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Selecting a Bath Treatment for the Marine Carpet Sea Squirt *Didemnum vexillum, Kott 2002* in Scottish Shellfish Aquaculture

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Summary

Following the identification of the invasive non-native species *Didemnum vexillum*, Kott 2002, on a Scottish shellfish aquaculture unit, a control method was needed to permit the legal movement of live Pacific oysters (*Magallana gigas*) off from the farm, and which would kill the pest species but result in acceptable mortality in the aquaculture species. Following a literature review, the most relevant control method in our case was determined to be a bath treatment. Twelve relevant published studies were found describing bath treatments for fouled shellfish, although some results were contradictory and their interpretation was complex. This report presents a review and analysis of the evidence needed to have a control method accepted for initial trial by the Scottish shellfish industry and by the relevant national regulatory authorities. The control method that will go forward for field trial is immersion in freshwater for a minimum of 24 hours.

Keywords: Shellfish aquaculture; Marine invasive non-native species; Control methods; Bath treatment; *Didemnum vexillum*; *Magallana gigas*

1. Introduction

Globally, the impact of Invasive Non-Native Species (INNS) ranks alongside climate change as a major threat to natural biodiversity, as well as having significant economic impact through loss of food production as well as control, containment and eradication costs. The UN Environment Programme (UNEP) estimates the annual cost of INNS currently to be about 5% of global Gross Domestic Product (UNEP, 2016).

In the marine environment, marine INNS are being spread through global shipping, recreational boating, aquarium releases, fishing, renewable energy, aquaculture and

other marine industry related activities. Additionally, increasingly drifting marine litter is also responsible for the transfer of INNS (Bax et al., 2003). It is estimated that at any one time 10,000 marine species are being transported in the seawater ballast tanks of global shipping (Bax et al., 2003). As climate change warms our waters, so the areas where thermal conditions are right for warm water species to survive and reproduce spread pole-wards.

Hence, marine INNS have both the introductory mechanisms to arrive in new habitats, and the increasing chance that those new habitats will be within their environmental range, particularly in high-latitude waters, such as the United Kingdom (UK). In the UK, situated on the northwest boundary of the European continental shelf, marine INNS is already a source of concern, introduced through all of the routes described above. European and national laws have been established to deal with INNS, and there are national coordinating mechanisms such as the GB Non-Native Species Secretariat (http://www.nonnativespecies.org/) and it's Marine Pathways project. It is estimated that the annual cost of marine INNS to the UK economy is already of the order of €45 million (Marine Pathways, 2015). These costs are incurred through dealing with fouling of structures, moorings, intakes, nets, fish farm cages and vessels, and loss of production when cleaning is needed or when natural exploited habitats or species are impacted.

UNEP (2016) notes that one of six key barriers, preventing action globally to reduce the impact of INNS, is that most countries emphasise INNS introduction prevention (e.g. through biosecurity) rather than trying to contain or control INNS once they are identified. This report describes a key component required in Scotland to contain the marine INNS *Didemnum vexillum* once it is discovered on a shellfish aquaculture unit, i.e. the selection of a bath treatment in order to allow the transport of live shellfish out from the farm.

1.1 *Didemnum vexillum*

Didemnum vexillum (*D. vexillum*) is a colonial ascidian tunicate, commonly known as carpet sea squirt. It is characterised by a tough outer rubbery tunic made from polysaccharide cellulose. *D. vexillum* is a filter feeder, feeding on phytoplankton and suspended organic matter (Fletcher et al., 2013). Its colonies have been described in some areas of the world as "growing aggressively on all manner of substrates" (Carman et al., 2009). It can smother other sessile species, modify habitats it colonises and foul man-made structures, infrastructure and vessels.

D. vexillum, like many other fouling organisms, can restrict water exchange through nets or bags used to contain shellfish aquaculture species, thereby reducing food supply and decreasing internal water quality (Sharp et al., 2006). Buoys and ropes can become weighed down with fouling. Infestations of marine INNS can lead to increased maintenance and management costs for aquaculture, as well as loss of income associated with reduced growth or increased mortality of the aquaculture species (Denny and Hopkins, 2007; Forrest et al., 2007). In addition, marine INNS can transport secondary pest species from area to area following transport by man (Forrest et al., 2007).

Some shellfish aquaculture businesses rely solely on the sale of product for human consumption. Here management options for the operator are focussed on the control of an INNS on the shellfish farm itself. However, some shellfish units supply live shellfish to other businesses for the purposes of on-growing in local conditions. If exporting live shellfish to areas where the marine INNS is currently not present, a treatment is required in order to contain the INNS on the source farm. In Scotland it is illegal to transport an INNS from one area to another, either knowingly or accidentally, unless all reasonable actions have been taken to avoid this. Hence a shellfish aquaculture business where a known marine INNS exists, such as *D. vexillum*, must treat live shellfish before transportation.

The purpose of this report is to present a comprehensive review of currently available studies of chemical bath treatments for *D. vexillum* (and similar organisms) fouling, as well as studies on the effect of such bath treatments on live shellfish. The report also presents the systematic method we used to reject some recommended bath treatments as unsuitable for our own requirements, and select one for onward field trial. It is hoped the review we have carried out, the meta-data we have generated, its analysis, the systematic assessment method (with a set of assessment criteria) and suggestions to improve future bath treatment trials may all be of interest to other practitioners faced with similar tasks as our own, although they may need to select different criteria to suit their own circumstances, and hence come to different conclusions on which treatments to use.

1.2 Treatment Options

Once *D. vexillum* is found on a shellfish farm, an operator has several treatment options to contain or control it. These include:

Air Drying – This method is most suitable for equipment that can be removed from the water for long periods of time (e.g. >48 hours) such as oyster bags, trestles,

mooring lines, buoys and ropes in order to kill fouling species. Long periods of air drying are not suitable for most shellfish species, although some species can survive some exposure to the air. Air exposure has been used in combination with bath treatments for live shellfish, and this option is considered in this review.

Chemical Spraying – This method of treatment is most suitable for managing *D. vexillum* within an intertidal shellfish farm. Spraying can be used in a prophylactic way, accompanied by bag turning on an oyster farm (O'Brien et al., 2015). When spraying fouling on live shellfish, treatment concentrations must be effectively monitored to avoid shellfish mortality. However, spraying is also suitable for equipment that cannot be taken out of the water for drying, such as intertidal trestles set into the foreshore. Where live shellfish are not present on equipment, stronger sprays can be used (although licensing and environmental impact considerations must be taken into account).

Wrapping – Here the idea is to smother the fouling organism using in impermeable membrane, or use such a membrane to hold treatment chemicals around the organism. This is mechanically complicated to apply, and is most suited for equipment that is always below the water surface. It is not suitable for live shellfish.

When we consider the options above, spraying and air drying are the only ones suitable for live shellfish, although both have drawbacks for this purpose.

Spraying may not result in 100% coverage of fouled surfaces, especially when applied to a large consignment of shellfish in a practical application on a commercial farm. Coverage is uncertain, and leaves doubt whether the treatment has been equally applied to all fouling.

Air drying can result in high shellfish mortality. In large quantities of wet shellfish with complex shells which can retain water, and under temperate maritime weather conditions, there is no certainty that all fouling has actually dried, especially over short periods of air exposure.

Finally, we must consider the scale of treatments required. For example, in our case a typical consignment of Pacific oysters for the purposes of "on growing" can be of the order of 200,000 shells. Any treatment applied for the purposes of the removal of *D. vexillum* fouling must be able to be applied on this commercial scale.

All of the considerations above result in the conclusion that only an immersive bath treatment is able to meet the standards required for the transport of live shellfish off

from a shellfish farm which is infected with *D. vexillum*. A bath treatment means that the shellfish is completely immersed in a specified concentration of treatment chemical for a specified length of time. Taking all of the above considerations into account, Table 1 summarises in which circumstance on a shellfish aquaculture unit each treatment option is most suitable.

Once a bath treatment is chosen as the most appropriate treatment option, it must pass certain criteria in order to be useful in a commercial sense.

1.3 Criteria for a Bath Treatment

In general, a bath treatment to remove fouling from live shellfish, in a commercial aquaculture context in Scotland, needs to meet a range of criteria:

1. Safe: Does not cause harm to human operators, when using the correct personal protective equipment and operating procedures.

2. Environmental: Does not cause unacceptable harm to the surrounding marine environment when released into it.

3. Legal: Is acceptable to all of the relevant licensing authorities.

4. Marketable: Does not impact the ability of the shellfish business to sell its product, e.g. does not jeopardise an “organic” status or its status as a high quality food product.

5. Cheap: Has an acceptable cost to the aquaculture business.

6. Available: Is readily available to aquaculture businesses.

7. Practical: Has a method of effective implementation that is practical within the operation of the aquaculture business (including practicalities of storage and handling of raw treatment chemicals).

8. Sensible: Does not cause an unacceptable level of harm to the aquaculture species to which it is applied.

9. Effective: Causes the required levels of mortality to the target pest species.

Apart from Criterion 1, all of the others require a level of judgment to determine their target attainment, based on a balance between benefits and costs, where costs not

only include financial ones, but also environmental “costs” associated with damage to naturally occurring habitats and species.

For example, in order to avoid the spread of a marine INNS from an infected area to a highly sensitive or protected habitat which might be severely impacted by the introduction of an INNS, the “level of mortality to the target pest species” (Criterion 9) which is “required” by a marine management organisation licensing a transfer of live shellfish, or for that matter by the industry itself wishing to avoid the costs of an invasive marine INNS, is 100% (Denny and Hopkins, 2007). To an aquaculture business, the “unacceptable level of harm” to a high cost, low volume aquaculture species might be <10% (Criterion 8).

In order to help select a suitable bath treatment for the purposes of moving live shellfish (in our case Pacific oysters, *Magallana gigas*) from an infected farm, we undertook a quantitative comparison (meta-analysis) between published assessments of bath treatments, that are of relevance to the control and management of *D. vexillum*, focusing on Criteria 8 (harm to aquaculture species) and Criteria 9 (harm to pest species).

2. Methods

2.1 Data Sources

Twelve previous studies of various bath treatments to remove fouling from live shellfish were used in this study, from which data on fouling organism mortality and aquaculture species survival following bath treatments were extracted. All data sources are summarised in Table 2.

Denny and Hopkins (2007; Data Source 1) examined treatments to control the spread of *D. vexillum* via the transport of live seed green-lipped mussels (*Perna canaliculus*). They wanted a treatment that resulted in 100% *D. vexillum* mortality owing to the ability of *D. vexillum* to asexually reproduce from small fragments which survive other treatments. Their studies built on the work of Forrest and Blakemore (2006; Data Source 11) who had found that freshwater immersion for 24 to 48 hours resulted in 100% mortality of the kelp *Undaria pinnatifada*.

Denny and Hopkins (2007) presented results of mortality in *D. vexillum* and green-lipped mussel seed for acetic acid, bleach and freshwater bath treatments. As Denny and Hopkins (2007) were trying to find a treatment to use on seed mussel being transported between farms, their analyses of treatments also included time in

air after immersion in a bath treatment. This was to simulate what happened in reality, during the transport process. Denny (2008; Data Source 2) looks at the same issue, and shares much of its information with Denny and Hopkins (2007). In our meta-analysis, any duplication of results between these two related publications has been eliminated.

Data Sources 03 (Switzer et al., 2011) and 04 (Rolheiser et al., 2012) both looked at bath treatments to remove *D. vexillum* from Pacific oysters in order to control fouling on shellfish farms. Switzer et al. (2011) only considered bath treatments using lime (calcium hydroxide), although they also considered mechanical and biological methods of removing *D. vexillum* fouling, whereas Rolheiser et al. (2012) went on to look at the efficacy of bath treatments using acetic acid, bleach, freshwater and brine. Switzer et al. (2011) used repeated treatments (lime, mechanical, biological) through a growing season, and so the efficacy of each treatment was hard to determine from the cumulative results. Hence, in this review, only the first lime treatment of the season is used.

Data Source 5 (McCann et al., 2013) did not consider the effect of the tested treatments on shellfish, as they were trying to find a general eco-friendly immersion treatment for moveable equipment in order to control and eradicate *D. vexillum* in a response to the discovery of the marine INNS in a harbour.

Data Source 6 (Carman et al., 2016) wanted to find an eco-friendly bath treatment for tunicate fouling on seed blue mussels (*Mytilus edulis*) which may be collected from sites where INNS such as *D. vexillum* occur. Other invasive tunicates present alongside *D. vexillum* included solitary (*Ascidiella aspersa*, *Styela clava*) and colonial species (*Botrylloides violaceus*, *Botryllus schlosseri* and *Diplosoma listerianum*). A treatment for tunicate fouling was needed as fouling on seed mussels could restrict their respiration, reduce their price at market and break regulations if the mussels were transported.

Data Sources 07, 08, 09 and 10 consider tunicates other than *D. vexillum*. However, their results are still considered useful owing to the similarities between the fouling species, and the results for the shellfish species in each trial are useful.

While Data Source 7 (Carver et al., 2003) looked at treatments for the tunicate *Ciona intestinalis* on oyster bags and trestles very similar to those used in Scotland, the investigation of treatments was not the main purpose of the paper, and hence few experimental details were given. Some values of mortality of the tunicate were presented, and these are included in the data analysis.

Data Source 8 (Forrest et al., 2007) investigated the use of acetic acid to treat seed mussels in order to remove INNS prior to transport and hence control a human vector of INNS dispersion in the environment. The initial target pest species in this study was Asian or Japanese kelp *(Undaria sp.)*, but these results are not considered here. However, they went on to look at general multi-species fouling on mussel ropes, which included solitary (*Cnemidocarpa bicornuata*, *Corella eumyota*) and colonial tunicates (*Botryllus schlosseri*, *Botrylloides leachi*), as well as bryozoa, serpulids, polycheates and macroalgae, but not *D. vexillum* itself. Forrest et al. (2007) were particularly explicit about including a transport phase into their treatment routine, using exposure to air to simulate the transport process. They examined the effect of air exposure both before and after bath treatments, as well as the effect of rinsing or not rinsing before air exposure.

Data Source 9 (LeBlanc et al., 2007) presented a detailed study of non-lethal effects of acetic acid and air drying on blue mussel seed. For the purposes of this review, only three values of seed mussel survival from this study could be used.

Locke et al. (2009, Data Source 10) investigated the effect of treatment chemicals on a number of non-target organisms that may be found in the marine environment around a shellfish farm, such as bacteria, shrimp and fish, but also briefly looked at the effect of treatments on the solitary tunicate *Ciona intestinalis*.

Data Sources 11 (Forrest and Blakemore, 2006) and 12 (Sharp et al., 2006) considered bath treatments to deal with marine plants (Japanese kelp and green algal mats respectively) as fouling organisms. However, these data sources have been included as they present valid and useful results for the aquaculture species used in each study (green-lipped mussel and blue mussel).

2.2 Data Extraction

The quantitative data extracted during this review was:

- Treatment Chemical (Formula);
- Strength of treatment chemical (% w/w active ingredient in water diluent);
- Immersion time in bath treatment (minutes);
- Time exposed to air before or after immersion (minutes) when relevant;
- Value of the mortality of the fouling species (0%-no mortality, 100%-full mortality);
- Value of the survival of the aquaculture species (0%-no survival, 100%-full survival).

Note the difference between fouling and aquaculture species effect quantification. We chose to present the extracted data as fouling organism mortality, and aquaculture species survival, as in both cases the ideal treatment, from the perspective of the shellfish aquaculture industry, would result in 100% values, e.g. 100% *D. vexillum* mortality with 100% shellfish survival.

Data was extracted from the text, table and graphs of each data source. Values of fouling mortality and aquaculture species survival were most often digitised from graphs presented in the published papers. On occasion some data manipulation (such as data inversion) was required in order to get all of the data into the same form. Tables A1 and A2 (Appendix) summarise all data conversions that were applied.

A range of ancillary information was also extracted, describing the experimental details relevant to each data point. Table A3 (Appendix) provides a description of all ancillary data extracted, and Table A4 summarises the experimental details of each data source. To accompany this review, there is an associated data set (https://doi.org/10.7489/12128-1) which contains all data extracted during the study. Table A3 also describes the contents of each column of data in the data set.

2.3 Assessing Mortality - Fouling

Few sources describe in detail how mortality was assessed, either in pest species or in aquaculture species. For *D. vexillum*, most studies apply a treatment and then place the treated fragments back into acceptable growing conditions for periods of between seven and 35 days in order to see if they regenerate or shows signs of recovery or growth.

Switzer et al. (2011) photographed oysters covered in *D. vexillum*, and used a quantitative scoring system for *D. vexillum* coverage, after ten days of recovery in the sea following treatment. Denny (2008) quotes percentage mortality of *D. vexillum* on large mussels. The mussels were treated and placed in the sea in bags attached to ropes at 1-2 m depth. Values are given for percent mortality but no indication how this was measured. A typical statement from Denny (2008) is “Samples were collected two weeks later, and seed-mussel and *Didemnum* mortality was recorded”. While it is possible to assume how seed-mussel mortality was measured (e.g. open shells, detached seed), there is no way of telling what the tests applied for *D. vexillum* mortality were, or how percentages were calculated.

McCann et al. (2013) specifically noted the importance of monitoring the fate of treated *D. vexillum* for at least three weeks, as seemingly dead colonies could regenerate within this period. They used visual mortality assessments, including tissue loss, change of colour, mucous coating, and perforated tunic. They also used the presence of foul odour as an indication of mortality.

McCann et al. (2013) make one other relevant observation. They suggest that tunicates may exhibit greater treatment tolerance in colder waters, and reference a Japanese study. This is perhaps a warning that treatments (and *D. vexillum* mortality) must be tested under the same ambient conditions in which they will be applied commercially.

Carman et al. (2016) examine *D. vexillum* mortality after just one week after treatment, and use the visual signs of absence, putrefaction, or detachment. In the discussion of their results, they note that *D. vexillum* placed into “aquaculture socks were all destroyed (dead or shredded into fragments)” in some of the treatments. This is an odd phrase. We know that fragments of *D. vexillum* can remain viable for several weeks (e.g. Morris and Carman, 2012), and hence being torn into fragments is not an indication of mortality.

Forrest et al. (2007) used the weight of mixed-species (solitary and colonial tunicates, bryozoa, serpulids, polycheates and macroalgae) fouling on a 1 m length of test rope as a fully quantitative way of assessing fouling reduction. However, the weight reduction on occasion was dominated by the loss of one species (*Ciona*), and the method could not differentiate between weight loss through mortality versus survival but with weight loss.

When assessing the health of the tunicate *C. intestilanis*, Locke et al. (2009) used attachment to substrate, whether the tunicate was still siphoning or whether it was “obviously dead and decomposing” as signs of mortality.

In summary, the method used by all studies to estimate *D. vexillum* mortality (Table S1) was to treat it, then place it back into conditions that would allow regeneration, in most cases being back into the sea. After a set period of time, varying between 7 and 35 days, visual assessments were made of *D. vexillum* health based on colour, size, attachment as well as, in one study, smell.

2.4 Assessing Mortality – Aquaculture Species

Most studies relied on the standard tests for shellfish mortality, including gaping shells, failure to close on touching (seed mussels), or easily opened (oysters). Forrest et al. (2007) used the percentage of seed mussels which reattached to a mussel rope via their byssus 24 hours after treatment as a short-term assessment of mortality, but simply assess “percent survival” for longer-term monitoring. No description of how “percent survival” was assessed was given.

Forrest and Blakemore (2006) thought that gaping tests could be equivocal. They preferred to test reattachment to mussel ropes by the byssus after a treated mussel was held in seawater for 24 hours. If seed mussels could not do this, they were of no use to the industry. However, this method was also validated using a six month trial of total mortality.

In general, descriptions of shellfish mortality estimation (Table S2) were poor, and papers often assume that the reader knows how it was achieved.

2.5 An Additional Treatment Step – Bath Treatment plus Air Drying

Several studies looked at the use of treatments to remove fouling from shellfish prior to transport. These studies recognised that shellfish would be transported out of the water, typically immediately after a bath treatment has been applied, and hence a test exposure to air was included as part of the treatment trial. Forrest et al. (2007) in particular examined the effect of air exposure both before and after the application of a bath treatment, but Carman et al. (2016), Denny and Hopkins (2007) and Denny (2008) all investigate air exposure only after the application of a chemical treatment.

2.6 An Additional Treatment Step – Rinsing

Several studies introduced a further variable into treatment trials which simulated the transport process using exposure to air; rinsing. The underlying idea was that the transport process could be used to expand the effect of treatment on *D. vexillum* by leaving the treatment chemical on the shellfish after removal from an immersion bath. Denny and Hopkins (2007) and Forrest et al. (2007) both include air exposure with shellfish both rinsed after the treatment and not rinsed. This treatment step is considered in the analyses presented below.

2.7 Replicates

Although this meta-analysis treats each value of either fouling mortality or aquaculture species survival as a single value, often in each study it is the average of many replicates, with the associated errors presented.

2.8 Clarity on Treatment Chemicals

Some of the Data Sources used in this study introduce ambiguity concerning the treatment chemicals applied in their trials. This is addressed further in the discussion below. However, care has been taken in this meta-analysis to remove any ambiguity. When there was any doubt, first authors of papers were contacted where possible in order to gain clarity concerning what was meant. Table 3 summarises the details of the treatment chemicals as referred to in this report.

2.9 Data Visualisation

A combination of graphical analytical techniques have been applied to the extracted data describing *D. vexillum* mortality and shellfish survival. The most basic variables required for this study were bath concentration, bath immersion time and then either *D. vexillum* mortality or shellfish survival. However, some studies have the complication of multiple variables; not simply bath concentration and immersion time but also air exposure time and rinsing/not rinsing. Each data set has been analysed in a way most appropriate for the type of data and the amount of data.

Three-Dimensional Analysis (Contour Plots)

Where three key variables are present (i.e. bath concentration, immersion time and *D. vexillum* mortality) a three dimensional graphical method of analysis has been used, involving contoured data plots (e.g. Figure 1). The extracted data values used, to fit the smoothed contours to, are shown on the plots as printed red figures. The software package *Surfer* (©Golden Software inc.) was then used to fit smoothed contours to the data using a kriging regression method (further details in Appendix).

This analysis demonstrates the properties which we would expect to see for an immersive treatment of a fouling species using a chemical as pesticide (in the case of Figure 1 - acetic acid). Fouling organism mortality increases with increasing immersion times, and with increasing bath concentrations. Hence contour lines of constant mortality slope from top left to bottom right in such an analysis. If a combination of treatment concentration and immersion time is recommended by the

authors of the study from which the data has been extracted, this is represented as a point on the contoured figure, but in reality there is most likely a range of different combinations of immersion times and concentrations which will all result in 100% fouling organism mortality, and these will all fall along a contour from top left to bottom right. The aim of any bath treatment study should be to pick the combination of immersion time and concentration which results in both 100% *D. vexillum* mortality, and the greatest shellfish survival.

Figure 6 shows the same graphical analysis for shellfish (Pacific oyster in this case) survival rates, again for acetic acid as the treatment. As survival rates are used, now contoured values decrease towards the top right of the figure. In both Figure 1 and Figure 6 there is only just sufficient span of data to allow contouring to be useful. Perhaps in the future trials of treatments should consider obtaining a sufficient range of values of concentrations and immersion times to allow such figures to be constructed, or statistical examinations such as two-way ANOVA to be run, rather than focussing on finding a single set of concentration and immersion time values to recommend. Such analyses would then tell us more about the general response of *D. vexillum* and shellfish to treatments, and would allow a business to optimise its selected treatment scenarios.

Two-dimensional Analysis (Regression Plots)

Where only two variables are relevant, (e.g. *D. vexillum* mortality and immersion time for a fixed treatment concentration, Figure 2), a two-dimensional graphical method has been used. In these plots, the extracted data are shown as solid symbols (circles). A least-squares-fit method is used in the graphics package *Grapher* (©Golden Software inc.) in order to find a best-fit regression through the data (further details in Appendix).

In Figure 2 we see *D. vexillum* mortality increasing with immersion time linearly in a natural log transformed space. This method allows extrapolation to be used to predict target values from a bulk of variable data. Obviously such predictions require validating in the field before they are used commercially.

3. Results

3.1 The Base Data Set

The base data set, extracted from the 12 data sources, consists of 355 individual values of either fouling species mortality or aquaculture species survival, along with

the other supporting information as described above. Treatments which were found to have useful numbers of quantitative data were acetic acid, bleach (sodium hypochlorite), brine, freshwater, and lime (calcium hydroxide). Thirteen results from additional treatments were also recorded for completeness. Table 4 presents a summary of the 355 data records extracted from the 12 Data Sources.

The majority of the 186 treatments extracted from the literature which reported the mortality of fouling species were applied to *D. vexillum* itself as the fouling agent (141), with a further nine applied to other tunicate species and a further 36 trials performed on mixed tunicate fouling which included *D. vexillum*.

In relation to aquaculture species, two thirds of the studies used the green-lipped mussel as the aquaculture test species (108), with 49 trials using Pacific oysters and 12 using blue mussels.

Acetic acid (1-10% w/w) was the most trialled treatment chemical applied to *D. vexillum* (79 trials), whereas bleach (0.1-1% w/w), brine, freshwater and lime (concn) all had about 20 trials each.

Whereas all tests on Pacific Oysters were on larger mature oysters, tests on both green-lipped and blue mussels were on juvenile seed shellfish. Green-lipped mussel seed, used in the data sources cited here, varied between 16 to 60 mm in length, and blue mussel seed between 15 to 30 mm in length (Table 2). One study used green-lipped mussel spat (4-5 mm) rather than seed. Seed mussels for the green lipped mussel in the New Zealand aquaculture industry can be anywhere in size from 15 mm up to 60 mm (Forrest and Blakemore, 2006).

3.2 Criterion 9 – Fouling Organism Mortality

Note that here we assume that the mortality of other test tunicates, or of multi-species test species (Table 2) which included *D. vexillum*, all represent *D. vexillum* equivalent mortality. We also have not just considered trials which resulted in 100% *D. vexillum* mortality, but also analysed results of trials which produced less than 100% *D. vexillum* death. These “contraindicating” trials give an indication of the possible variability in treatment outcomes that a treatment chemical may produce, or that the published trial methodologies produced, as well as looking at possible contradictions between studies. In some cases there is enough data to look at the dependency between variables such as bath concentration, immersion durations and *D. vexillum* mortality. In a few cases extrapolation has been used from results of

<100% mortality to predict what treatment variables may be needed to produce total *D. vexillum* death.

Acetic Acid

In all, 15 trials using acetic acid resulted in 100% *D. vexillum* mortality (Table S5). One trial which used 10% w/w acetic acid treatment is dismissed owing to the high degree of shellfish mortality this would produce. The remaining 14 acetic acid trials resulting in 100% *D. vexillum* mortality all came from one reference, DS08 (Forrest et al., 2007). These trials all included 24 hours of air exposure, either before or after immersion in the acetic acid bath. Hence, from the 14 trials that resulted in 100% *D. vexillum* mortality (excluding the trial using 10% w/w acetic acid), acetic acid treatments were of concentrations between 1 to 4% w/w, with immersion times of 1 to 4 minutes, and involved 24 hours air exposure as part of the treatment (Table S5).

In the 12 Data Sources, there were 64 acetic acid treatment trials which resulted in <100% *D. vexillum* mortality (Tables S6 and S7). From the analysis above, we are interested in contra-indicating trials using concentrations between 1% and 4%. Eighteen such trials used concentrations between 2% and 4%, and one to four minutes immersion with air exposure as part of the routine, and 21 trials used treatments in this same range but without air exposure. It should be noted that the 24 acetic acid trials by Data Source 8 (Forrest et al., 2007) also used rinsing or not rinsing following treatment as part of the variables tested for significance (Tables S6 and S7).

In summary, the contra-indicating trials using acetic acid suggest that in order to reliably reach mortality values of ≥97%, acetic acid treatment concentration should be at least 4%, and immersion times at least three minutes (no data was available for immersion times of two minutes). Air exposure should be included in the treatment protocol following bath immersion.

Generalised *D. vexillum* Response to Acetic Acid - No Air Exposure

When there is no air exposure used as part of the acetic acid treatment, extracted data showing 100% *D. vexillum* mortality (Figure 1) was only obtained for bath treatments of 10% concentration for ten minutes (Figure 1- top right apex of diagram). The suggested treatment of 4% acetic acid for two minutes (but with air exposure) results in about 80% *D. vexillum* mortality without air exposure. From Figure 1 we would conclude that air exposure as part of an acetic acid treatment in the range 0.5% to 5% w/w is needed if more than 80% *D. vexillum* mortality is to be

achieved. This means that, from the currently available data, air exposure is an essential part of a treatment at ≥4% w/w concentration and ≥2 minutes immersion time.

Closer examination of the acetic acid results show that the trials conducted by Forrest et al. (2007) which included a stage of air exposure but without rinsing off the treatment chemical resulted in high *D. vexillum* mortalities. As these also resulted in high aquaculture species mortalities (see Section 3.3) these are not considered further.

Hence, the final group of trials to be examined in more detail are the ones where 24 hour air exposure was used as part of the trial, and either *D. vexillum* was placed straight back in to the sea after treatment, or it was rinsed of any remaining treatment chemical. There were only eight trials in this last group, and only two treatment concentrations: 2% and 4% w/w. Examination of the data reveals that *D. vexillum* mortality was not sensitive to bath concentration in the range 2% to 4% w/w, but varied principally with immersion time. If both concentrations are combined, four combinations of immersion time and *D. vexillum* mortality are identical.

When 24 hour air exposure is used as part of an acetic acid treatment regime (Figure 2), in the range of bath treatment concentrations of 2% to 4% w/w, bath immersion times should be 3.5 minutes or longer.

Hence, we can conclude that acetic acid treatments that produced 100% *D. vexillum* mortality were of concentrations between 1 to 4% w/w, with immersion times of one to four minutes, and involved 24 hours air exposure as part of the treatment. The contraindicating trials suggest that in order to reliably reach *D. vexillum* mortality values of ≥97%, acetic acid treatment concentration should be at least 4% w/w, and immersion times at least three minutes. Air exposure should be included in the treatment protocol following bath immersion. Air exposure as part of an acetic acid treatment in the range 0.5% to 5% w/w is needed if >80% *D. vexillum* mortality is to be achieved. When 24 hour air exposure is used as part of an acetic acid treatment regime, in the range of bath treatment concentrations of 2% to 4% w/w, bath immersion times should be 3.5 minutes or longer.

Hence, our overall summary for acetic acid bath treatments for *D. vexillum* are:

- Acetic acid was used in 79 treatment trials, and 14 of these resulted in 100% *D. vexillum* mortality (excluding one trial which used an extreme concentration).

- The large number of contraindicating trials using acetic acid provide evidence to suggest there may be potential variability in the outcomes of treatments when using this compound as the active ingredient.

- The currently available evidence suggests that bath treatments using acetic acid should be of at least 4% w/w strength for at least 3.5 minutes, followed by at least 24 hours air exposure.

Authors that recommended acetic acid as a treatment for *D. vexillum* were McCann et al. (2013 - 10% w/w for two minutes), Carver et al. (2003 – 5% w/w, 15 to 30 seconds) and Forrest et al. (2007 - 4% w/w, 1 minute + 24 hours air).

Bleach

Three studies (Denny and Hopkins, 2007; Denny, 2008; and McCann et al., 2013) found a total of 15 bleach treatments which returned 100% *D. vexillum* mortality (Table S5). Bleach concentrations ranged from 0.1% to 1% w/w and immersion times from 20 seconds to 10 minutes, with and without exposure to air as part of the treatment. Eleven trials resulted in less than 100% *D. vexillum* mortality (Table S8).

Taking into account the fact that bleach had less trials than acetic acid, the impression given by the results is that bleach treatments give high (>90%) *D. vexillum* mortality more consistently than acetic acid treatments over a range of treatment concentrations. Acetic acid treatments appear more variable in outcome for a given set of concentration and immersion times. However, this observation needs further trials to confirm.

From the contraindicating trials, nine of the 11 contraindicating trials used concentrations less than 0.5% w/w, while six of the contra-indicating trials used immersion times less than two minutes. Figure 3 shows the graphical interpretation of the bleach data for zero air exposure. From this diagram it is clear that 0.5% w/w is the minimum treatment concentration which reliably results in 100% *D. vexillum* mortality (blue vertical line in Figure 3). Note that the contours in Figure 3 are only valid for the lower right quadrant of the figure. The data are too sparse in the remainder of the figure to draw any further conclusions from the contouring.

Given that this analysis suggests that, for no air exposure, 0.5% w/w is the minimum bath concentration which reliably results in 100% *D. vexillum* mortality, we can return to the of trials which resulted in 100% *D. vexillum* mortality. Here four trials used

0.5% w/w bleach with no associated air exposure, with immersion times of 20 seconds, 30 seconds, two minutes and two minutes respectively giving an average of 1.2 ± 0.9 minutes. Therefore, given the possible variability in immersion times, the suggestion is that, as concluded by Denny (2008) and Denny and Hopkins (2007), two minutes is the minimum immersion time that should be applied for 0.5% w/w bleach.

In summary, for bleach:

- Bleach (sodium hypochlorite) was used in 26 treatment trials, and 15 of these resulted in 100% *D. vexillum* mortality.

- The currently available evidence suggests that bath treatments using bleach should be of at least 0.5% w/w concentration, with immersion times of at least two minutes. The evidence suggests that no additional air exposure is necessary.

This conclusion concurs with the recommendation of Denny and Hopkins (2007), and Denny (2008).

Brine

Three bath treatments using brine (62 ppt) resulted in 100% *D. vexillum* mortality, two for eight hour immersion times and one for 24 hour immersion. No air exposure was used (Table S5). In terms of contra-indicating results (Table S9), 25 trials using brine found less than 100% *D. vexillum* mortality. Twenty two trials actually resulted in *D. vexillum* growth rather than death. All contraindicating trials used immersion times of three hours or less. Immersion times of one hour or less produced *D. vexillum* growth.

The graphical method used for acetic acid and bleach is not directly applicable to brine treatments as although different studies report different concentrations, we assume all used saturated brine solutions. Hence in this case only immersion time is a relevant parameter and concentration is invariant. Figure 4 presents *D. vexillum* mortality versus ln(Immersion Time). The best-fit regression indicates 100% mortality for an immersion time of 1800 minutes or 30 hours.

The summary for brine is :

- Brine (sodium chloride) was used in 28 treatment trials, and 3 of these resulted in 100% *D. vexillum* mortality.

- The currently available evidence suggests that bath treatments using brine should be of at least 62ppt concentration (i.e. saturated), with immersion times of at least 30 hours, although shorter immersion times may be possible if more trial data confirms this. The evidence suggests that no additional air exposure is necessary.

Freshwater

Only three trials are available which used freshwater and returned 100% *D. vexillum* mortality (Table S5). One was for 24 hours immersion, one with 24 hours immersion followed by an hour of air exposure, and one of eight hours immersion followed by an hour of air exposure.

In all, 19 treatment trials using freshwater returned less than 100% *D. vexillum* mortality (Table S10). All contra-indicating freshwater trials were for immersion times of ten minutes or less, except one of eight hours. The one contra-indicating eight hour immersion trial resulted in only 80% *D. vexillum* mortality.

Figure 5 presents the regressions for bath immersion times against *D. vexillum* mortality for trials using freshwater that used air exposure (blue symbols and lines) and no air exposure (red symbols and lines). It is clear that air exposure as part of the treatment regime results in higher *D. vexillum* mortalities for the same immersion times. The best-fit regressions suggest that bath immersion times in freshwater should be of at least eight hours when air exposure is used as part of the treatment regime. Bath immersion times in freshwater should be of at least 17 hours when no air exposure is used as part of the treatment regime. However, this is from the regression alone. As only three trials actually resulted in 100% *D. vexillum* mortality, 24 hours immersion is suggested as a minimum as one trial did achieve this level of mortality.

In summary, for freshwater bath treatments;

- Freshwater was used in 22 treatment trials, and three of these resulted in 100% *D. vexillum* mortality.

- The currently available evidence suggests that bath treatments using freshwater should use immersion times of at least 24 hours, although more trial data are needed to confirm this. The evidence suggests that no additional air exposure is necessary.

Lime

Lime was used in 19 treatment trials, and none resulted in 100% *D. vexillum* mortality (Table S5). The currently available evidence does not support lime cannot being used as a bath treatment for *D. vexillum*.

Other Treatments

Twelve treatment trials used chemicals other than those described above. These were caustic soda (NaOH), citric acid ($C_6H_8O_7.H_2O$), waterglass (Na_2SiO_3) and hypoxia. Only two treatments resulted in 100% *D. vexillum* mortality, both using caustic soda (Table S5). Ten treatment trials using other chemicals resulted in less than 100% *D. vexillum* mortality (Table S11). Two of these were using caustic soda at immersion times and concentrations the same as those producing 100% *D. vexillum* mortality in the two previously noted trials. Hence, the currently available evidence is not enough to support caustic soda (NaOH), citric acid ($C_6H_8O_7.H_2O$), water-glass (Na_2SiO_3) or hypoxia as bath treatments for *D. vexillum*.

3.3 Criterion 8 - Treatments Effects on Aquaculture Species Survival

We now consider the available evidence of the impact of bath treatments on the various aquaculture species used in the published treatment trials. In all, 169 treatment trials reported survival rates for aquaculture species (Table S13).

Acetic Acid

Acetic acid was used in 98 trials, with the aquaculture species being Pacific oyster, green-lipped mussel seed and blue mussel seed used in 12, 81 and four trials respectively. One additional trial used blue mussel spat.

Acetic Acid - Pacific Oysters

All values of survival rates (%) of Pacific Oysters (Table S14) treated with acetic acid immersion baths were from Data Source 4 (Rolheiser et al., 2012). None of the trials included air exposure. It is evident that as immersion time increases, and as concentration increases, survival rates decrease (Figure 6). The treatment

combination of 4% w/w bath concentration and 3.5 minutes immersion time (the combination suggested above as effective for treating *D. vexillum*) results in a Pacific Oyster survival rate of approximately 25%.

Acetic Acid - Blue Mussel Seed

Only four trials used blue mussel seed, all using an acetic acid concentration of 5% w/w. Immersion times of longer than five minutes resulted in total mussel mortality, while immersion times of two minutes and 30 seconds resulted in survival rates of 86% and 74% respectively.

Acetic Acid - Green-lipped Mussel Seed

Eighty one trials used green-lipped mussels, but with a large variety of air exposure routines. Three trials using acetic acid concentrations of 10% w/w all had survival rates of <30%. Obviously this high treatment strength had an unacceptable impact on green-lipped mussel seed. The remaining 78 trials split into two distinct groups; “no rinse” trials (14) and the remainder (64).

The 14 “no rinse” trials were performed by Data Source 2 (Denny, 2008) and Data Source 8 (Forrest et al., 2007). They consisted of trials to simulate transport after bath treatments when the aquaculture species was not rinsed with either fresh or salt water. The idea behind these treatments was to enhance *D. vexillum* mortality during transport. However, they invariably resulted in low mussel seed survival. Two concentrations were used; 4% and 8% w/w. Immersion times varied from one to four minutes. Survival rates varied between 11% and 76%, with an average of 41% ± 20%.

The remaining 64 trials used concentrations between 0.1% and 8% w/w, with immersion times of between one and ten minutes. Average mussel seed survival was 96% ± 4%. In summary, green-lipped mussel seed exhibit high (>90%) survival rates for bath treatments up to 8% w/w, although rinsing after immersion is necessary.

The published trials reveal a puzzling difference between the reaction of Pacific oysters and green-lipped mussel seed to acetic acid. Oysters exhibited a survival response that was related both to bath concentration and immersion time (Figure 6), whereas green-lipped mussel seed exhibited survival rates, at least up to bath concentrations of 8% w/w, which were high whatever the bath concentration or the immersion time. If the results are correct, one possible explanation is that oysters

did not completely seal in a bath of acetic acid (at least in the published trials analysed here) and ingested some of the compound, whereas mussel seed ingested none of the treatment bath.

Bleach

No trials reported survival rates for Pacific oysters or blue mussel seed treated with bleach immersion baths. Eighteen published trials used bleach to treat green-lipped mussel seed. Bath concentrations varied between 0.5% and 2% w/w, with immersion times of between 0.5 and two minutes. High survival rates were reported for all trials, with an average of 96% ± 2%.

Brine

Twenty published trials examined the effect of brine bath treatments on Pacific oysters. Bath immersion times varied between 0.5 minutes and ten minutes. All treatments resulted in 100% oyster survival.

No trials reported survival rates for green-lipped mussel seed treated with brine immersion baths.

Two published trials examined the effect of brine bath treatments on blue mussel seed, and two on blue mussel spat. Bath immersion times were ten and 30 seconds. The treatments resulted in 92% and 94% mussel seed survival and 99% and 100% mussel spat survival.

Freshwater

In all, four published trials examined the effect of freshwater immersion on Pacific oysters. Immersion times were 30 seconds, one minute, five minutes and ten minutes and survival rates were 83%, 100%, 84% and 83% respectively.

A further four published trials examined the effect of a ten minute immersion in freshwater on green-lipped mussel seed, but with air exposure periods ranging from one to 24 hours. All resulted in 99% mussel survival.

Five trials examined the effect of long term immersion on green-lipped mussel seed. Immersion times were 24, 48, 72, 96 and 120 hours. Survival rates varied between 70% and 100% with an average of 87% ± 14%.

Two published trials examined the effect of freshwater immersion on blue mussel seed. Immersion times were eight and 24 hours and survival rates were 98% and 94% respectively.

Lime

A total of 13 published trials examined the effect of lime on Pacific oysters. Bath concentrations varied from 1% to 4% w/w, and immersion times from 30 seconds to ten minutes. Survival rates were variable, ranging from 44% to 100%. Although the lowest survival figures were for the 4% w/w baths, the relationship between immersion concentration and time and survival was varied.

No trials reported survival rates for green-lipped mussel seed or blue mussel seed treated with lime immersion baths.

3.4 Recommendations of Data Sources

Finally, the recommended bath treatments of each of the 12 papers used as Data Sources are presented in Table 5. These are the results of the authors' own consideration of their data, and the balance between Criteria 8 and 9.

3.5 A Brief Analysis of Criteria 1 to 7

Four treatment chemicals, acetic acid, bleach, brine and freshwater, are now examined in relation to Criteria 1 to 7, from the perspective of a shellfish farmer who wishes to treat a commercial quantity of shellfish. Table 6 summarises our conclusions for each of these criteria for these four chemicals.

Using vinegar to make up 5% w/w acetic acid bath treatments is probably safe to human operators using moderate care and personal protective equipment. Environmentally there is probably little concern, although discharging a large volume of spent treatment may in some jurisdictions need a licence. Vinegar is obviously a food-grade product. Commercial vinegar is certainly readily available from suppliers. Using vinegar means it is difficult to exceed the safe working concentration for many aquaculture species.

However, two aspects may rule vinegar out as a practical treatment chemical. One is cost, and the other is the practicality of transport. Transporting one tonne (or more) of vinegar onto a remote farm site in order to create bath treatments on a

commercial scale may not be straight forward, or cheap. However, cost versus benefit can only be judged by individual businesses, based on their own business model, suppliers etc..

Using glacial acetic acid to make up an acetic acid treatment bath may also not be practical. Fifty litres would be needed to make 1000 L of 5% w/w treatment. Transporting and handling 50 L of such a dangerous chemical is not trivial, and should not be undertaken without thorough training and extensive precautions. Additionally, small errors in making up treatment solutions could result in safe working concentrations being exceeded, thereby leading to excessive shellfish mortality. However, again the consideration of these factors is up to the individual aquaculture business, and their goods and services suppliers. The phrase “glacial acetic acid” may not be as easily understood as being a food product by the public as the term vinegar.

Moving onto bleach, just 33 L of 15% w/w NaClO domestic/commercial bleach is needed to make up a treatment bath of 1000 L of 0.5% w/w NsClO strength. This has a reasonable cost, and no severe environmental impact or legislative restrictions anticipated, although this may vary in different jurisdictions. Bleach is used extensively in other food processing industries, although for some businesses might not be acceptable from a food quality perspective. Making up treatment baths using bleach has the same problems as with using glacial acetic acid, i.e. if mistakes are made the safe working concentrations can easily be exceeded, resulting in high shellfish mortality.

However, two properties of bleach may mean it is impractical for on-farm use. The strength of bleach decays in storage, and is difficult to measure in the field. Hence knowing exactly what the concentration of a treatment bath is, and monitoring its strength through a treatment procedure may be very difficult. Sodium hypochlorite powder is not readily available to the general public, hence this rules out this way of avoiding the storage issues of liquid bleach. Finally, one of the breakdown products of bleach can be chlorine gas. Thus this could pose a severe risk to human health.

The third treatment option, brine is safe to use, has no detrimental environmental effects (if used within reason), is a “natural” product and probably in all jurisdictions has no legislative issues. However, the one main restriction in the use of brine may be the long treatment times needed (i.e. ≥30 hours, or perhaps ≥8 hours of further trials are performed to confirm this). These long times may not be practical in some business models.

Finally, freshwater, is obviously safe to use, generally free, and has no food quality, environmental or legislative restrictions. One restriction is that it is not always available at some remote coastal sites (in Scotland at least), and generally a flow of freshwater is needed in order to remove any salt from the system introduced by the immersed shellfish. Also, as with brine, long treatment times are needed (≥24 hours) which may not suit all businesses.

4. Discussion

While there are a whole range of treatment options available to an aquaculture business to control *D. vexillum* on a site (e.g. drying, spraying, wrapping), there is probably only one serious contender as a treatment for the removal of *D. vexillum* from live shellfish, and that is immersion in a chemical bath.

Switzer et al. (2011) examined the efficacy of mechanical cleaning (i.e. removing *D. vexillum* from oysters with soft wire brushes). Although the method worked well, it did not consistently result in 100% removal. They also noted that the method took approximately one minute 20 seconds per oyster. Hence a consignment of 100,000 oysters would take approximately 2,200 working hours, or 93 person-days, to manually clean. Hence manual cleaning of shellfish is clearly impractical on a commercial scale.

Additionally, Switzer et al. (2011) amongst others, also warn of the danger of creating small fragments of *D. vexillum*, and other fouling colonial organisms, which were found to stay viable and can disperse from the cleaning site and re-establish colonies. Hence, mechanical cleaning cannot currently be recommended to treat live shellfish carrying *D. vexillum* fouling.

Switzer et al. (2011) also reviewed the use of biological treatment methods (i.e. using an introduced predator of *D. vexillum*), and trialled the use of green sea urchins (*S. droebachiensis*) as a biological treatment for *D. vexillum* fouling on oysters. No cited study found fouling removal greater than 75%, and Switzer et al. (2011) concluded that biological treatments for fouling on shellfish were of limited potential. Carman et al. (2009) reviewed the use of the common periwinkle (*Littorina littorea*) as a biological control, but found it to be ineffective in an aquacultural context. Hence, biological controls can also not currently be recommended for the treatment of *D. vexillum* fouling on shellfish for the purposes of shellfish movements.

Carman et al. (2016) noted that air drying was used in the North American east coast aquaculture industry to rid live shellfish of fouling, including fouling by tunicates and

D. vexillum. They noted that exposure to the sun, variations in air temperature and relative humidity all affected the degree of shellfish mortality incurred by this method, and they did not investigate drying times any further.

It would be supposed that precipitation or high atmospheric humidity, may not only affect shellfish mortality, but might also compromise the efficiency of air drying for killing *D. vexillum* on shells. Air exposure was included in Denny and Hopkins (2007) but as part of a bath treatment operation. Hence air drying alone was not assessed. Owing to the possible variability of air drying under various atmospheric conditions, and the absence of any quantitative evidence as to its efficiency, air drying can also currently not be recommended for the removal of *D. vexillum* fouling from live shellfish.

Hence, chemical treatment must be considered. Spraying large quantities of live shellfish must have an associated risk of not dosing all surfaces with the required amount of treatment chemical, and expose the *D. vexillum* to this chemical for the required length of time, especially when one considers the complex shell morphology of some species along with the presence of other fouling organisms, both of which could shelter *D. vexillum* from spray. Thus we would conclude that the only consistent way to deliver a treatment is by immersion into a bath of the selected chemical.

Owing to the range of criteria a chemical treatment needs to meet in our situation in Scotland, there is a relatively limited range of chemicals available to the shellfish industry to control *D. vexillum* when it is present on a farm. The shellfish industry produces high quality, high value food products with an emphasis on the fresh and “green”, chemical-free origins of the product which must not be jeopardised either by the presence of *D. vexillum* itself, or by the treatment used to control it (Sharp et al., 2006). Hence the chemicals used are generally ones which have a proven record of safe use elsewhere in food production industries. These are acetic acid (the active ingredient of vinegar), bleach, brine (i.e. salt), freshwater and lime. Some other compounds have been experimented with (e.g. caustic soda, caustic acid, water-glass) but not in sufficient numbers of trials to demonstrate they are of any use in the treatment of *D. vexillum*.

Hence this review focused on five bath immersion treatments for the removal or killing of *D. vexillum* on live shellfish; acetic acid, bleach, brine, freshwater and lime.

Acetic Acid

From our analysis of 79 published treatment trials using acetic acid, we conclude that immersion in diluted acetic acid can kill *D. vexillum* (concentration ≥4% w/w, immersion times ≥3.5 minutes, 24 hours air exposure). However, at the concentrations required unacceptable shellfish mortality may occur, especially for Pacific oysters, and this should certainly be tested before commercial application. Additionally there are problems with the two methods of making up acetic acid baths. Vinegar is expensive and cumbersome, and glacial acid dangerous.

Forrest et al. (2007) made some important points about acetic acid as a treatment. They noted that the efficacy of a bath of acetic acid treatment may decline with use either through dilution, or consumption of the active ingredient. They suggest acetic acid concentrations may decay with time in storage, and they recommend that methods are developed to measure acetic acid levels in bath treatments on site during use. To do this, they are the only authors to explicitly describe pH measurements associated with acetic acid treatments, using a pH electrode device on-site as well as taking samples for subsequent analysis using titration.

From their results, Table 6 has been extracted. It can be seen that the pH range for the working concentrations of acetic acid bath treatments is small, and hence accurate measurements are needed. Forrest et al. (2007) found greatest error at the high pH values, and suggested that dilution of test samples could improve accuracy by moving towards lower concentrations.

Forrest et al. (2007) went on to confirm that acetic acid was stable when stored as a dilute treatment. However, they did find that through treatment the pH as measured by the electronic pH meter increased, whereas the acetic acid concentration as measured by titration remained stable. They suggested this was the effect of the dissolution of calcium carbonate from the treated mussel shells. Hence in-situ electronic pH may not be a good measure of bath efficacy, and they suggested the development of in-situ titration kits. Forrest et al. (2007) conclude by suggesting that acetic acid treatments do not work through lowering pH per se, but rather through the action of the compound itself.

Acetic Acid - Ambiguity of Chemical Treatment Specification

Several papers were rather ambiguous concerning the concentrations of chemical treatments used. For example, Sharp et al. (2006) refer to their acetic acid treatment as "5% acetic acid, $C_2H_4O_2$, vinegar". However, in the text and figure legends, treatments are described as "5% vinegar" as well as ""vinegar 5%". Hence it is not

clear whether undiluted vinegar was used (i.e. 5% w/w acetic acid), or vinegar diluted to 5% w/w (i.e. 5% vinegar or 0.25% w/w acetic acid).

McCann et al. (2013) note that they make up their acetic acid treatment (10% acetic acid) from “reagent grade” acetic acid in seawater, hence there is no ambiguity. Forrest et al. (2007) also make up a solution of acetic acid to a strength of 4% as that is “equivalent to the content of domestic vinegar”. When using vinegar, Carman et al. (2016) are the only authors which specify a type of vinegar, white vinegar, but they note its strength can vary from 3% to 5%. Rolheiser et al. (2012) say they use “regular-strength (5%) household vinegar”.

Acetic Acid - Use of Vinegar

Data sheets for white distilled vinegar, or cleaning vinegar, generally say the contents are acetic acid and water, with acetic acid concentrations generally stated as “1 to 5%”, although some brands state “<10%”. Operators should, therefore, note that if they use vinegar as a bath treatment for *D. vexillum* they should ascertain its true acetic acid content, or be confident that the concentration and duration of immersion they will use has the target effect on *D. vexillum* and on the aquaculture species. It is recommended that operators trial made-up treatments before they use them on a large scale.

Bleach

In our analysis we conclude that immersion in treatment baths made up with bleach can kill *D. vexillum* (concentration ≥0.5% w/w, immersion times ≥2 minutes, no air exposure needed), but there are problems storing bleach and measuring its strength during treatment. Additionally, there is limited information on the effects of immersion in diluted bleach on shellfish, and hence this needs to be tested before this treatment is applied commercially.

In industrial-scale trials, where 500 kg of seed mussels were lowered by crane into 1000 L of 0.5% w/w sodium hypochlorite, operators found unacceptable seed mussel mortality (>50%) especially when mussels were not rinsed after dipping (Denny and Hopkins, 2007). Another problem reported was maintaining chlorine levels in the treatment during use, as well as actually measuring the chlorine levels using commercially available tests which proved to be unreliable. It was suggested that further work was needed in order to provide “buffers” around treatment times and concentrations so that these were not so highly critical to the success of the treatment.

Bleach - Ambiguity of Chemical Treatment Specifications

As with acetic acid, some published studies were ambiguous with respect to the concentration of active ingredient in bleach baths. Denny et al. (2008) were unambiguous with regards some treatments. Bleach is referred to as bleach rather than sodium hypochlorite (NaClO). In fact, the paper trials two makes of domestic bleach, so are obviously aware that domestic bleach can have varying contents.

Domestic bleach is actually a solution that can contain various concentrations of sodium hypochlorite typically below 10% w/w, normally 5% w/w, along with other elements such as surfactants, soaps and perfumes (Table S17, Appendix). As with acetic acid, an operator using domestic or industrial bleach to make up a bath treatment must reassure themselves of the chlorite concentration in the selected product, remembering that chlorite content decays with time in storage. Once again, tests are recommended before use.

Brine

In our analysis, we conclude that immersion in brine can kill *D. vexillum* (concentration ≥62ppt, immersion time ≥30 hours). The analysis of all available data suggests long immersion times are needed, but shorter immersion times may be possible if more trial data confirms this. Trials of the effect of brine on shellfish mortality are all for short (<10 minutes) immersion, and hence further trials are needed for the long duration immersion times needed to kill *D. vexillum*.

Although brine is recommended in the USA for the treatment of boring sponges in oyster culture (Carman et al., 2016), *D. vexillum* was noted to grow following brine treatments (Rolheiser et al. 2012). Carman et al. (2016) make the point that the tolerance of shellfish, specifically mussels, is generally unknown.

Freshwater

For freshwater bath treatments we conclude that freshwater baths can kill *D. vexillum* (immersion time ≥24 hours). However, the published evidence is limited and further trials are needed before application in a commercial setting. Shellfish mortality using freshwater seems acceptable, although should be tested prior to any commercial application.

Carman et al. (2016) make the comment that freshwater baths should be kept aerated. Forrest and Blakemore (2006) also note that their freshwater treatments were aerated. This implies that shellfish may still respirate in freshwater, and users may wish to take this into consideration.

Lime

From the evidence we found in our review, we ruled out lime as a treatment chemical for *D. vexillum,* as no published trial resulted in 100% *D. vexillum* mortality.

Others

Some authors also mention other treatments that have been tried: Cement powder, burning (petrogen torch), and hypoxia (McCann et al. 2013); formalin, detergents, UV light, steam, hot water and electricity (Rolheiser et al., 2012); high pressure washing and hot water (Forrest and Blakemore, 2006); citric acid (Locke et al. (2009); citric acid and sucrose (Sharp et al., 2006); puncturing (Locke et al., 2009). As these have not been followed up on by further trials, nor their use documented in the shellfish industry, we should consider them ineffective or have disadvantages which make them unusable.

Rinsing

Several studies noted the importance of rinsing, or not rinsing, treated shellfish after a bath treatment, but the evidence that rinsing is needed is not clear or unequivocal (Denny and Hopkins, 2007). Some studies recommended not rinsing in order to allow the bio-cidal effect of the treatment on *D. vexillum* to continue after leaving the bath. However, Denny and Hopkins (2007) point out that at a commercial scale, the lack of rinsing to stop the effect of treatments, especially acetic acid and bleach, could lead to high shellfish mortalities.

As leaving shellfish un-rinsed following a bath treatment introduces a random element (i.e. depending on how long a fouled shellfish is left covered by the treatment after leaving the bath), then it is suggested here that shellfish are rinsed, preferably in freshwater if available but in sea water if not (see below), after a chemical bath treatment. At least this way mortalities are the result of known exposure times, which can be adjusted, rather than some random effect dependent on the drainage and drying characteristics of the treatment chemical and the target shellfish. However, this suggestion is yet to be fully tested in a commercial trial.

Freshwater or Seawater Solute

Several studies explicitly test the difference between using freshwater or sea water to dilute down acetic acid or bleach to treatment concentrations (e.g. Denny and Hopkins, 2007;). Both sea water and freshwater can be used, but it is suggested here that if freshwater is available at a site, it is used instead of sea water. This suggestion is based on the results of Denny and Hopkins trials by commercial shellfish farms that reported higher shellfish mortalities when sea water was used. It might be speculated that shellfish are more likely to open or take in water in salt water than in freshwater. However, this suggestion is yet to be fully tested in a commercial trial.

Environmental Impact of Treatments

Few studies considered the environmental impact of releasing bath treatments into the marine environment. As Locke et al. (2009) point out, maintaining good water quality in the vicinity of aquaculture sites is particularly important, from the perspective of both the industry and the regulators.

Locke et al. (2009) looked extensively at the lethal and non-lethal impact of treatment chemicals (acetic acid, lime and other acids) on non-target organisms including bacteria, shrimp and fish. They also attempted to estimate the change in pH resulting from the discharge of lime from all aquaculture within a water body, such as an estuary. Switzer et al. (2011) did measure the pH of receiving marine waters when they released lime into the environment, but they could detect no change in local pH and concluded the chemical was quickly dispersed into the ambient seawater. Rolheiser et al. (2012) note that there is a need for the proper disposal of treatments after use, and that many constitute controlled substances which will require to meet various thresholds and rules for use.

Treatment Scales

All studies of bath treatments used in this review operated on an experimental scale, where few tens of shellfish were treated. Rolheiser et al. (2012) concluded that more work was needed on the problems of scaling up treatments to an industrial scale. Denny and Hopkins (2007) did try the industrial scale application of a bleach treatment, with 500 kg of fouled mussels lowered into a 1000 L bath by crane, but found problems with treatment stability, treatment strength measurement and unexpectedly high shellfish mortalities.

Recommendations for Future Studies

Finally, while conducting this review of bath treatments, some additional conclusions and recommendations became evident which may help future practitioners, study authors and publishers:

Treatment Ambiguity: Published trials of bath treatments, and spray treatments, should be explicit and accurate concerning the content of the treatments they use. While it is to be hoped that the outcome of a treatment is not dependent on exact concentrations of treatment chemical, as this will make them difficult to implement in a real commercial setting, trials should still be unambiguous concerning the concentration of the active ingredient in a treatment. For example, vinegar and bleach are not the chemical elements of concern, acetic acid and sodium hypochlorite are.

Chemical Bath Details: Published trials should also provide measurements of treatment content and properties during use. For example, the salinity, pH, acetic acid content or chlorine content of bath treatments should be monitored through the duration of a treatment and published, as should treatment temperatures. Currently just the target concentration of a dilution is listed in most reports of trials, not the concentrations actually achieved and maintained through the trial.

Sources of Chemicals: If operators use domestic or industrial bleach or vinegar to make up bath treatments they are advised to 1) find out what the true contents are from the manufacturer, 2) remember that the strength of bleach decays with time in storage, 3) perform pre-use tests on a small scale, to confirm *D. vexillum* and shellfish mortalities, before trialling treatments on a large scale.

Measurement of Bath Strength: Field kits and test methods need to be developed in order to monitor the efficacy of bath treatments through use in a commercial aquaculture setting. Such field measurement systems are particularly needed for acetic acid and bleach.

Freshwater or Saltwater Diluent: If available, freshwater is the preferred diluent to sea water when making up large volume bath treatments. It is suggested that freshwater diluent may result in lower shellfish mortalities. When water is used as a diluent, it should be stated if this was freshwater or salt water (and, if used, of what salinity).

Recording Mortality: Studies of all treatments in general should be more explicit, accurate and quantitative concerning the methodologies used to record fouling and shellfish mortalities.

Assessing D. vexillum Mortality: *D. vexillum* mortality currently can only be judged by providing it with ideal regenerating conditions and monitoring it for at least three weeks. However, a more direct test of mortality for *D. vexillum* should be developed (e.g. a cell viability assay).

Basic Biological Studies: Almost no published study considers the mechanism by which the various treatments kills *D. vexillum*. Basic biology and chemistry should be used to understand the mechanisms leading to mortality. This may suggest alternative treatments, or optimum applications of treatments.

Scaled-up Trials: Few published trials exist of commercial scale applications of bath treatments. More such studies should be performed and published, although these require the full cooperation of the business concerned, as applying the rigour scientific trials need may conflict with how an operation is performed on a commercial farm.

5. Conclusions

The use of the nine criteria related to immersive bath treatments for removing invasive non-native species fouling from shellfish aquaculture species provided us with a useful framework to analyse proposed treatments in the context of the aquaculture industry and regulatory framework in Scotland, and select one for field trial.

Acetic Acid: For our own purposes, owing to the low degree of survival of Pacific oysters at the treatment concentrations which kill *D. vexillum*, the possible large variability of the outcomes for *D. vexillum* and the practical difficulties associated with using either vinegar or glacial acetic acid on a commercial scale, this treatment was not selected for field trial.

Bleach: We rejected bleach as a trial treatment owing to the practical difficulties associated with its use on a commercial scale, as well as the negative connotations the use of this chemical might have for a business focusing on high quality “organic” produce.

Brine: Owing to the long soak times needed, and the equipment needed on site to manufacture and maintain brine baths on a commercial scale, brine has been initially rejected for commercial trial in Scotland, but this decision may be revisited.

Freshwater: Freshwater treatment baths of commercial size probably require a local source of running freshwater in order to remove any introduced salt. In our own case such a source of freshwater is supplied on site by a deep freshwater fast-flowing stream and the immersion of commercial numbers of shellfish in freshwater was considered by the shellfish farm as entirely practical. Hence we have selected this treatment for onwards testing, despite the limited number of published trials. By conducting field trials we hope to add to the body of knowledge concerning the use of a freshwater bath treatment to remove *D. vexillum* from live Pacific oysters.

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8. Tables

Table 1: Summary of treatments available to a shellfish farm for the control and containment of *D. vexillum*, and the circumstances under which each one might be used. The terms “Non-Lethal” and “Lethal” are with reference to the aquaculture species under consideration. The concentration of the active ingredient in a spray treatment can be increased when used on equipment devoid of the aquacultural species, as opposed to equipment still containing live shellfish, where excessive mortality of the shellfish must be avoided.

Circumstance	Treatment
Live shellfish moving off a farm (e.g. for “growing on”)	Bath Treatments
Live shellfish on a farm	Bath Treatments Spray Treatments (Non-Lethal)
Moveable equipment	Air Drying Spray Treatments (Non-Lethal and Lethal)
Immoveable equipment (exposed for some of the time)	Spray Treatments (Non-Lethal and Lethal) Wrapping
Immoveable equipment (always submerged)	Wrapping

Table 2: Summary of the published Data Sources (DS) used in the meta-analysis of bath treatments. AA - Acetic acid - $C_2H_4O_2$; BL - Bleach - NaClO; BR - Brine – NaCl; FW - Freshwater – H_20; LI - Lime – $Ca(OH)_2$; OT – Other treatments (see text). Source DS12 tried air drying and heat treatment. Note (*) that DS1 is a data report which formed the basis of DS2, but it presented additional data. Shellfish species are: Green-lipped mussels - *Perna canaliculus*; blue mussels - *Mytilus edulis*; Pacific oysters - *Magallana gigas*.

DS	Source	Region	Aim of Treatments	Pest Species	Aquaculture Species	AA	BL	BR	FW	LI	OT
1	Denny and Hopkins (2007)*	New Zealand	To reduce spread of INNS via transport of seed mussels.	*Didemnum vexillum*	Green-lipped mussel (seed, 20-60 mm)	Y air	Y air		Y air		
2	Denny (2008)	New Zealand	To reduce spread of INNS via transport of seed mussels	*Didemnum vexillum*	Green-lipped mussel (seed, 20-60 mm)	Y	Y			Y	Y
3	Switzer et al. (2011)	British Columbia	To control *D. vexillum* and other fouling of oysters internally on an oyster farm	*Didemnum vexillum (mixed with other tunicates)*	Pacific oyster (110-150 mm)					Y	
4	Rolheiser et al (2012)	British Columbia	To control *D. vexillum* and other fouling of oysters internally on an oyster farm	*Didemnum vexillum*	Pacific oyster (75-90 mm)	Y		Y	Y	Y	
5	McCann et al. (2013)	Alaska	Control or eradication method for response to an INNS	*Didemnum vexillum*	None	Y	Y	Y	Y		Y
6	Carman et al. (2016)	New England	To control tunicates (including *D. vexillum*) on juvenile mussels	*Didemnum vexillum*	Blue mussel (seed, 15-25 mm)	Y air		Y air	Y air		
7	Carver et al. (2003)	Nova Scotia	To control biofouling (solitary ascidian)	*Ciona intestinalis*	Mussel (>20 mm) Oyster (>20 mm)	Y	Y	Y	Y	Y	
8	Forrest et al. (2007)	New Zealand	Test of acetic acid on multi-species fouling in mussel aquaculture	Multiple species inc. tunicates	Green-lipped mussel (seed, 26-56 mm)	Y air					
9	LeBlanc et al. (2007)	Prince Edward Island	Managing tunicates in shellfish (mussel) aquaculture	*Styela clava*	Blue mussel (seed, 30 mm)	Y					Y
10	Locke et al. (2009)	Prince Edward Island	Effect of fouling treatments in aquaculture on non-target organisms in the environment	*Ciona Intestinalis*	None	Y					Y
11	Forrest and Blakemore (2006)	New Zealand	To control the spread of INNS (seaweed) via transport of seed mussels and equipment	*(Undaria pinnatifida* Not used here)	Green-lipped mussel (seed, 16-36 mm)				Y		
12	Sharp et al. (2006)	Prince Edward Island	To find environmentally friendly treatments to remove biofouling from spat collectors	(Green algae, Not used here)	Green-lipped mussel (spat, 4-5 mm)	Y		Y			

Table 3: Chemical names used in this report.

Common Name	Chemical Formula	Comments	Concentrations
Acetic Acid	$C_2H_4O_2$	- The active ingredient in domestic vinegar - In its pure form often referred to as glacial acetic acid	X% w/w acetic acid in this report means X g of glacial acetic acid diluted with (100-X)g of freshwater
Bleach	NaClO	- Sodium hypochlorite - This is the active ingredient in domestic “bleach”, which is a compound itself (See Appendix for details of the composition of domestic bleach).	X% w/w bleach in this report means X g of sodium hypochlorite diluted with (100-X)g of water
Brine	NaCl	- Salt water - Brine is assumed to be water that is saturated in salt, i.e. no more salt can be dissolved in the solution of brine at the temperature at which it is being used.	Various authors have reported concentrations ranging from 2% w/w NaCl (i.e. 20 parts per thousand) to 7% w/w NaCl (70 parts per thousand)
Freshwater	H_2O		Assumed to be kept below 2 ppt, i.e. 2g of sodium chloride diluted in 998g pure H2O. This is an arbitrary value taken to account for any salt introduced during the shellfish immersion process.

Table 4: Summary of numbers of data points extracted from the twelve data sources used in the review, by fouling species and aquaculture species. *Dvex* – *Didemnum vexillum*.

Treatment		Fouling				Aquaculture Species			
		Total	***Dvex***	**Other Tunicate**	**Mixed Fouling**	**Total**	**Pacific Oyster**	**Green-Lipped Mussel (seed)**	**Blue Mussel (seed)**
Acetic acid	$C_2H_4O_2$	79	43	3	33	98	12	81	5
Bleach	NaClO	26	25	1	0	18	0	18	0
Brine	NaCl	28	26	1	1	24	20	0	4
Freshwater	H_2O	22	20	1	1	15	4	9	2
Lime	$Ca(OH)_2$	19	17	1	1	13	13	0	0
Other		12	10	2	0	1	0	0	1
Total		186	141	9	36	169	49	108	12

Table 5: Summary of the final recommendations made by the authors of the 12 Data Sources used in the meta-analysis of bath treatments.

DS	Source	Region	Recommended Treatment	Quoted Summary Efficacy
1	Denny and Hopkins (2007)	New Zealand	Dip seed mussels in 0.5% w/w bleach for 2 minutes - But, at commercial scale there may be problems with bleach - Freshwater treatments need to be examined further – drawback is long soak times	"100% Effective"
2	Denny (2008)	New Zealand	Dip seed mussels in 0.5% w/w bleach for 2 minutes	"100% Effective"
3	Switzer et al. (2011)	Canada	Lime and mechanical treatments of oysters both reduced *D. vexillum* coverage, but both required further development. - Neither resulted in 100% *D. vexillum* mortality.	"85% to 96%"
4	Rolheiser et al (2012)	Canada	Dip oysters into 3-5% w/w lime for 5 minutes	"Removes total biofouling, *D. vexillum* fouling and predatory starfish"
5	McCann et al. (2013)	Alaska	Dip *D. vexillum* in 1) 10% w/w acetic acid 2 minutes 2) 1% w/w bleach for 10 minutes 3) FW for 4 hours 4) 62ppt Brine for 1 day - But effect on shellfish was not a concern of this study	"100% treatment efficacy"
6	Carman et al. (2016)	USA	Dip seed mussels in FW for 8 hours	"removes tunicates while maintaining high survivorship among juvenile mussels"
Other Tunicates				
7	Carver et al. (2003)	Canada	Dip mussel/oyster in 5% w/w acetic acid for 15 to 30s - For *Ciona intestinalis*	"Total Mortality"

8	Forrest et al. (2007)	New Zealand	Dip mussels into 4% w/w acetic acid for at least 1 minute, rinse in seawater, and transport (in air) - For mixed tunicates - Transport adds to stress on biofouling – an essential step - If don't rinse then unacceptable mussel mortality	"Eliminate many soft-bodied fouling organisms"
9	LeBlanc et al. (2007)	Canada	Not purpose of study	
Unrelated Fouling Organisms				
10	Locke et al. (2009)	Canada	Not purpose of study	
11	Forrest and Blakemore (2006)	New Zealand	Immerse seed mussels in freshwater for 2 days - For *Undaria pinnatifida*	"complete *Undaria* mortality"
12	Sharp et al. (2006)	Canada	Dip mussel ropes into 300 ppt brine for 20 seconds - For green algal mats - May need 2 or 3 treatments per season	"Effective reducing fouling"

Table 6: List of four selected treatments used in the studies reviewed here, alongside an assessment against seven of the nine criteria for a bath treatment to control *Didemnum vexillum*. Text highlighted in red note criteria conditions that may mean that chemical is unusable for a standard application in industry. Text highlighted in green note criteria that may be unacceptable in certain circumstances (i.e. long treatment times). Notes: 1 – Cost of chemicals in Euro as at November 2016, based on internet shopping prices (excl P+P). Does not include costs of tanks, filters etc. Volume given in brackets is that needed to make up 1000 L of bath treatment, solute is freshwater). 2 – available from public shopping outlets on the internet (in UK). 3 – This assumes discharge into the environment of a treatment bath of 4000 L or less into a tidal environment. 4 – Operators must check the situation in each regulatory area. 5 - In solution, strength (efficacy) decays in storage. 6 - Can contain other chemicals (e.g. NaOH, soaps, perfumes etc.) with unknown effects. 7 - Strength of treatment declines with use as chlorine oxidises with organic matter. 8 – decay products can pose hazards. Chlorine-based disinfectants can form non-effective chloramines in the presence of organic matter, whilst all oxidisers are chemically reduced by organic matter in the water. Increased pH and dilution of the active compound by addition of salt water entrained in the oyster bags will also affect the efficacy of the treatment. In some circumstances, chlorine based products can produce chlorine gas. 9 - Exchange rates at time of study 05/11/16 - 1.00 GBP: 1.25 USD:1.12 EUR. (See Table S16 for details of cost calculations). 10 – Difficulty monitoring chlorine levels. 11 – Need Personal Protective Equipment to handle (gloves, face mask, goggles etc.). Needs COSHH (Control of Substances Hazardous to Health regulations in UK) and risk assessments. 12 – SWC is a safe working concentration for aquaculture species. 13 - this is the volume needed to make up a 1000 L immersion bath of the stated concentration.

Chemical	Formula	Concentration	Criterion 1 Safe	Criterion 2 Environmental	Criterion 3 Legal[3,4]	Criterion 4 Marketable	Criterion 5 Acceptable Cost[1] Cost / 1000L	Criterion 6 Available	Criterion 7 Practical
Acetic acid	**$C_2H_4O_2$**	5% w/w (Using Vinegar)	Needs moderate care[11]	None expected	May need discharge licence	A natural food product	**650GBP[9] (1000L[13])**	Yes[2]	**Large volumes need to be transported** Can exceed SWC[12]
		5% w/w (Using diluted glacial acetic acid)	**Severe hazards to operator[11]**	None expected	May need discharge licence	Might have a negative view by public	170GBP[9] (50L[13])	Yes[2]	Can be diluted in seawater Can exceed SWC[12]
Bleach	**NaClO**	0.5% w/w - From domestic/commercial bleach	Needs moderate care[11] **Decay products[8]**	None expected	Will need discharge licence	Used extensively in food industry but some businesses may object as being a man made chemical	41GBP[9] (33L[13])	Yes[2] Strengths available: Domestic, 5% w/w Industrial, 15% w/w	**Storage decay[5]** Chemical mixture[6] **Use decay[7]** **Difficult to monitor[10]** Can exceed SWC[12]
		0.5% w/w - from sodium hypochlorite powder	Needs moderate care[11] **Decay products[8]**	None expected			**Unavailable**	**No**	**Use decay[7]** **Difficult to monitor[10]** Can exceed SWC[12]
Brine	**NaCl**	Saturated	Yes	None expected	Yes	A natural food product	30GBP[9] (62kg[13])	Yes	Yes **But may need 30 hours** No SWC concerns[12]
Freshwater	**H_20**	N/A	Yes	None expected	Yes	No issue	Zero (1000L[13])	Yes If farm has freshwater source	Yes **But needs 24 hours** No SWC concerns[12]

Table 7: The pH values of various acetic acid concentrations, with sea water as the diluent.

Acetic Acid Concentration % w/w (in seawater)	pH
2	2.54
3	2.43
4	2.35
5	2.29
6	2.24
7	2.20
8	2.17
9	2.14
10	2.12

9. Figures

Figure 1: Interpolated values of *D. vexillum* mortality rates (%) for variable acetic acid treatment concentrations (%) and immersion times (minutes) with anomalies removed. No treatment used air exposure as part of the routine. Concentrations and immersion times have been natural log transformed. Mortality rates (%) have been contoured in 20% intervals. Red values of mortality rate give the raw data (see Tables S5 and S6). The blue vertical and horizontal lines are drawn at a bath concentration of 4% and an immersion time of 3.5 minutes. The 0% contour is also highlighted in blue and 100% in red. See Figure S1 (Appendix) for details of data smoothing.

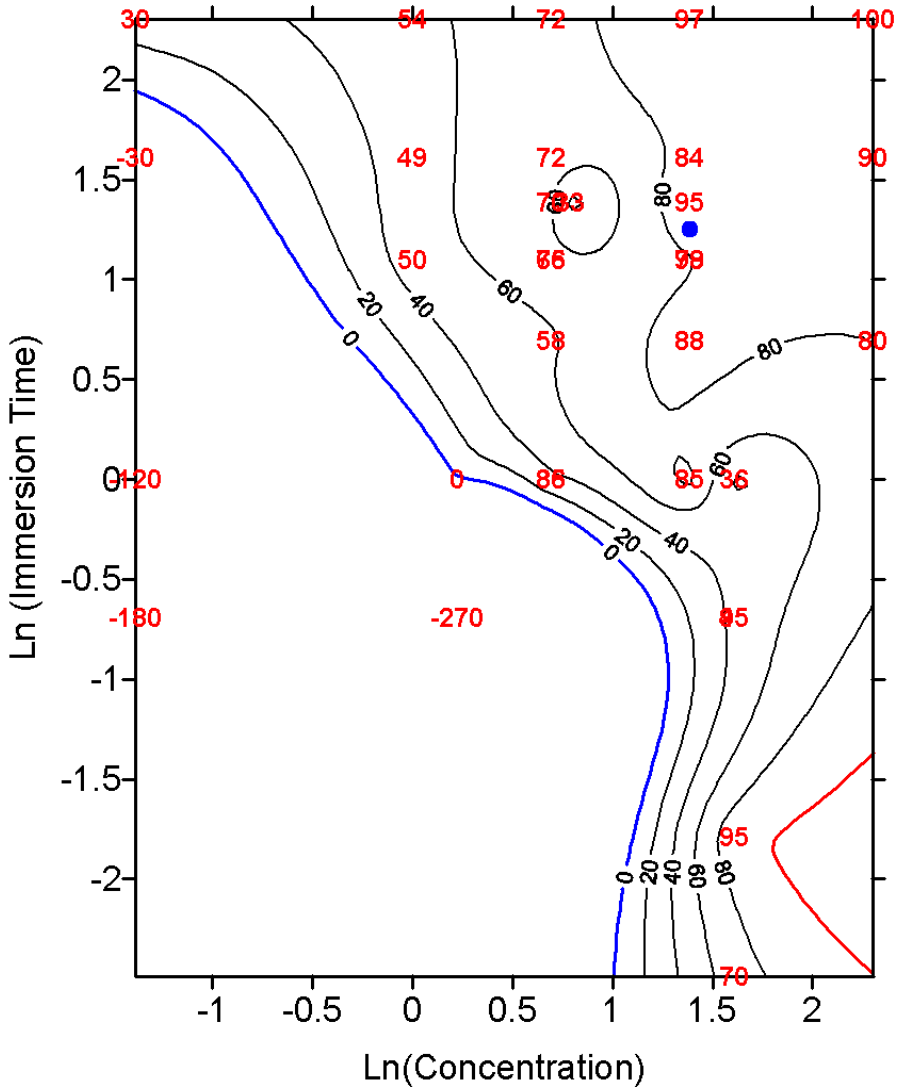


Figure 2: Relationship between bath immersion time and *D. vexillum* mortality for acetic acid trials with concentrations in the range 2% to 4% w/w, using 24 hour air exposure and post-treatment rinsing. Immersion time (minutes) has been transformed by natural logarithms. The thin line is a best-fit regression. The best-fit regression indicates 100% mortality at $e^{1.24}$ = 3.5 minutes. Dashed lines show 95% confidence intervals.

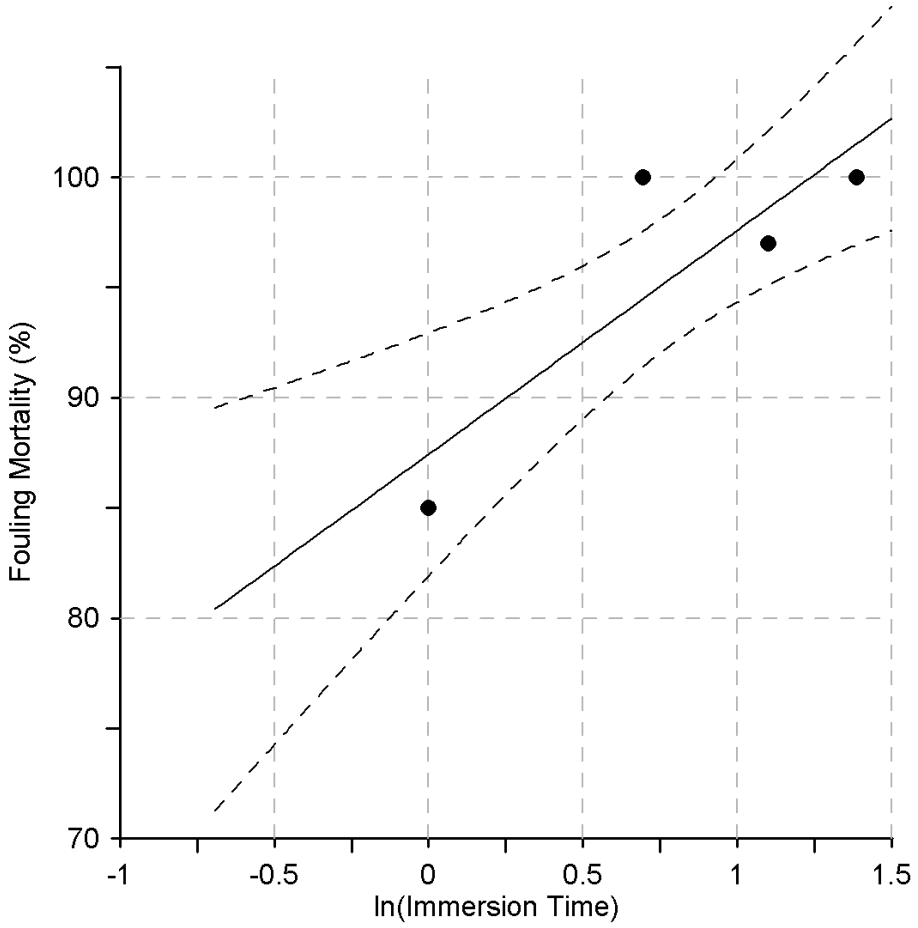


Figure 3. Interpolated values of *D. vexillum* mortality rates (%) for variable bleach treatment concentrations (% w/w) and immersion times (minutes). No treatment used air exposure as part of the routine. Concentrations and immersion times have been natural log transformed. Mortality rates (%) have been contoured in 20% intervals. Red values of survival rate give the raw data (see Table S5 and Table S8). The blue vertical and horizontal lines are drawn at a bath concentration of 0.5% w/w and an immersion time of two minutes. The 100% contour is highlighted in red.

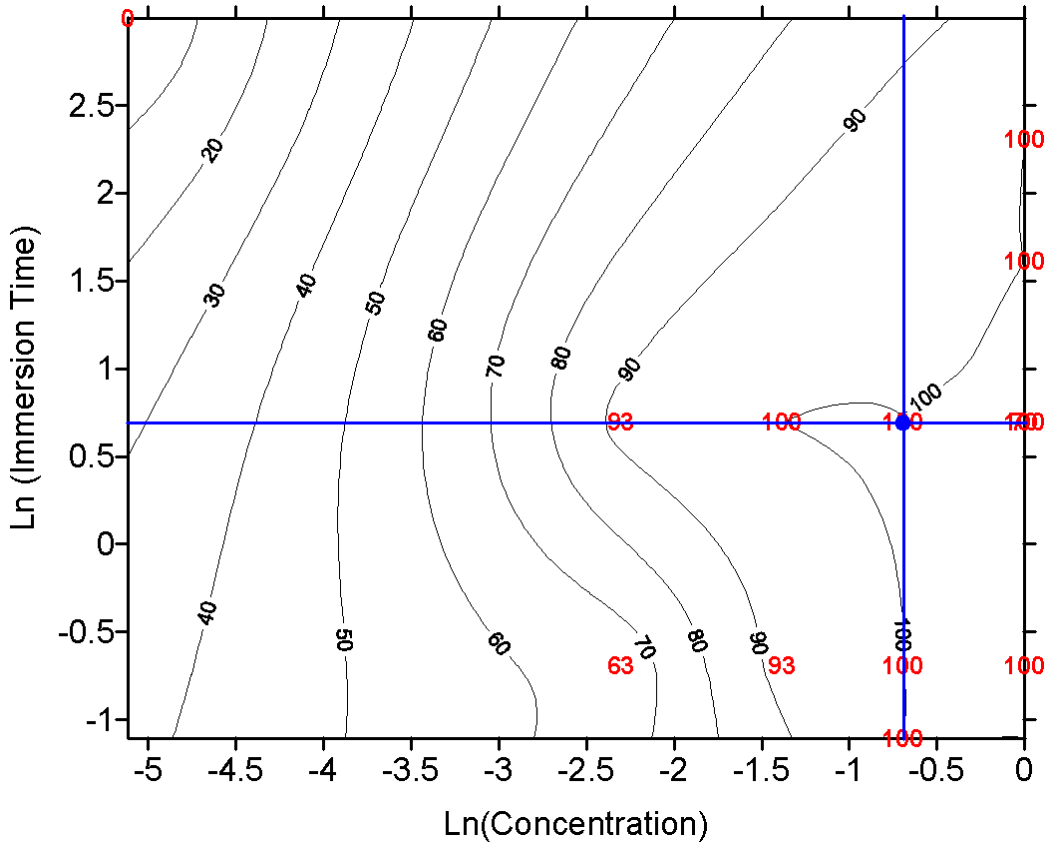


Figure 4: Relationship between bath immersion time and *D. vexillum* mortality for saturated brine trials. Immersion time (minutes) has been transformed by natural logarithms. The thin line is a best-fit regression. The best-fit regression indicates 100% mortality at $e^{7.5}$ = 1800 minutes or 30 hours. Dashed lines show 95% confidence intervals.

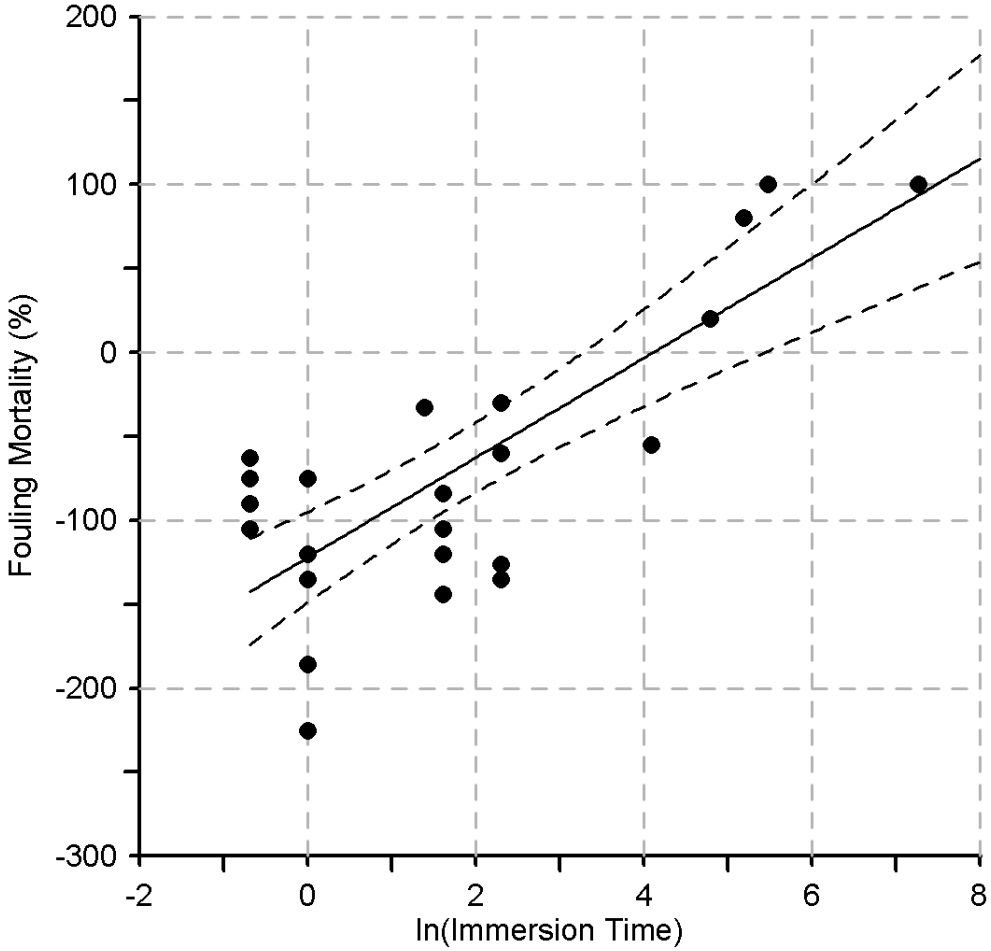


Figure 5: Relationship between bath immersion time and *D. vexillum* mortality for freshwater trials. Immersion time (minutes) has been transformed by natural logarithms. The thin line is a best-fit regression. Red symbols and fits lines are for trials with no air exposure. Blue symbols and lines are for trials with some degree of air exposure (see Table S10). The best-fit regression for trials with no air exposure indicates 100% mortality at $e^{6.9}$ = 1040 minutes or 17 hours. Dashed coloured lines indicate 95% confidence limits. (The air exposure regression results in 100% mortality at $e^{6.2}$ minutes, or 8 hours).

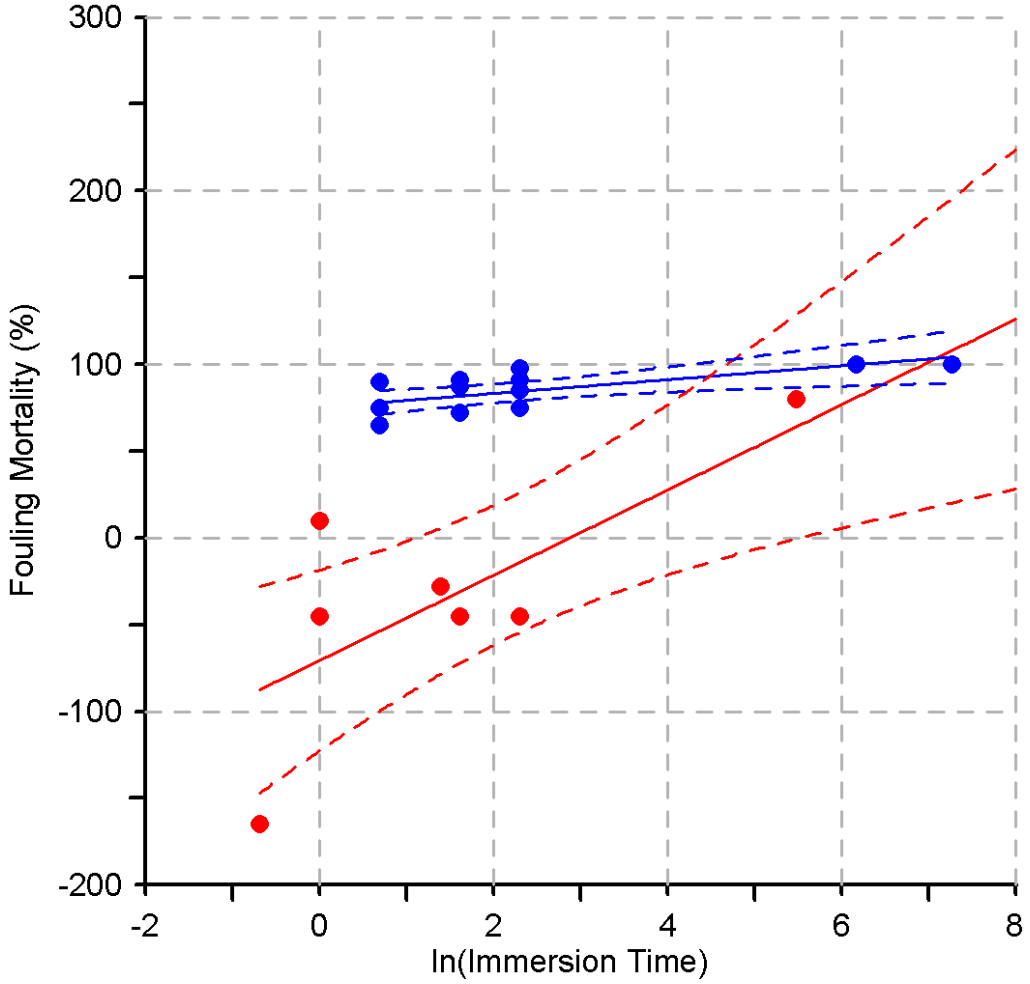
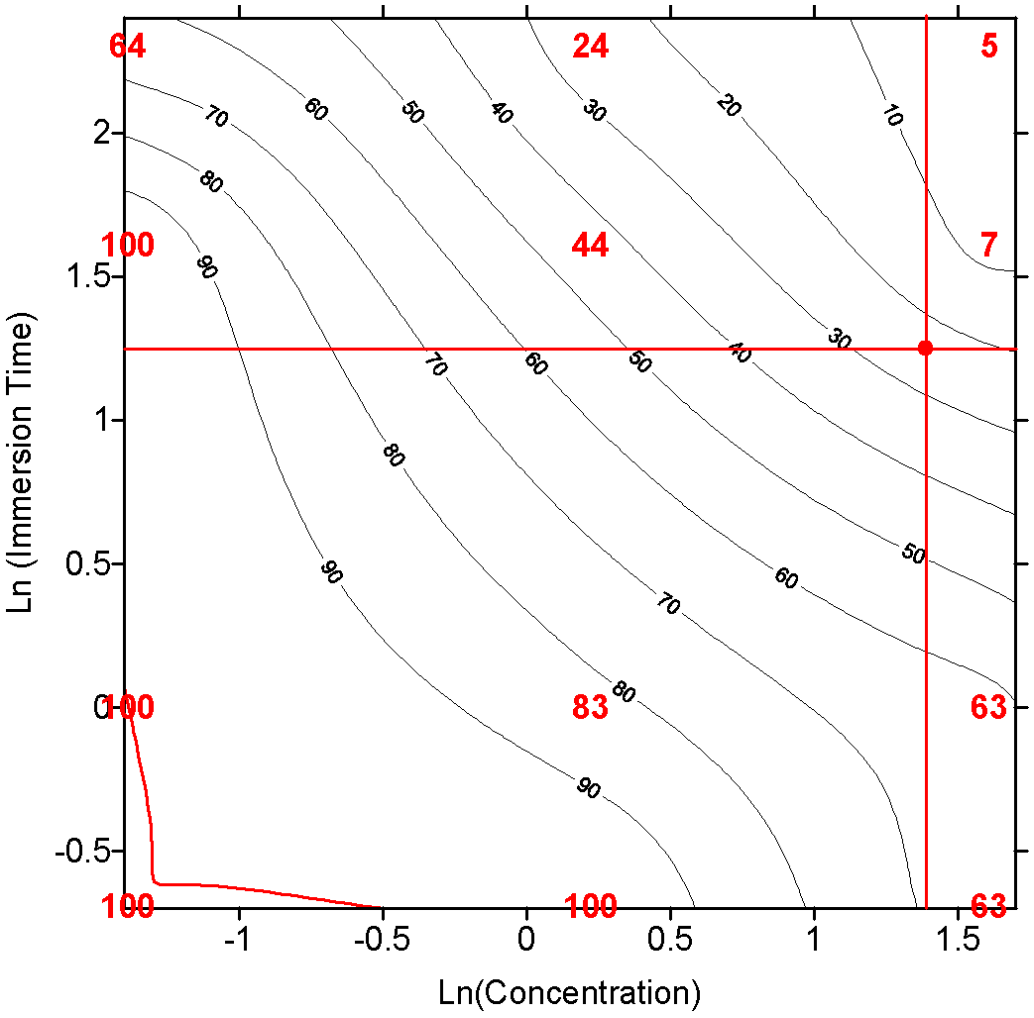


Figure 6: Interpolated values of Pacific oyster survival rates (%) for variable acetic acid treatment concentrations (% w/w) and immersion times (minutes). No treatment used air exposure as part of the routine. Concentrations and immersion times have been natural log transformed. Survival rates (%) have been contoured in 10% intervals. Red values of survival rate give the raw data (see Table 13). The red lines are drawn at bath concentrations of 4% w/w and immersion time of 3.5 minutes. The 100% contour is also highlighted in red.



Appendix

Table A1 - Summary of the tests for fouling species mortality determination in the published Data Sources (DS)
Table A2 - Summary of the tests for aquaculture species mortality determination in the published Data Sources (DS)
Table A3 - Description of all ancillary data extracted from the 12 data sources
Table A4 -. Summary of the experimental set ups in the published Data Sources (DS)

Table A5 - All trials which resulted in 100% *D. vexillum* (Foul Code - D) or multi-species (Foul Code - M) mortality
Table A6 - Summary of contraindicating acetic acid trials with treatment concentrations between 2% and 4%
Table A7 - Acetic acid treatments which resulted in <100% *D. vexillum* mortality

Figure S1 - The evidence for the effect of acetic acid on D. vexillum is further examined using a graphical method

Table A8 - Bleach contraindications
Table A9 - Brine contraindications
Table A10 - Freshwater contraindications
Table A11 - Other treatments contraindications
Table A12 - Summary of conclusions from the currently available evidence relating to bath treatments which result in 100% *D. vexillum* mortality.

Table A13 - Effect of treatments on aquaculture species.
Table A14 - The survival rates (%) of Pacific Oysters treated with acetic acid immersion baths.
Table A15 - Summary of conclusions from the currently available evidence relating to the effect of bath treatments on various aquaculture species.

Table A16 - Details of cost / dilution calculations used in Table 16, main text

Table A1: Summary of the tests for fouling species mortality determination in the published Data Sources (DS) used in the meta-analysis of bath treatments. "Where" indicates where in the Data Source the mortality test results were extracted from. "Days" is number of days between treatment and mortality test. "Converted Units" is a description of what the Data Source units were converted to. This is further described by "What 0% means" and "What 100% means", which is self-explanatory. "Conversion" finally confirms what conversion was needed to move from the Data Source units to the units used in this study.

DS	Source	Where	Mortality Test details	Days	Converted Units	What 0% means	What 100% means	Conversion
1	Denny and Hopkins (2007)	Figs 3, 6, 14, 15	No details of mortality test provided. Assume it is same as DS02 (Text).	14	% mortality	All *D. vexillum* alive	All *D. vexillum* dead	No conversion needed
2	Denny (2008)	Text	% *D. vexillum* mortality after 2 weeks back in sea	14	% mortality	All *D. vexillum* alive	All *D. vexillum* dead	No conversion needed
		Figs 2, 4	% *D. vexillum* mortality after 2 weeks back in sea	14	% mortality	All *D. vexillum* alive	All *D. vexillum* dead	No conversion needed
		Figure 3	% *D. vexillum* mortality after 10 days back in sea	10	% mortality	All *D. vexillum* alive	All *D. vexillum* dead	No conversion needed
3	Switzer et al. (2011)	Figure 1	*D. vexillum* fouling coverage (score 1 - 10 from photos). Aug value compared to July, 1 month after July treatment	30	% *D. vexillum* coverage reduction	No *D. vexillum* removed	All *D. vexillum* removed	July values = 100%. Aug values compared to them. Sites A and B averaged.
4	Rolheiser et al (2012)	Figure 2	General biofouling coverage (score 1 - 10 from digital photos). 5 weeks after treatment applied.	35	% fouling coverage change	No fouling removed	All fouling removed	x=(100-x)
		Figure 3	*D. vexillum* fouling coverage (score 1 - 10 from digital photos). 5 weeks after treatment applied.	35	% *D. vexillum* coverage change	No change in *D. vexillum* coverage	All *D. vexillum* removed	x=(100-x)
5	McCann et al. (2013)	Figure 4	*D. vexillum* fouling coverage (Proportion from photos) 3 weeks after treatment. Note that results text does not match	21	% *D. vexillum* coverage	No change in *D. vexillum* coverage	All *D. vexillum* removed	Inverted (i.e. -1 in Fig 4 becomes +100%)

			3 weeks results for 5 min bleach.					
		Figure 5	*D. vexillum* fouling coverage (Proportion from photos) 5 weeks after treatment.	35	% *D. vexillum* coverage	No change in *D. vexillum* coverage	All *D. vexillum* removed	Inverted (i.e. -1 in Fig 5 becomes +100%)
6	Carman et al. (2016)	Text	% *D. vexillum* "dead or shredded into fragments" visually inspected for presence, putrefaction, attachment 1 week after treatment	7	% *D. vexillum* dead	No *D. vexillum* dead or shredded into fragments	All *D. vexillum* dead or shredded into fragments	No conversion needed
Other Tunicates								
7	Carver et al. (2003)	Table 3	% tunicate mortality - no other details given	Not given	% tunicate (*C. intestinalis*) mortality	All tunicates die	All tunicates live	No conversion needed
8	Forrest et al. (2007)	Figure 2	% biomass removal after 4 weeks in seawater	28	% biomass removal	No biomass removed	All biomass removed	No conversion needed
9	LeBlanc et al. (2007)	No fouling tests used in review						
10	Locke et al. (2009)	Figure 2	% survival of *Ciona* Attachment to substrate, siphoning action, decomposure	13	% mortality	All tunicates die	All tunicates live	x=(100-x)
Unrelated Fouling Organisms								
11	Forrest and Blakemore (2006)	No fouling tests used in review						
12	Sharp et al. (2006)	No fouling tests used in review						

Table A2: Summary of the tests for aquaculture species mortality determination in the published Data Sources (DS) used in the meta-analysis of bath treatments. "Where" indicates where in the Data Source the mortality test was used. "Days" is number of days between treatment and mortality test. "Converted Units" is a description of what the Data Source units were converted to. This is further described by "What 0% means" and "What 100% means", which is self-explanatory. "Conversion" finally confirms what conversion was needed to move from the Data Source units to the units used in this study.

DS	Source	Where	Mortality Test details	Days	Units of Mortality	What 0% means	What 100% means	Conversion
1	Denny and Hopkins (2007)	Figs 4, 7, 9, 16, 17	Same as DS02 - % seed mussel mortality 2 weeks after treatment. No other details given.	14	% seed mussel survival	All mussels die	All mussels still alive	x=(100-x)
2	Denny (2008)	Figs 1, 5	% seed mussel mortality 2 weeks after treatment. No other details given.	14	% seed mussel survival	All mussels die	All mussels still alive	x=(100-x)
3	Switzer et al. (2011)	Figure 2	% oyster survival after 1 month	30	% oyster survival	All oysters die	All oysters still alive	Aug value used. No conversion needed. Sites A and B averaged
4	Rolheiser et al (2012)	Figure 4	% oyster survival after 5 weeks	35	% oyster survival	All oysters die	All oysters still alive	No conversion needed
5	McCann et al. (2013)	No shellfish tests						
6	Carman et al. (2016)	Text	% seed mussel mortality 1 week after treatment. Empty shell, tissue putrefied, shell did not close on touch, shell did not close when gently opened.	7	% seed mussel survival	All mussels die	All mussels still alive	No conversion needed
Other Tunicates								
7	Carver et al. (2003)	No shellfish tests						
8	Forrest et al. (2007)	Figure 3	% mussel attachment 24 hours after treatment	1	% spat attachment	No mussel spat attached	100% of mussel spat attached	No conversion needed

		Figure 4	% mussel survival after 1 month	30	% mussel survival	All mussels die	All mussels still alive	No conversion needed
9	LeBlanc et al. (2007)	Table 1	Weight of 0.61m mussel sock 7 months after treatment and compared to control	210	% weight lost compared to control	No weight loss	All mussels gone	Comparison to control performed
10	Locke et al. (2009)		No shellfish tests					
Unrelated Fouling Organisms								
11	Forrest and Blakemore (2006)	Figure 4	% Reattachment to mussel ropes by the byssus after 24 hours in seawater	1	% mussel reattached	No mussels reattached	100% Mussels reattached	No conversion needed
12	Sharp et al. (2006)	Figure 5	Number of attached (attached and not open) mussel spat (4-5mm) after 24 hours in seawater recovery tank	1	% mussel not disrupted	All spat dead	No difference to control	Reference to control value

Table A3: Description of all ancillary data extracted from the 12 data sources, along with the column headings used in accompanying data set.

Column Heading	Description
ID	Unique ID code 1 to 355
Biofoul	Y = This test was for fouling organism
Aquaculture	Y = This test was for aquaculture species
Ref	Data Source number
Source	Journal citation
Region	Region where study was conducted
Country	Country where study was conducted
Aquaculture Species (Common Name)	Aquaculture Species (Common Name)
Aquaculture Species (Species Name)	Aquaculture Species (Species Name)
Aqua (Code)	Three letter code for aquaculture species
Fouling Organism (Common Name)	Fouling Organism (Common Name)
Fouling Organism (Species Name)	Fouling Organism (Species Name)
Fouling Organism (Second Species)	Fouling Organism (Second Species)
Foul (Code)	Three letter code for fouling species
Data Source (Fig/Table)	Where in the cited data source the data was extracted from
Overall Aim of Trial	Overall Aim of Trial
Treatment Chemical (Common Name)	Treatment Chemical (Common Name)
Treatment Chemical (Formula)	Treatment Chemical (Formula)
Treatment - Why was this chemical chosen ?	Why the cited reference said it choise this chemical
TR1 (Code)	Two letter code for treatment chemical (see table of codes below)
TR2 (%)	Strength of treatment chemical (% w/w active ingredient in water diluent)
TR3 (Type)	Whether the treatment was just an immersion in chemical (Dip) or included also exposure to air (Dip+Air)
TR4 dip (mins)	Immersion time in bath treatment (minutes)
TR5 air (mins)	Time exposed to air before or after immersion (minutes)
Lab or Field	Whether test was performed in a laboratory or in the field
Treatment Overall Description	Overall description of how the treatment was applied
Treatment Details (Times, Rinses etc)	Secondary treatment details such as whether a rinse was applied
Outcome from Summary of Paper	Any notes from the paper itself with respect to the treatment - if it was finally recommended or not
Mort Test (Days)	Number of days between treatment and mortality/survival test
Mortality Test Fouling Species Effect Used (Description of Test)	Description of test for mortality of fouling species

Mortality Test Fouling Effect Measurement used by study (Units)	Description of what units were used by the cited reference for fouling species mortality
VALfoul (%)	Value of the mortality of the fouling species (0%-no mortality, 100%-full mortality)
VALfoul What % means	Confirmation of what the measurement of mortality means
Mortality Test Aquaculture Species Effect Used (Description of Test)	Description of test for survival of aquaculture species
Mortality Test Aquaculture Effect Measurement used by study (Units)	Description of what units were used by the cited reference for aquaculture species mortality/survival
VALaqua (%)	Value of the survival of the aquaculture species (0%-full mortality/no survival, 100%-full survival/no mortality)
VALaqua What % means	Confirmation of what the measurement of survival means
What 0% Means	Secondary confirmation of what 0% of mortality/survival index means
What 100% Means	Secondary confirmation of what 100% of mortality/survival index means
NOTES	Any text notes

Two letter treatment codes (TR1):

AA	Acetic acid	$C_2H_4O_2$
BL	Bleach	NaClO
FW	Freshwater	H_20
BR	Brine	NaCl
CS	Caustic Soda	NaOH
LI	Lime	$Ca(OH)_2$
SA	Silicic acid	Na_2SiO_3
SU	Sucrose	$C_{12}H_{22}O_{11}$
CA	Citric acid	$C_6H_8O_7.H_2O$
WG	Waterglass	Na_2SiO_3

Table A4: Summary of the experimental set ups in the published Data Sources (DS) used in the meta-analysis of bath treatments.

DS	Source	Region	Source	Treatment	Rationale for Experiment	Details of Experiment
1	Denny and Hopkins (2007)	New Zealand	Figure 3 Figure 4	Freshwater	Mussels can tolerate FW, while ascidians have a limited tolerance. Wanted to test if short FW baths that were suitable for industry operations could provide enough osmotic shock to kill *D. vexillum*.	130 seed mussels (20-60 mm) in mesh bags, with 3 large mussels covered in *D. vexillum* in same bag. Immersed in a bin of freshwater, then held in air to simulate transport. Performed in the field.
			Figure 6 Figure 7 Figure 8 Figure 9	Acetic acid	To verify Forrest et al (2007)	130 declumped seed mussels (20-60 mm) + 2 to 3 large mussels covered in *D. vexillum* in a mesh bag. Dipped in treatment bath in field, then immediately put back into the sea suspended on ropes 1-2 m deep for 2 weeks.
			Figure 14 Figure 15 Figure 16	Bleach	To further trial bleach as a treatment	10cm x 10cm piece of *D. vexillum* in bag with 60 seed mussels (20-60 mm) – dipped in treatment - then immersed in seawater suspended on a rope - analysed after 2 weeks
2	Denny (2008)	New Zealand	Figure 1	Acetic acid	Find an AA method that doesn't affect seed mussels and to confirm work of DS08	Dip in treatment bath in laboratory, then 24 hrs in air, no rinse - then bags suspended in marina for 2 weeks in the sea before mortality test.
			Figure 2	Acetic acid		130 declumped seed mussels (20-60 mm) + 2 to 3 large mussels covered in *D. vexillum* in a mesh bag. Dipped in treatment bath in field, then immediately put back into the sea suspended on ropes 1-2 m deep for 2 weeks.
			Figure 3	Bleach Lime Silicic acid Sodium hydroxide	Acetic acid was unable to produce 100% *D. vexillum* mortality – hence looked for alternative treatments	Dipped in treatment, put back in sea suspended on a rope at 1.5 m, assessed after 10 days

			Figure 4	Bleach	To further trial bleach as a treatment	10 cm x 10 cm piece of *D. vexillum* in bag with 60 seed mussels (20-60 mm) – dipped in treatment - then immersed in seawater suspended on a rope - analysed after 2 weeks
3	Switzer et al. (2011)	Canada	Figure 1	Lime	Lime traditionally used to control fouling in Canadian shellfish industry	15 fouled oysters, declumped, in a submerged oyster tray, with replicates. Dipped in treatment, put back in sea, analysed 1 month later.
4	Rolheiser et al (2012)	Canada	Figure 2 Figure 3 Figure 4	Brine Freshwater Lime Acetic Acid	To extend the work of Switzer et al. (2011) who concluded by recommending more work on environmentally friendly treatments.	5 heavily fouled oysters, declumped, in a submerged oyster tray, with replicates. Dipped in treatment, put back in sea, analysed 5 weeks later.
5	McCann et al. (2013)	Alaska	Figure 4 Figure 5	Acetic Acid Freshwater Bleach Brine	To try to find an eco-friendly bath treatment to control or eradicate a newly discovered INNS in a region – not specifically a shellfish farm	5 cm square nylon net covered in *D. vexillum* Dipped in treatment, put back in sea, analysed 3 to 5 weeks later
6	Carman et al. (2016)	USA	Text Figure 1	Acetic Acid Brine Freshwater	To find an eco-friendly treatment for fouled seed blue mussels to allow transport, culturing and sale.	3 square cm pieces of *D. vexillum* with ~60 seed mussels (15-25 mm) in a sock. Dipped in treatment, air dryed for 1 hour, put back into the sea
Other Tunicates						
7	Carver et al. (2003)	Canada	Table 3	Bleach Brine Lime Freshwater Acetic acid	To find treatments to eliminate the solitary tunicate *C. intestinalis* from oyster aquaculture.	No experimental details given
8	Forrest et al. (2007)	New Zealand	Figure 2	Acetic acid	Test of acetic acid on multi-species fouling in mussel aquaculture	1m long fouled rope - tunicates (solitary and colonial), bryozoa, serpulids, polycheates and macroalgae - Dipped in treatment, various air exposures, put back in sea for 4 weeks, then weighed.

			Figure 3	Acetic Acid	Test of acetic acid on multi-species fouling in mussel aquaculture	Percentage of seed mussels (26-56 mm) which reattached via their byssus to a 1m long mussel rope 24 hours after treatment.
			Figure 4	Acetic Acid	Test of acetic acid on multi-species fouling in mussel aquaculture	Percentage of mussels which survive after being treated and kept in the sea for 1 month. “Survival” estimation is not described.
9	LeBlanc et al. (2007)	Canada	Table 1	Acetic Acid Air	Managing tunicates in shellfish (mussel) aquaculture	0.61 m of mussel sock, 200 seed mussels in each sock (30 mm length), treated, put back into sea, weighed 7 months after treatment.
10	Locke et al. (2009)	Canada	Figure 2	Acetic acid Citric acid	Effect of fouling treatments in aquaculture on non-target organisms in the environment	30 tunicates on a piece of styrofoam buoy, dipped in treatment, 10 secs air drying, put back into sea in a cage, visually assessed 8/13 days later
Unrelated Fouling Organisms						
11	Forrest and Blakemore (2006)	New Zealand	Figure 4	Freshwater	To control the spread of INNS (seaweed) via transport of seed mussels and equipment	Seed mussels (16-36 mm), unknown number, in 1L pots of aerated tap water at 10 Deg C. Held in 4L buckets of seawater after treatment for 24 hours.
12	Sharp et al. (2006)	Canada	Figure 5	Acetic acid Brine	To find environmentally friendly treatments to remove biofouling from spat collectors.	30 test mussel spat (4-5 mm) dipped into trays of treatment, rinsed in seawater, placed in recovery tanks. Inspected for attachment to rope via byssus, or gaping open.

Table A5: All trials which resulted in 100% *D. vexillum* (Foul Code - D) or multi-species (Foul Code - M) mortality. ID – Unique data identifier. TR2 (% w/w) - Strength of treatment chemical (% active ingredient in water diluent); TR3 (Type) - Whether the treatment was just an immersion in chemical (Dip) or included also exposure to air (Dip+Air); TR4 dip (mins) - Immersion time in bath treatment (minutes); TR5 air (mins) - Time exposed to air before or after immersion (minutes). TR1 (Code) – CS is Caustic Soda.

ID	Ref	Foul (Code)	TR2 (% w/w)	TR3 (Type)	TR4 dip (mins)	TR5 air (mins)	Lab or Field	Mort Test (Days)	TR1 (Code)
Acetic Acid									
275	DS08	M	2	Dip+Air	1	1440	Laboratory	28	
272	DS08	M	2	Dip+Air	2	1440	Laboratory	28	
276	DS08	M	2	Dip+Air	2	1440	Laboratory	28	
280	DS08	M	2	Air+Dip	2	1440	Laboratory	28	
277	DS08	M	2	Dip+Air	3	1440	Laboratory	28	
274	DS08	M	2	Dip+Air	4	1440	Laboratory	28	
278	DS08	M	2	Dip+Air	4	1440	Laboratory	28	
291	DS08	M	4	Dip+Air	1	1440	Laboratory	28	
288	DS08	M	4	Dip+Air	2	1440	Laboratory	28	
292	DS08	M	4	Dip+Air	2	1440	Laboratory	28	
296	DS08	M	4	Air+Dip	2	1440	Laboratory	28	
293	DS08	M	4	Dip+Air	3	1440	Laboratory	28	
290	DS08	M	4	Dip+Air	4	1440	Laboratory	28	
294	DS08	M	4	Dip+Air	4	1440	Laboratory	28	
242	DS05	D	10	Dip	10	0	Field	21	
Bleach									
68	DS01	D	0.1	Dip+Air	2	1440	Field	14	
69	DS01	D	0.25	Dip+Air	0.5	1440	Field	14	
125	DS02	D	0.25	Dip	2	0	Field	14	
70	DS01	D	0.25	Dip+Air	2	1440	Field	14	
108	DS02	D	0.5	Dip	0.33	0	Field	10	
126	DS02	D	0.5	Dip	0.5	0	Field	14	
71	DS01	D	0.5	Dip+Air	0.5	1440	Field	14	
109	DS02	D	0.5	Dip	2	0	Field	10	
127	DS02	D	0.5	Dip	2	0	Field	14	
66	DS01	D	0.5	Dip+Air	2	360	Field	14	
72	DS01	D	0.5	Dip+Air	2	1440	Field	14	
128	DS02	D	1	Dip	0.5	0	Field	14	
129	DS02	D	1	Dip	2	0	Field	14	
244	DS05	D	1	Dip	5	0	Field	21	
245	DS05	D	1	Dip	10	0	Field	21	
Brine									
238	DS05	D	6.2	Dip	240	0	Field	21	

253	DS05	D	6.2	Dip	240	0	Field	35	
239	DS05	D	6.2	Dip	1440	0	Field	21	
Freshwater									
247	DS05	D	0	Dip	1440	0	Field	21	
254	DS06	D	0	Dip+Air	480	60	Field	7	
255	DS06	D	0	Dip+Air	1440	60	Field	7	
Lime									
None									
Other									
116	DS02	D	6	Dip	0.33	0	Field	10	CS
117	DS02	D	6	Dip	2	0	Field	10	CS

Table A6: Summary of contraindicating acetic acid trials with treatment concentrations between 2% and 4%. Trials are separated into those which used air exposure as part of the treatment regime (lower table) and those which did not (upper table). Air exposures used varied from one to 40 hours. All data are presented in Table A7.

Conc. (%)	1 min		2 mins		3 mins		4 mins	
	Min	Max	Min	Max	Min	Max	Min	Max
Plus Air Exposure								
4	85	92	-	-	97	97	97	97
2	85	92	-	-	97	97	80	97
No Air Exposure								
4	85	85	88	88	79	98	84	95
2	36	85	58	58	65	76	70	70

Table A7: Acetic acid treatments which resulted in <100% *D. vexillum* mortality.

ID	Ref	Foul (Code)	TR2 (% w/w)	TR3 (Type)	TR4 dip (mins)	TR5 air (mins)	Lab or Field	Mort Test (Days)	VALfoul (%)
101	DS08	M	4	Dip	3	0	Laboratory	28	98
289	DS08	M	4	Dip+Air	3	1440	Laboratory	28	97
297	DS08	M	4	Air+Dip	3	1440	Laboratory	28	97
298	DS08	M	4	Air+Dip	4	1440	Laboratory	28	97
273	DS08	M	2	Dip+Air	3	1440	Laboratory	28	97
281	DS08	M	2	Air+Dip	3	1440	Laboratory	28	97
282	DS08	M	2	Air+Dip	4	1440	Laboratory	28	97
344	DS02	D	4	Dip	10	0	Field	14	97
266	DS10	T	5	Dip	0.167	0	Field	13	95
286	DS07	T	5	Dip	0.5	0	Laboratory	NA	95
103	DS08	M	4	Dip	4	0	Laboratory	28	95
295	DS08	M	4	Air+Dip	1	1440	Laboratory	28	92
279	DS08	M	2	Air+Dip	1	1440	Laboratory	28	92
24	DS01	D	2	Dip+Air	4	360	Field	14	91
28	DS01	D	2	Dip+Air	4	1080	Field	14	90
107	DS05	D	10	Dip	5	0	Field	21	90
22	DS01	D	0.5	Dip+Air	4	360	Field	14	89
240	DS08	M	4	Dip	2	0	Laboratory	28	88
23	DS01	D	1	Dip+Air	4	360	Field	14	87
287	DS08	M	4	Dip+Air	1	1440	Laboratory	28	85
271	DS08	M	2	Dip+Air	1	1440	Laboratory	28	85
20	DS01	D	2	Dip+Air	4	60	Field	14	85
29	DS01	D	0.1	Dip+Air	4	2460	Field	14	85
267	DS08	M	4	Dip	1	0	Laboratory	28	85
104	DS02	D	4	Dip	1	0	Field	14	85
100	DS08	M	2	Dip	1	0	Laboratory	28	85
106	DS02	D	4	Dip	5	0	Field	14	84
27	DS01	D	1	Dip+Air	4	1080	Field	14	83
17	DS01	D	0.1	Dip+Air	4	60	Field	14	83
19	DS01	D	1	Dip+Air	4	60	Field	14	82
26	DS01	D	0.5	Dip+Air	4	1080	Field	14	82
31	DS01	D	1	Dip+Air	4	2460	Field	14	81
25	DS01	D	0.1	Dip+Air	4	1080	Field	14	81
32	DS01	D	2	Dip+Air	4	2460	Field	14	80
285	DS05	D	10	Dip	2	0	Field	21	80
269	DS02	D	4	Dip	3	0	Field	14	79
96	DS08	M	2	Dip	3	0	Laboratory	28	76
30	DS01	D	0.5	Dip+Air	4	2460	Field	14	72
153	DS02	D	2	Dip	5	0	Field	14	72
177	DS02	D	2	Dip	10	0	Field	14	72
18	DS01	D	0.5	Dip+Air	4	60	Field	14	70
345	DS10	T	5	Dip	0.083	0	Field	13	70
102	DS08	M	2	Dip	4	0	Laboratory	28	70
99	DS02	D	2	Dip	3	0	Field	14	65
21	DS01	D	0.1	Dip+Air	4	360	Field	14	64

105	DS08	M	2	Dip	2	0	Laboratory	28	58
176	DS02	D	1	Dip	10	0	Field	14	54
187	DS02	D	1	Dip	1	0	Field	14	50
188	DS02	D	1	Dip	3	0	Field	14	50
164	DS02	D	1	Dip	5	0	Field	14	49
241	DS04	D	5	Dip	0.5	0	Field	35	45
283	DS04	D	5	Dip	5	0	Field	35	45
284	DS04	D	5	Dip	1	0	Field	35	36
268	DS02	D	2	Dip	1	0	Field	14	36
270	DS04	M	2.2	Dip	4	0	Field	35	33
98	DS04	D	5	Dip	10	0	Field	35	30
152	DS04	D	0.25	Dip	10	0	Field	35	30
189	DS04	D	1.25	Dip	10	0	Field	35	15
97	DS04	D	1.25	Dip	1	0	Field	35	0
141	DS04	D	1.25	Dip	5	0	Field	35	0
151	DS04	D	0.25	Dip	5	0	Field	35	-30
163	DS04	D	0.25	Dip	1	0	Field	35	-120
175	DS04	D	0.25	Dip	0.5	0	Field	35	-180
165	DS04	D	1.25	Dip	0.5	0	Field	35	-270

Fouling codes:

D – *D. vexillum*

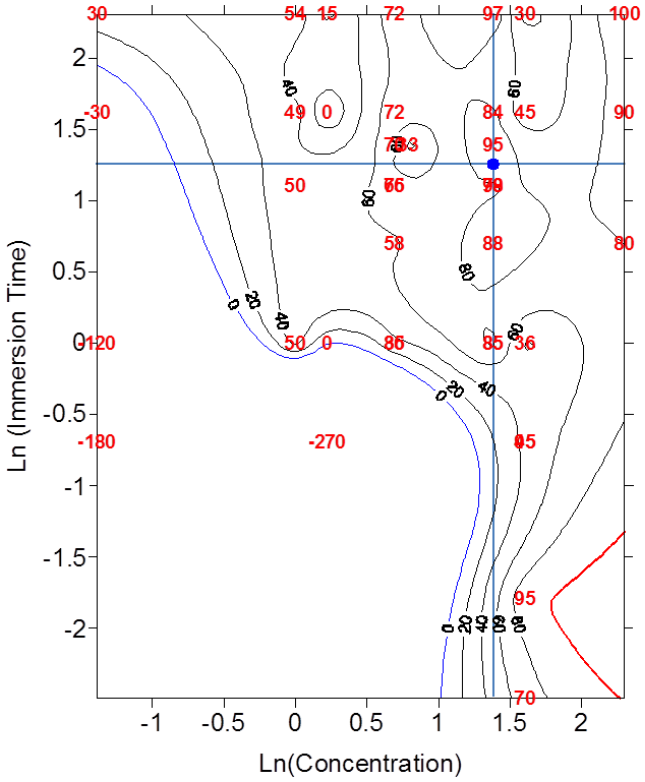
M – Mixed tunicates including *D. vexillum*

T – Tunicates other than *D. vexillum*

Figure S1

The evidence for the effect of acetic acid on *D. vexillum* is further examined using a graphical method. Concentrations and immersion times for all treatment trials which did not use air exposure (39 in all – all of these trials placed treated *D. vexillum* back into seawater hence rinsing was not a necessary step) are first transformed using natural logarithms. Percentage mortality values (z) are then gridded in the three-dimensional space (x,y,z) using a krigging technique, where x is ln(concentration) and y is ln(Immersion time). This was performed in the software package Surfer (Golden Software inc.). The gridded data set was then contoured.

Interpolated values of *D. vexillum* mortality rates (%) for variable acetic acid treatment concentrations (%) and immersion times (minutes). No treatment used air exposure as part of the routine. Concentrations and immersion times have been natural log transformed. Mortality rates (%) have been contoured in 20% intervals. Red values of mortality rate give the raw data (see Tables S5 and S6). The blue vertical and horizontal lines are drawn at a bath concentration of 4% and an immersion time of 3.5 minutes. The 0% contour is also highlighted in blue, and the 100% contour in red).



From Figure S1 we can see that although the data from a variety of unrelated sources are variable, they can be sensibly contoured on one diagram. *D. vexillum* mortality increases from left to right in the diagram (increasing treatment

concentrations) and from bottom to top (increasing immersion times) as one would expect. A few closed contours ("bulls eyes") indicate unexpected or non-uniform data, but on the whole anomalies are rare.

However, if we remove five data points which appear anomalous, i.e.

Conc	Time	ln(Conc)	ln(Time)	VALfoul (%)
1.25	5	0.223144	1.609438	0
1.25	10	0.223144	2.302585	15
5	5	1.609438	1.609438	45
5	10	1.609438	2.302585	30
1	1	0	0	50

We get a smoothed version of Figure S1 (Figure 1 in main paper).

Table A8: Bleach contraindications - treatments using sodium hypochlorite which did not result in 100% *D. vexillum* mortality.

ID	Ref	Foul (Code)	TR2 (% w/w)	TR3 (Type)	TR4 dip (mins)	TR5 air (mins)	Lab or Field	Mort Test (Days)	VALfoul (%)
262	DS07	T	0.006	Dip	20	0	Laboratory	NA	0
122	DS02	D	0.1	Dip	0.5	0	Field	14	63
61	DS01	D	0.1	Dip+Air	0.5	360	Field	14	94
67	DS01	D	0.1	Dip+Air	0.5	1440	Field	14	99
123	DS02	D	0.1	Dip	2	0	Field	14	93
62	DS01	D	0.1	Dip+Air	2	360	Field	14	97
124	DS02	D	0.25	Dip	0.5	0	Field	14	93
63	DS01	D	0.25	Dip+Air	0.5	360	Field	14	94
64	DS01	D	0.25	Dip+Air	2	360	Field	14	93
65	DS01	D	0.5	Dip+Air	0.5	360	Field	14	99
243	DS05	D	1	Dip	2	0	Field	21	70

Table A9: Brine contraindications - treatments using concentrated salt (sodium chloride) which did not result in 100% *D. vexillum* mortality.

ID	Ref	Foul (Code)	TR2 (% w/w)	TR3 (Type)	TR4 dip (mins)	TR5 air (mins)	Lab or Field	Mort Test (Days)	VALfoul (%)
252	DS05	D	6.2	Dip	180	0	Field	35	80
251	DS05	D	6.2	Dip	120	0	Field	35	20
250	DS05	D	6.2	Dip	60	0	Field	35	-55
178	DS04	D	4	Dip	10	0	Field	35	-30
182	DS04	D	0.5	Dip	10	0	Field	35	-60
179	DS04	D	5	Dip	10	0	Field	35	-60
180	DS04	D	7	Dip	10	0	Field	35	-126
183	DS04	D	2	Dip	10	0	Field	35	-135
263	DS07	T	SAT	Dip	8	0	Laboratory	NA	25
171	DS04	D	2	Dip	5	0	Field	35	-84
168	DS04	D	7	Dip	5	0	Field	35	-84
167	DS04	D	5	Dip	5	0	Field	35	-105
170	DS04	D	0.5	Dip	5	0	Field	35	-120
166	DS04	D	4	Dip	5	0	Field	35	-144
138	DS04	M	5	Dip	4	0	Field	35	-33
158	DS04	D	0.5	Dip	1	0	Field	35	-75
154	DS04	D	4	Dip	1	0	Field	35	-120
155	DS04	D	5	Dip	1	0	Field	35	-135
159	DS04	D	2	Dip	1	0	Field	35	-186
156	DS04	D	7	Dip	1	0	Field	35	-225
142	DS04	D	4	Dip	0.5	0	Field	35	-63
146	DS04	D	0.5	Dip	0.5	0	Field	35	-75
147	DS04	D	2	Dip	0.5	0	Field	35	-90
143	DS04	D	5	Dip	0.5	0	Field	35	-105
144	DS04	D	7	Dip	0.5	0	Field	35	-105

Table A10: Freshwater contraindications - treatments using freshwater (H_2O) which did not result in 100% *D. vexillum* mortality.

ID	Ref	Aqua (Code)	Foul (Code)	TR2 (%)	TR3 (Type)	TR4 dip (mins)	TR5 air (mins)	Lab or Field	Mort Test (Days)	VALfoul (%)
12	DS01	x	D	0	Dip+Air	10	1440	Field	14	98
6	DS01	x	D	0	Dip+Air	10	300	Field	14	91
8	DS01	x	D	0	Dip+Air	5	720	Field	14	91
10	DS01	x	D	0	Dip+Air	2	1440	Field	14	90
5	DS01	x	D	0	Dip+Air	5	300	Field	14	87
11	DS01	x	D	0	Dip+Air	5	1440	Field	14	87
9	DS01	x	D	0	Dip+Air	10	720	Field	14	85
246	DS05	x	D	0	Dip	240	0	Field	21	80
3	DS01	x	D	0	Dip+Air	10	60	Field	14	75
4	DS01	x	D	0	Dip+Air	2	300	Field	14	75
2	DS01	x	D	0	Dip+Air	5	60	Field	14	72
1	DS01	x	D	0	Dip+Air	2	60	Field	14	65
7	DS01	x	D	0	Dip+Air	2	720	Field	14	65
265	DS07	x	T	0	Dip	1	0	Laboratory	NA	10
139	DS04	x	M	0.8	Dip	4	0	Field	35	-28
157	DS04	x	D	0	Dip	1	0	Field	35	-45
169	DS04	x	D	0	Dip	5	0	Field	35	-45
181	DS04	x	D	0	Dip	10	0	Field	35	-45
145	DS04	x	D	0	Dip	0.5	0	Field	35	-165

Table A11: Other treatments contraindications - treatments using caustic soda (CS), citric acid (CA), waterglass (WG) and hypoxia (HY).

ID	Ref	Foul (Code)	TR2 (% w/w)	TR3 (Type)	TR4 dip (mins)	TR5 air (mins)	Lab or Field	Mort Test (Days)	VALfoul (%)	TR1 (Code)
115	DS02	D	3	Dip	2	0	Field	10	71	CS
114	DS02	D	3	Dip	0.33	0	Field	10	67	CS
119	DS02	D	3	Dip	2	0	Field	10	62	WG
121	DS02	D	6	Dip	2	0	Field	10	58	WG
118	DS02	D	3	Dip	0.33	0	Field	10	38	WG
120	DS02	D	6	Dip	0.33	0	Field	10	12	WG
347	DS10	T	5	Dip	0.167	0	Field	13	5	CA
249	DS05	D	0	Dip	1440	0	Field	21	-10	HY
346	DS10	T	5	Dip	0.083	0	Field	13	-13	CA
248	DS05	D	0	Dip	240	0	Field	21	-120	HY

Table A12: Summary of conclusions from the currently available evidence relating to bath treatments which result in 100% *D. vexillum* mortality.

Acetic Acid	• Acetic acid was used in 79 treatment trials, and 14 of these resulted in 100% *D. vexillum* mortality. • The large number of trials using acetic acid provide evidence to suggest potential variability in the outcomes of treatments when using this compound as the active ingredient. • The currently available evidence suggests that bath treatments using acetic acid should be of at least 4% w/w strength, with immersion times of at least 3.5 minutes, followed by at least 24 hours air exposure.
Bleach	• Bleach (sodium hypochlorite) was used in 26 treatment trials, and 15 of these resulted in 100% *D. vexillum* mortality. • The currently available evidence suggests that bath treatments using bleach should be of at least 0.5% w/w NaClO concentration, with immersion times of at least 2 minutes. The evidence suggests that no additional air exposure is necessary.
Brine	• Brine (sodium chloride) was used in 28 treatment trials, and 3 of these resulted in 100% *D. vexillum* mortality. • The currently available evidence suggests that bath treatments using brine should be of at least 62ppt concentration, with immersion times of at least 30 hours, although shorter immersion times may be possible if more trial data confirms this. The evidence suggests that no additional air exposure is necessary.
Freshwater	• Freshwater was used in 22 treatment trials, and 3 of these resulted in 100% *D. vexillum* mortality. • The currently available evidence suggests that bath treatments using freshwater should use immersion times of at least 24 hours, although more trial data are needed to confirm this. The evidence suggests that no additional air exposure is necessary.
Lime	• Lime was used in 19 treatment trials, and none resulted in 100% *D. vexillum* mortality. • The currently available evidence suggests that lime cannot be used as a bath treatment for *D. vexillum*.
Others	• Other treatments were tested in 12 trials. • The currently available evidence suggests that caustic soda (NaOH), citric acid (C6H8O7.H2O), waterglass (Na2SiO3) and hypoxia can not be used as bath treatments for *D. vexillum*.

Table A13: Effect of treatments on aquaculture species.

ID	Ref	Aqua (Code)	TR1 (Code)	TR2 (% w/w)	TR3 (Type)	TR4 dip (mins)	TR5 air (mins)	Lab or Field	Mort Test (Days)	VALaqua (%)
Acetic Acid										
237	DS04	PO	AA	5	Dip	10	0	Field	35	5
225	DS04	PO	AA	5	Dip	5	0	Field	35	7
213	DS04	PO	AA	5	Dip	1	0	Field	35	63
201	DS04	PO	AA	5	Dip	0.5	0	Field	35	63
236	DS04	PO	AA	1.25	Dip	10	0	Field	35	24
224	DS04	PO	AA	1.25	Dip	5	0	Field	35	44
212	DS04	PO	AA	1.25	Dip	1	0	Field	35	83
200	DS04	PO	AA	1.25	Dip	0.5	0	Field	35	100
235	DS04	PO	AA	0.25	Dip	10	0	Field	35	64
223	DS04	PO	AA	0.25	Dip	5	0	Field	35	100
211	DS04	PO	AA	0.25	Dip	1	0	Field	35	100
199	DS04	PO	AA	0.25	Dip	0.5	0	Field	35	100
257	DS06	sBM	AA	5	Dip+Air	10	60	Field	7	0
256	DS06	sBM	AA	5	Dip+Air	5	60	Field	7	0
342	DS09	sBM	AA	5	Dip	2	0	Field	210	86
341	DS09	sBM	AA	5	Dip	0.5	0	Field	210	74
95	DS02	sGM	AA	10	Dip+Air	1	1440	Laboratory	14	21
94	DS02	sGM	AA	10	Dip+Air	0.333	1440	Laboratory	14	28
93	DS02	sGM	AA	10	Dip+Air	0.083	1440	Laboratory	14	23
314	DS08	sGM	AA	8	Air+Dip	2	1440	Laboratory	1	97
315	DS08	sGM	AA	8	Air+Dip	2	1440	Laboratory	1	97
316	DS08	sGM	AA	8	Air+Dip	2	1440	Laboratory	1	96
308	DS08	sGM	AA	8	Dip+Air	2	1440	Laboratory	1	91
309	DS08	sGM	AA	8	Dip+Air	2	1440	Laboratory	1	83
310	DS08	sGM	AA	8	Dip+Air	2	1440	Laboratory	1	54
311	DS08	sGM	AA	8	Dip+Air	2	1440	Laboratory	1	26
313	DS08	sGM	AA	8	Dip+Air	2	1440	Laboratory	1	20
312	DS08	sGM	AA	8	Dip+Air	2	1440	Laboratory	1	11
60	DS01	sGM	AA	4	Dip	10	0	Field	14	97
57	DS01	sGM	AA	4	Dip	5	0	Field	14	94
334	DS08	sGM	AA	4	Dip+Air	4	1440	Field	30	97
340	DS08	sGM	AA	4	Air+Dip	4	1440	Field	30	97
328	DS08	sGM	AA	4	Air+Dip	4	1440	Field	30	90
322	DS08	sGM	AA	4	Dip+Air	4	1440	Field	30	84
337	DS08	sGM	AA	4	Dip+Air	4	1440	Field	30	53
325	DS08	sGM	AA	4	Dip+Air	4	1440	Field	30	27
331	DS08	sGM	AA	4	Dip	4	0	Field	30	95
319	DS08	sGM	AA	4	Dip	4	0	Field	30	92

54	DS01	sGM	AA	4	Dip	3	0	Field	14	95
305	DS08	sGM	AA	4	Air+Dip	2	1440	Laboratory	1	100
306	DS08	sGM	AA	4	Air+Dip	2	1440	Laboratory	1	100
299	DS08	sGM	AA	4	Dip+Air	2	1440	Laboratory	1	97
307	DS08	sGM	AA	4	Air+Dip	2	1440	Laboratory	1	97
300	DS08	sGM	AA	4	Dip+Air	2	1440	Laboratory	1	96
327	DS08	sGM	AA	4	Air+Dip	2	1440	Field	30	96
333	DS08	sGM	AA	4	Dip+Air	2	1440	Field	30	96
339	DS08	sGM	AA	4	Air+Dip	2	1440	Field	30	96
321	DS08	sGM	AA	4	Dip+Air	2	1440	Field	30	88
301	DS08	sGM	AA	4	Dip+Air	2	1440	Laboratory	1	80
302	DS08	sGM	AA	4	Dip+Air	2	1440	Laboratory	1	76
336	DS08	sGM	AA	4	Dip+Air	2	1440	Field	30	67
303	DS08	sGM	AA	4	Dip+Air	2	1440	Laboratory	1	57
91	DS02	sGM	AA	4	Dip+Air	2	1440	Laboratory	14	42
304	DS08	sGM	AA	4	Dip+Air	2	1440	Laboratory	1	39
324	DS08	sGM	AA	4	Dip+Air	2	1440	Field	30	36
92	DS02	sGM	AA	4	Dip+Air	2	1440	Laboratory	14	24
330	DS08	sGM	AA	4	Dip	2	0	Field	30	96
318	DS08	sGM	AA	4	Dip	2	0	Field	30	92
332	DS08	sGM	AA	4	Dip+Air	1	1440	Field	30	100
338	DS08	sGM	AA	4	Air+Dip	1	1440	Field	30	96
326	DS08	sGM	AA	4	Air+Dip	1	1440	Field	30	93
320	DS08	sGM	AA	4	Dip+Air	1	1440	Field	30	92
335	DS08	sGM	AA	4	Dip+Air	1	1440	Field	30	64
323	DS08	sGM	AA	4	Dip+Air	1	1440	Field	30	37
329	DS08	sGM	AA	4	Dip	1	0	Field	30	99
51	DS01	sGM	AA	4	Dip	1	0	Field	14	97
317	DS08	sGM	AA	4	Dip	1	0	Field	30	93
59	DS01	sGM	AA	2	Dip	10	0	Field	14	97
56	DS01	sGM	AA	2	Dip	5	0	Field	14	93
48	DS01	sGM	AA	2	Dip+Air	4	2460	Field	14	99
44	DS01	sGM	AA	2	Dip+Air	4	1080	Field	14	98
40	DS01	sGM	AA	2	Dip+Air	4	360	Field	14	98
36	DS01	sGM	AA	2	Dip+Air	4	60	Field	14	99
53	DS01	sGM	AA	2	Dip	3	0	Field	14	97
50	DS01	sGM	AA	2	Dip	1	0	Field	14	98
90	DS02	sGM	AA	1	Dip+Air	10	1440	Laboratory	14	91
58	DS01	sGM	AA	1	Dip	10	0	Field	14	99
89	DS02	sGM	AA	1	Dip+Air	5	1440	Laboratory	14	89
55	DS01	sGM	AA	1	Dip	5	0	Field	14	96
47	DS01	sGM	AA	1	Dip+Air	4	2460	Field	14	97
43	DS01	sGM	AA	1	Dip+Air	4	1080	Field	14	98
39	DS01	sGM	AA	1	Dip+Air	4	360	Field	14	99

35	DS01	sGM	AA	1	Dip+Air	4	60	Field	14	99
52	DS01	sGM	AA	1	Dip	3	0	Field	14	99
88	DS02	sGM	AA	1	Dip+Air	2	1440	Laboratory	14	93
49	DS01	sGM	AA	1	Dip	1	0	Field	14	94
46	DS01	sGM	AA	0.5	Dip+Air	4	2460	Field	14	99
42	DS01	sGM	AA	0.5	Dip+Air	4	1080	Field	14	99
38	DS01	sGM	AA	0.5	Dip+Air	4	360	Field	14	99
34	DS01	sGM	AA	0.5	Dip+Air	4	60	Field	14	100
87	DS02	sGM	AA	0.5	Dip+Air	2	1440	Laboratory	14	99
86	DS02	sGM	AA	0.1	Dip+Air	10	1440	Laboratory	14	100
45	DS01	sGM	AA	0.1	Dip+Air	4	2460	Field	14	99
41	DS01	sGM	AA	0.1	Dip+Air	4	1080	Field	14	99
37	DS01	sGM	AA	0.1	Dip+Air	4	360	Field	14	99
33	DS01	sGM	AA	0.1	Dip+Air	4	60	Field	14	99
85	DS02	sGM	AA	0.1	Dip+Air	2	1440	Laboratory	14	100
353	DS12	spBM	AA	5	Dip	0.33	0	Laboratory	1	35
Bleach										
78	DS01	sGM	BL	0.5	Dip+Air	2	360	Field	14	98
84	DS01	sGM	BL	0.5	Dip+Air	2	360	Field	14	90
135	DS02	sGM	BL	0.5	Dip	2	0	Field	14	94
83	DS01	sGM	BL	0.5	Dip+Air	0.5	360	Field	14	96
77	DS01	sGM	BL	0.5	Dip+Air	0.5	360	Field	14	94
134	DS02	sGM	BL	0.5	Dip	0.5	0	Field	14	94
82	DS01	sGM	BL	0.25	Dip+Air	2	360	Field	14	95
76	DS01	sGM	BL	0.25	Dip+Air	2	360	Field	14	94
133	DS02	sGM	BL	0.25	Dip	2	0	Field	14	95
75	DS01	sGM	BL	0.25	Dip+Air	0.5	360	Field	14	98
81	DS01	sGM	BL	0.25	Dip+Air	0.5	360	Field	14	97
132	DS02	sGM	BL	0.25	Dip	0.5	0	Field	14	97
74	DS01	sGM	BL	0.1	Dip+Air	2	360	Field	14	98
80	DS01	sGM	BL	0.1	Dip+Air	2	360	Field	14	96
131	DS02	sGM	BL	0.1	Dip	2	0	Field	14	97
73	DS01	sGM	BL	0.1	Dip+Air	0.5	360	Field	14	98
79	DS01	sGM	BL	0.1	Dip+Air	0.5	360	Field	14	98
130	DS02	sGM	BL	0.1	Dip	0.5	0	Field	14	98
Brine										
228	DS04	PO	BR	7	Dip	10	0	Field	35	100
216	DS04	PO	BR	7	Dip	5	0	Field	35	100
204	DS04	PO	BR	7	Dip	1	0	Field	35	100
192	DS04	PO	BR	7	Dip	0.5	0	Field	35	100
227	DS04	PO	BR	5	Dip	10	0	Field	35	100
215	DS04	PO	BR	5	Dip	5	0	Field	35	100
203	DS04	PO	BR	5	Dip	1	0	Field	35	100
191	DS04	PO	BR	5	Dip	0.5	0	Field	35	100

226	DS04	PO	BR	4	Dip	10	0	Field	35	100
214	DS04	PO	BR	4	Dip	5	0	Field	35	100
202	DS04	PO	BR	4	Dip	1	0	Field	35	100
190	DS04	PO	BR	4	Dip	0.5	0	Field	35	100
231	DS04	PO	BR	2	Dip	10	0	Field	35	100
219	DS04	PO	BR	2	Dip	5	0	Field	35	100
207	DS04	PO	BR	2	Dip	1	0	Field	35	100
195	DS04	PO	BR	2	Dip	0.5	0	Field	35	100
230	DS04	PO	BR	0.5	Dip	10	0	Field	35	100
218	DS04	PO	BR	0.5	Dip	5	0	Field	35	100
206	DS04	PO	BR	0.5	Dip	1	0	Field	35	100
194	DS04	PO	BR	0.5	Dip	0.5	0	Field	35	100
258	DS06	sBM	BR	7	Dip+Air	0.33	60	Field	7	92
259	DS06	sBM	BR	7	Dip+Air	0.17	60	Field	7	94
355	DS12	spBM	BR	30	Dip	0.5	0	Laboratory	1	100
354	DS12	spBM	BR	30	Dip	0.33	0	Laboratory	1	99
Freshwater										
229	DS04	PO	FW	0	Dip	10	0	Field	35	83
217	DS04	PO	FW	0	Dip	5	0	Field	35	84
205	DS04	PO	FW	0	Dip	1	0	Field	35	100
193	DS04	PO	FW	0	Dip	0.5	0	Field	35	83
261	DS06	sBM	FW	0	Dip+Air	1440	60	Field	7	94
260	DS06	sBM	FW	0	Dip+Air	480	60	Field	7	98
352	DS11	sGM	FW	0	Dip	7200	0	Laboratory	1	97
351	DS11	sGM	FW	0	Dip	5760	0	Laboratory	1	94
350	DS11	sGM	FW	0	Dip	4320	0	Laboratory	1	100
349	DS11	sGM	FW	0	Dip	2880	0	Laboratory	1	70
348	DS11	sGM	FW	0	Dip	1440	0	Laboratory	1	72
16	DS01	sGM	FW	0	Dip+Air	10	1440	Field	14	99
15	DS01	sGM	FW	0	Dip+Air	10	720	Field	14	99
14	DS01	sGM	FW	0	Dip+Air	10	300	Field	14	99
13	DS01	sGM	FW	0	Dip+Air	10	60	Field	14	99
Lime										
234	DS04	PO	LI	4	Dip	10	0	Field	35	44
222	DS04	PO	LI	4	Dip	5	0	Field	35	85
137	DS03	PO	LI	4	Dip	4	0	Field	30	85
210	DS04	PO	LI	4	Dip	1	0	Field	35	64
198	DS04	PO	LI	4	Dip	0.5	0	Field	35	44
233	DS04	PO	LI	2	Dip	10	0	Field	35	100
221	DS04	PO	LI	2	Dip	5	0	Field	35	100
209	DS04	PO	LI	2	Dip	1	0	Field	35	100
197	DS04	PO	LI	2	Dip	0.5	0	Field	35	100
232	DS04	PO	LI	1	Dip	10	0	Field	35	100
220	DS04	PO	LI	1	Dip	5	0	Field	35	85

208	DS04	PO	LI	1	Dip	1	0	Field	35	100
196	DS04	PO	LI	1	Dip	0.5	0	Field	35	85
Other										
343	DS09	sBM	AR	x	Exposure	0	2400	Field	210	26

Aquaculture species codes:

PO – Pacific oysters
sBM – Blue mussel seed
sGM – Green-lipped mussel seed
spBM – Blue mussel spat

Table A14: The survival rates (%) of Pacific Oysters treated with acetic acid immersion baths.

Conc. (% w/w)	Treatment Times (Minutes)			
	0.5	**1**	**5**	**10**
0.25	100	100	100	64
1.25	100	83	44	24
5	63	63	7	5

Table A15: Summary of conclusions from the currently available evidence relating to the effect of bath treatments on various aquaculture species.

Acetic Acid	• *Pacific Oysters:* From 12 treatment trials using acetic acid on Pacific oysters, interpolation of the results suggests that immersion in bath treatments of 4% w/w concentration or more, for immersion times of 3.5 minutes or more, will result in survival rates of 25% or less. • *Blue Mussel Seed:* Only 4 trials used blue oyster seed, all using an acetic acid concentration of 5% w/w. Immersion times of longer than 5 minutes resulted in total mussel mortality, while immersion times of 2 minutes and 30 seconds resulted in survival rates of 86% and 74% respectively. • *Green-lipped Mussel Seed:* Green-lipped mussel seed exhibit high (>90%) survival rates for bath treatments up to 8% w/w, although rinsing after immersion is necessary.
Bleach	• *Pacific Oysters:* No published trials. • *Blue Mussel Seed:* No published trials. • *Green-lipped Mussel Seed:* 18 published trials used bleach to treat green-lipped mussel seed. Bath concentrations varied between 0.5% and 2% w/w, with immersion times of between 0.5 and 2 minutes. High survival rates were reported for all trials, with an average of 96% ± 2%.
Brine	• *Pacific Oysters:* 20 published trials examined the effect of brine bath treatments on Pacific oysters. Bath immersion times varied between 0.5 minutes and 10 minutes. All treatments resulted in 100% oyster survival. • *Blue Mussel Seed:* 2 published trials examined the effect of brine bath treatments on blue mussel seed. Bath immersion times were 10 and 20 seconds. The treatments resulted in 92% and 94% survival. • *Blue mussel spat:* 2 published trials examined the effect of brine bath treatments on blue mussel spat. Bath immersion times were 20 and 30 seconds. the treatments resulted in 99% and 100% mussel survival. • *Green-lipped Mussel Seed:* No published trials.
Freshwater	• *Pacific Oysters:* 4 published trials examined the effect of freshwater immersion on Pacific oysters. Immersion times were 30 seconds, 1 minute, 5 minutes and 10 minutes and survival rates were 83%, 100%, 84% and 83% respectively. • *Blue Mussel Seed:* 2 published trials examined the effect of freshwater immersion on blue mussel seed. Immersion times were 8 and 24 hours and survival rates were 98% and 94% respectively. • *Green-lipped Mussel Seed:* 4 published trials examined the effect of a 10 minute immersion in freshwater on green-lipped mussel seed, but with air exposure periods ranging from 1 to 24 hours. All resulted in 99% mussel survival. 5 trials examined the effect of long term immersion on green-lipped mussel seed. Immersion times were 24, 48, 72, 96 and 120 hours. Survival rates varied between 70% and 100% with an average of 87% ± 14%.
Lime	• *Pacific Oysters:* 13 published trials examined the effect of lime on Pacific oysters. Bath concentrations varied from 1% to 4% w/w, and immersion times from 30 seconds to 10 minutes. Survival rates were variable, ranging from 44% to 100%. Although the lowest survival figures were for the 4% w/w baths, the relationship between immersion concentration and time and survival was varied. • *Blue Mussel Seed:* No published trials. • *Green-lipped Mussel Seed:* No published trials.

Table A16: Details of cost/dilution calculations used in Table 16, main text. GBP – UK Pound. USD – US Dollar. EUR – Euro. L – litres. kg – kilograms. ppt – parts per thousand.

Chemical	**Formula**	**Concentration**	**Exchange rates 05/11/16** **1.00GBP = 1.25USD = 1.12EUR**
Acetic acid	$C_2H_4O_2$	5% w/w - Vinegar	20L 5% w/w acetic acid (vinegar) = £13 **1000L@5% w/w = 650GBP**,
		5% w/w - diluted glacial acetic acid	5L 99% w/w glacial acetic acid (ethanoic acid) = £17 1000L@5% w/w = 50L@99% w/w + 950L water **50L@99% w/w = 170GBP,**
Bleach	NaClO	0.5% w/w - From domestic/commercial bleach	20L 15% w/w industrial bleach = £25 1000L@0.5% w/w = 33L@15% w/w + 967L water **33L@15% w/w = 41GBP**
		0.5% w/w - from powder sodium hypochlorite	None readily available to public
Brine	NaCl	Saturated (at least 62ppt)	25 kg industrial salt = £12 1000L@62ppt= 62kg NaCL + 938L water 62kg = £30 (Note – half this cost of sea water of >30ppt used as diluent)

Table A17: Domestic Bleach: Strength and Contents.

Product	Published Data Sheet	Conc.
Domestic		
Janola Premium Bleach	1. Sodium hypochlorite	<5%
	2. Sodium hydroxide	<3%
30 Seconds Outdoor Cleaner	1. Sodium hypochlorite by weight (exact percentage trade secret)	1-5%
30 Seconds Outdoor Cleaner Concentrate	1. Sodium hypochlorite	5%
	2. Trisodium phosphate	2%
Domestos	1. Sodium hypoclorite solution, % Cl Active	1-5%
	2. c12-18 alkyl dimethylamine oxide	1-5%
	3. Sodium hydroxide	<1%
Tesco Everyday Bleach	1. Sodium hypochlorite 1.5g per 100g	1.5%
Parazone	1. Sodium hypoclorite solution, % Cl Active	1-5%
Industrial		
Premier Liquid Bleach	1. Sodium hypoclorite solution, % Cl Active	1-5%
Bonnymans Industrial Bleach	1. Sodium hypochlorite solution	11%
	2. Sodium hydroxide	NA
	3. Viscosity stabilisers	NA
	4. Anionic surfactant	NA
	5. Perfume	NA
Swimmingpool chemicals.co,uk	1. Sodium hypoclorite solution, % Cl Active	10-15%
Champion Pool Shock	1. Sodium hypoclorite	10-11.5%
	2. Sodium hydroxide	<1.5%

Table A18: Summary of Treatment Conclusions - Acetic Acid.

D. vexillum	• Acetic acid was used in 79 treatment trials, and 14 of these resulted in 100% *D. vexillum* mortality. • The large number of trials using acetic acid provide evidence to suggest potential variability in the outcomes of treatments when using this compound as the active ingredient. • The currently available evidence suggests that bath treatments using acetic acid should be of at least 4% w/w strength, with immersion times of at least 3.5 minutes, followed by at least 24 hours air exposure.
Aquaculture Species	• *Pacific Oysters:* From 12 published treatment trials using acetic acid on Pacific oysters, interpolation of the results suggests that immersion in bath treatments of 4% w/w concentration or more, for immersion times of 3.5 minutes or more, will result in survival rates of 25% or less. No published trials using oyster seed were found. • *Blue Mussel Seed:* Blue mussel seed was used in 4 trials using an acetic acid concentration of 5% w/w. Immersion times of longer than 5 minutes resulted in 100% mussel seed mortality, while immersion times of 30 seconds and 2 minutes resulted in survival rates of 74% and 86% respectively. • *Green-lipped Mussel Seed:* Green-lipped mussel seed exhibit high (>90%) survival rates for bath treatments up to 8% w/w, although rinsing after immersion is necessary.
Limitations	• Acetic acid baths of commercial size, made up using vinegar, would be expensive and require large volumes of vinegar to be transported to a farm site. Assessment of the concentration of the active component in bath treatment made up from diluted acetic acid may also be an issue. • Acetic acid baths of commercial size, made up using glacial acetic acid, would require unsafe amounts of the acid, posing hazards during transportation, storage and handling.

Authors reporting acetic acid treatments were:

Bath Concentration*	**Immersion Time**	**Authors**	**Aquaculture Species**
10%	2 minutes	McCann et al. (2013)	None cited
5%	15 to 30 seconds	Carver et al. (2003)	Mussel seed / oysters
4%	1 minute + 24 hours air	Forrest et al. (2007)	Green-lipped mussel seed

* - w/w - Assumed freshwater diluent.

Note: Pacific oysters - *Crassostrea gigas.* Green-lipped mussels - *Perna canaliculus.* Blue mussels - *Mytilus edulis*

Table A19: Summary of Treatment Conclusions – Bleach.

D. vexillum	• Bleach (sodium hypochlorite) was used in 26 treatment trials, and 15 of these resulted in 100% *D. vexillum* mortality. • The currently available evidence suggests that bath treatments using bleach should be of at least 0.5% NaClO w/w concentration, with immersion times of at least 2 minutes. The evidence suggests that no additional air exposure is necessary.
Aquaculture Species	• *Pacific Oysters:* No published trials. • *Blue Mussel Seed:* No published trials. • *Green-lipped Mussel Seed:* 18 published trials used bleach to treat green-lipped mussel seed. Bath concentrations varied between 0.5% and 2% NaClO w/w, with immersion times of between 0.5 and 2 minutes. High survival rates were reported for all trials, with an average of 96% ± 2%.
Limitations	• Bleach baths of commercial size, made up using domestic or industrial bleach, could pose hazards during storage, and require careful measurement of strength as the product decays with time. Treatment bath strength also decays during use.

Authors recommending bleach treatments were:

Bath Concentration w/w	**Immersion Time**	**Authors**	**Aquaculture Species**
0.5%	2 minutes	Denny and Hopkins (2007) Denny (2008)	Green-lipped mussel seed
1%	10 minutes	McCann et al. (2013)	None
4%	1 minute + 24 hours air	Forrest et al. (2007)	Green-lipped mussel seed

Table A20: Summary of Treatment Conclusions – Brine.

D. vexillum	• Brine (sodium chloride) was used in 28 treatment trials, and 3 of these resulted in 100% *D. vexillum* mortality. • The currently available evidence suggests that bath treatments using brine should be of at least 62ppt concentration, with immersion times of at least 30 hours, although shorter immersion times may be possible if more trial data confirms this. The evidence suggests that no additional air exposure is necessary.
Aquaculture Species	• *Pacific Oysters:* 20 published trials examined the effect of brine bath treatments on Pacific oysters. Bath immersion times varied between 0.5 minutes and 10 minutes. All treatments resulted in 100% oyster survival. • *Blue Mussel Seed:* 2 published trials examined the effect of brine bath treatments on blue mussel seed. Bath immersion times were 10 and 20 seconds. The treatments resulted in 92% and 94% survival. • *Blue mussel spat:* 2 published trials examined the effect of brine bath treatments on blue mussel spat. Bath immersion times were 20 and 30 seconds. the treatments resulted in 99% and 100% mussel survival. • *Green-lipped Mussel Seed:* No published trials.
Limitations	• None

Authors reporting brine treatments were:

Bath Concentration	**Immersion Time**	**Authors**	**Aquaculture Species**
Saturated	24 hours	McCann et al. (2013)	None

Note: Sharp et al. (2006) recommended a brine bath for 20 seconds to remove green algal mats from mussel ropes.

Table A21: Summary of Treatment Conclusions – Freshwater.

D. vexillum	• Freshwater was used in 22 treatment trials, and 3 of these resulted in 100% *D. vexillum* mortality. • The currently available evidence suggests that bath treatments using freshwater should use immersion times of at least 24 hours, although more trial data are needed to confirm this. The evidence suggests that no additional air exposure is necessary.
Aquaculture Species	• *Pacific Oysters:* 4 published trials examined the effect of freshwater immersion on Pacific oysters. Immersion times were 30 seconds, 1 minute, 5 minutes and 10 minutes and survival rates were 83%, 100%, 84% and 83% respectively. • *Blue Mussel Seed:* 2 published trials examined the effect of freshwater immersion on blue mussel seed. Immersion times were 8 and 24 hours and survival rates were 98% and 94% respectively. • *Green-lipped Mussel Seed:* 4 published trials examined the effect of a 10 minute immersion in freshwater on green-lipped mussel seed, but with air exposure periods ranging from 1 to 24 hours. All resulted in 99% mussel survival. 5 trials examined the effect of long term immersion on green-lipped mussel seed. Immersion times were 24, 48, 72, 96 and 120 hours. Survival rates varied between 70% and 100% with an average of 87% ± 14%.
Limitations	• Freshwater treatment baths of commercial size probably require a local source of running freshwater.

Authors reporting freshwater treatments were:

Bath Concentration	**Immersion Time**	**Authors**	**Aquaculture Species**
Freshwater	4 hours	McCann et al. (2013)	None
Freshwater	8 hours	Carman et al. (2016)	Blue mussel seed

Note: Forrest and Blakemore (2006) recommended a bath treatment of freshwater for 48 hours to remove the alga *Undaria pinnatifida* from green-lipped mussel seed.

Table A22: Summary of Treatment Conclusions – Lime.

D. vexillum	• Lime was used in 19 treatment trials, and none resulted in 100% *D. vexillum* mortality. • The currently available evidence suggests that lime cannot be used as a bath treatment for *D. vexillum*.
Aquaculture Species	• *Pacific Oysters:* 13 published trials examined the effect of lime on Pacific oysters. Bath concentrations varied from 1% to 4% w/w, and immersion times from 30 seconds to 10 minutes. Survival rates were variable, ranging from 44% to 100%. Although the lowest survival figures were for the 4% w/w baths, the relationship between immersion concentration and time and survival was varied. • *Blue Mussel Seed:* No published trials. • *Green-lipped Mussel Seed:* No published trials.
Limitations	• Cannot currently be recommended for use

Authors reporting lime treatments were:

Bath Concentration (w/w)	**Immersion Time**	**Authors**	**Aquaculture Species**
3-5%	5 minutes	Rolheiser et al. (2012)	Pacific Oysters

Table A23: Summary of Treatment Conclusions – Others.

D. vexillum	• Other treatments were tested in 12 trials. • The currently available evidence suggests that hypoxia treatments, and caustic soda (NaOH), citric acid ($C_6H_8O_7.H_2O$), waterglass (Na_2SiO_3) bath treatments, can not produce 100% mortality in *D. vexillum*.