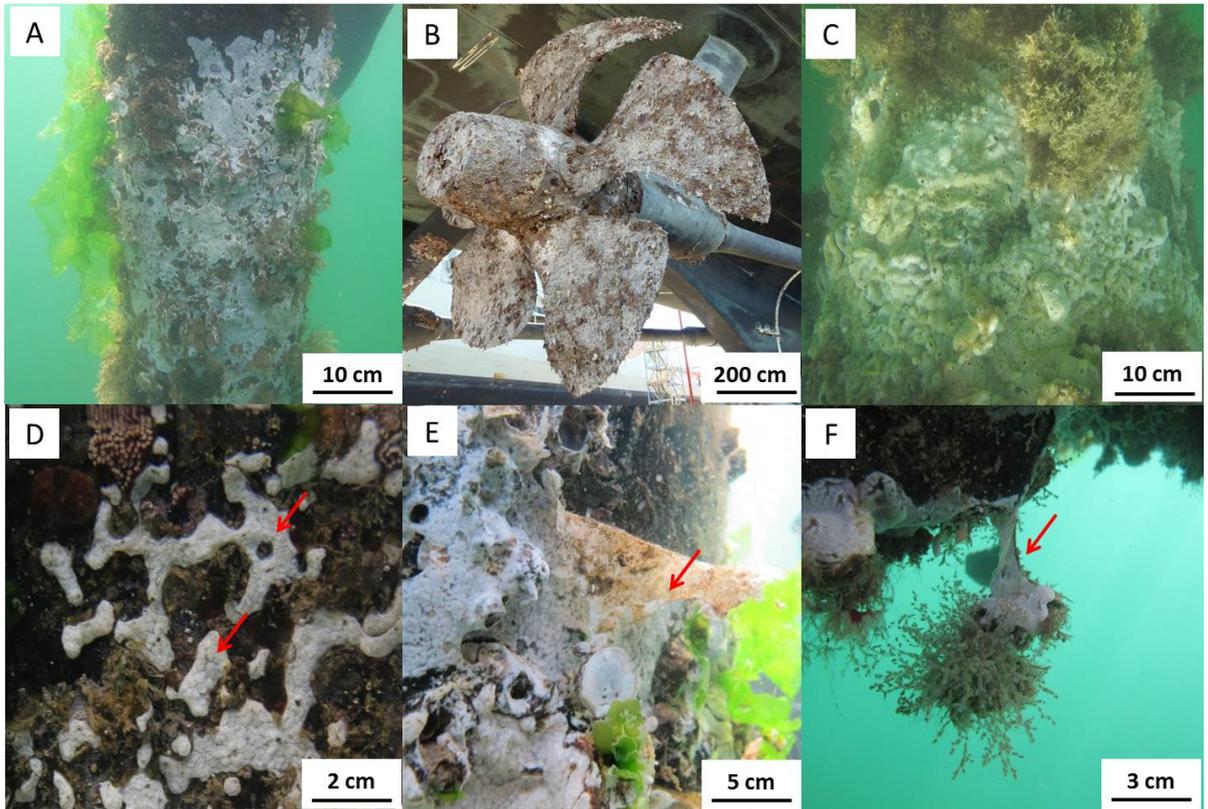


Figure 3. *Didemnum perlucidum* external appearance in summer (A-C) and winter (D-F). A) Colonies growing on pylons forming extensive mats up to 2 m depth; B) Colonies covering a vessel's propeller; C) Plump and healthy colonies growing on pylons with bumpy appearance; D) Winter colony showing a retraction and reduction in colony size; E) Colonies detaching from substrate (indicated with arrow); F) Detached colony hanging from its substrate. Photographs by the Department of Fisheries.

collected from cooler temperate waters. In this instance colonies were thicker in summer (3–5 mm: December to February, mean water 22°C) compared to winter (1–2 mm: June to August, mean water 16°C). These results have significant implications for monitoring and eradication programs targeting *D. perlucidum*. For example monitoring programs undertaken only in winter could quite easily miss the small colonies; however, if eradication is to be attempted, the best opportunity would be when the ascidian is beginning to senesce.

Where the species has displayed invasive characteristics in its tropical distribution, *D. perlucidum* is highly fecund and reproductively active throughout the year, even in cooler water temperatures of 18 to 23°C (Kremer et al. 2010). Our findings, although preliminary, were similar for specimens from the cooler temperate WA waters with specimens containing both eggs and larvae year round.

As *D. perlucidum* has shown invasive tendencies in other locations (Lambert 2002; Kremer et al.

2010), there is potential for the species to negatively impact the WA coastal environment. DoF is currently undertaking research into the potential impacts this species is having on WA habitats and other species as, to date, there is only anecdotal information on impacts to shellfish aquaculture facilities. In Brazil, *D. perlucidum* has been described as a common fouler of cultivated mussels (*Perna perna* Linnaeus, 1758) (Kremer 2008; Rocha et al. 2009) and oysters (*Crassostrea gigas* Thunberg, 1793) (Rosana Rocha, Universidade Federal do Paraná, Curitiba, Paraná, Brazil, pers. comm.). There, farmers often report the presence of *D. perlucidum* as beneficial, as it controls the level fouling by other unwanted organisms (Kremer 2008). The impacts of *D. perlucidum* on aquaculture facilities in WA are varied. A mussel farm (*Mytilus galloprovincialis*) in temperate WA waters (Henderson) reported a white growth that, when present in moderate amounts, appeared to limit the growth of other

foulers such as barnacles (Glenn Dibbin, Blue Lagoon Mussel Farm, WA, pers. comm. 2012). However, when the white growth became abundant, it caused the demise of over 80 % of the season's harvestable mussels (Glenn Dibbin, pers. comm.). This white growth was later identified (in 2012) by Gretchen Lambert, University of Washington as *D. perlucidum*. In contrast the presence of *D. perlucidum* at a pearl farm (*Pinctada maxima*) in tropical waters (Broome) has had no recognisable impact on the oysters to date (James Brown, Cygnet Bay Pearls, WA, pers. comm. 2013).

Clearly, *D. perlucidum* is widely established in WA. It is therefore highly unlikely that the species could be completely eradicated from WA waters. However, implementing local control measures aimed at excluding the species from high value environmental areas, such as marine parks or protected areas, may be feasible. These measures could include developing and instigating vessel management protocols to prevent translocation of *D. perlucidum* from known infected locations by limiting vessel movements between areas with *D. perlucidum* colonies and areas of high environmental value, or by requiring inspection of vessels prior to the journey. For the management to be robust, regular monitoring for the species in the high value environmental areas would need to occur in conjunction with vessel management protocols. Regular monitoring provides the best opportunity for early detection of the species. It is widely accepted that detection of an invasive species early in its invasion history gives the best chance of a successful eradication (Myers et al. 2000). If *D. perlucidum* was detected early in its invasion history in a high value environmental area, then our results show that the best opportunity to attempt eradication would be in winter when the species is starting to display the signs of senescence.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Record of verified detections of *Didemnum perlucidum* along the WA coast in 2011/2012.

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2014/Supplements/BIR_2014_Bridgwood_et_al_Supplement.xls