



Assessment of the Effects of Household Cleaners for the Treatment of Marine Pests

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1. Introduction

The Fiordland (Te Moana o Atawhenua) Marine Area (FMA) is a region of outstanding natural character and biodiversity and supports a range of coastal industries important to regional economies (DOC 2006; Batstone *et al.* 2009; Sinner *et al.* 2009). These important values can be put at risk through the unintentional introduction of non-indigenous marine species into this environment, and recognition of this has led to the development of a biosecurity management operational plan recommendations for the region (see Sinner *et al.* 2009). The operational plan identifies the need to focus on preventing marine invasive species from reaching Fiordland, particularly given the remoteness of the FMA and the corresponding difficulty of surveillance and response (Sinner *et al.* 2000).

Accordingly, MAF Biosecurity New Zealand (MAFBNZ) has prioritised the need to further explore treatment options to mitigate the potential marine biosecurity risks posed by marine equipment (*e.g.* anchors, mooring ropes, fishing nets and pots, kayaks, dive equipment and wetsuits) to the FMA. Previous research and experimentation has identified numerous effective chemicals and treatment systems (*e.g.* acetic acid, heat treatment) to manage invasive marine species (Wotton *et al.* 2004; Forrest & Blakemore 2006; Forrest *et al.* 2007). While chemical (*e.g.* acetic acid, lime, bleach) treatment methods can be very effective in pest eradication, they have limitations in that they are not always practical or can be potentially hazardous to gear and people. In contrast, simple household detergents are more accepted (*e.g.* used on some gear types routinely), generally readily available and cost effective to use. Cawthron has already trialled detergents in freshwater environments in the control of *Didymo* and elucidated effective dose-response scenarios for that species (Kuhajek & Wood 2009a,b; Tonkin & Taylor 2010). Similarly, preliminary work conducted in Australia against target marine pests including the Asian kelp *Undaria pinnatifida*, the fanworm *Sabella spallanzanii* and the seastar *Asterias amurensis* highlights the potential efficacy of detergent solutions for managing pathway risks (Gunthorpe *et al.* 2001). However, the effectiveness of these solutions in treating other groups of marine taxa (including both micro- and macroscopic life stages) is yet to be thoroughly evaluated.

In May 2010, MAFBNZ commissioned the Cawthron Institute (Cawthron) to research the effectiveness of hot soapy water (and/or other untested measures) for gear treatment (RFP 11815). Following a preliminary assessment of the available options (Appendix 1) and subsequent consultation with MAFBNZ, two common household chemicals were chosen for further assessment. The dishwashing liquid *Palmolive OriginalTM* (Palmolive) and the liquid disinfectant *DettolTM* (Dettol) were selected for a series of laboratory-based trials to evaluate their effectiveness in eliminating microscopic (juvenile) and macroscopic (adult) life stages of common marine fouling taxa. Heat treatment (*i.e.* hot water immersion, steam application) was not the focus of the trials due to several operational issues associated with its use (see Appendix 1). The results from the trials, along with findings from previous studies, were then used to produce robust guidelines on treatment options for the mitigation of biosecurity risks posed to the FMA from marine equipment (appended to this report).

2. Methods

2.1 TREATMENT SOLUTIONS, DELIVERY MECHANISMS AND EXPOSURE TIMES

Prior to the commencement of full-scale trials, a pilot study was undertaken to evaluate the efficacy of Palmolive and Dettol on a broad range of biofouling taxa (*i.e.* bryozoans, hydroids, anemones, bivalves, ascidians, and macroalgae) to ensure that satisfactory end-points could be achieved (*i.e.* mortality of selected taxa). The promising results from the pilot study (Appendix 2) led to full-scale trials that aimed to examine the effects of delivery method, concentration, and exposure times on macroscopic and microscopic fouling organisms (summarised in Table 1).

Macroscopic test organisms included general fouling taxa (including invertebrates and algae) and the sea squirt *Didemnum vexillum* (*Didemnum*). Microscopic test organisms included gametophytes of the Asian kelp *Undaria pinnatifida* (*Undaria*), and post-settlement larvae of the seasquirts *Ciona* spp. (*Ciona*) and the arborescent bryozoan *Bugula flabellata* (*Bugula*).

Two concentrations (1 and 5%) and exposure times (1, 10 and 60 min) were selected based on the pilot work and published studies (Kilroy 2005, Lewis and Dimas 2007, see Appendix 1). Chemicals were applied using two methods, full immersion and spray treatment, based on earlier work undertaken by Piola *et al.* (2010). Freshwater was used as the dilution medium for all chemical solutions, as it was envisaged that visitors to the Fiordland region would be reluctant to use saltwater when cleaning dive equipment and kayaking gear. Two chemical-free treatments, comprising freshwater only and seawater only, were used to control for the dilution medium and handling effects (*e.g.* air exposure), respectively. A smaller-scaled laboratory experiment (immersion only) was conducted to test the efficacy of hot water solutions of 5% Dettol and Palmolive, and heated freshwater and seawater on *Didemnum*.

Table 1: Treatment solution, delivery method and exposure time applied to the range of macro- (adult) and micro- (juvenile) organisms (n = 3 replicates per treatment combination)

Size class	Taxa	Solution	Delivery method	Exposure time (min)	Treatment Date	Assessment times (days)
Macroscopic organisms	Invertebrate and algal assemblages	1, 5% Palmolive	Spray + immersion	1, 10, 60	15/07/10	0, 7, 14
		1, 5 % Dettol				
		100% Freshwater 100% Seawater				
Macroscopic organisms	<i>Didemnum vexillum</i> (cold water solutions)	1, 5% Palmolive	Spray + immersion	1, 10, 60	05/08/10	0, 7, 14
		1, 5 % Dettol				
		100% Freshwater 100% Seawater				
Macroscopic organisms	<i>Didemnum vexillum</i> (hot water solutions)	Cold 100% seawater	Immersion only	1, 10, 60	19/08/10	0, 7, 14
		Hot 100% seawater				
		Hot 100% freshwater 1, 5 % hot Dettol 1, 5 % hot Palmolive				
Microscopic organisms	<i>Undaria pinnatifida</i> gametophytes	1, 5% Palmolive	Spray + immersion	1, 10, 60	19/08/10	0, 1, 4, 7
		1, 5 % Dettol				
		100% Freshwater 100% Seawater				
Microscopic organisms	<i>Ciona</i> sp. post-settlement larvae	1, 5% Palmolive	Spray + immersion	1, 10, 60	27/09/10	0, 4, 7
		1, 5 % Dettol				
		100% Freshwater 100% Seawater				
Microscopic organisms	<i>Bugula flabellata</i> post-settlement larvae	1, 5% Palmolive	Spray + immersion	1, 10, 60	27/09/10	0, 4, 7
		1, 5 % Dettol				
		100% Freshwater 100% Seawater				

2.2 EXPERIMENTAL SETUP AND APPLICATION OF CHEMICAL TREATMENTS

Experiments on invertebrate and algal assemblages were conducted at Port Nelson Marina (S41°15'32.52" E173°16'52.96"), while trials on *Didemnum* were conducted at Cawthron laboratories and on a floating pontoon in Nelson's commercial port (S41°15'23.62" E173°16'32.64"). Trials on *Undaria* gametophytes, *Ciona* spp. and *Bugula* post-settlement larvae were all conducted at Cawthron laboratories.

2.2.1 Macroscopic organisms

Due to differences in factors such as the morphology and attachment biology of the various macroscopic taxa trialled, it was necessary to cultivate experimental units using a variety of methods. Perspex settlement plates (20 x 20 cm, n = 108) that had been suspended from a floating marina pontoon for a period of ~ 12 months provided general fouling assemblage experimental units. Plates had developed diverse and prolific fouling assemblages (including known pest organisms and non-indigenous species), with representatives of bryozoans (encrusting and arborescent), colonial and solitary ascidians (sea squirts), bivalves, serpulids (tube worms) and macroalgae present (Figure 1). Use of these units allowed flexibility in experimental manipulations and consistency across treatments.

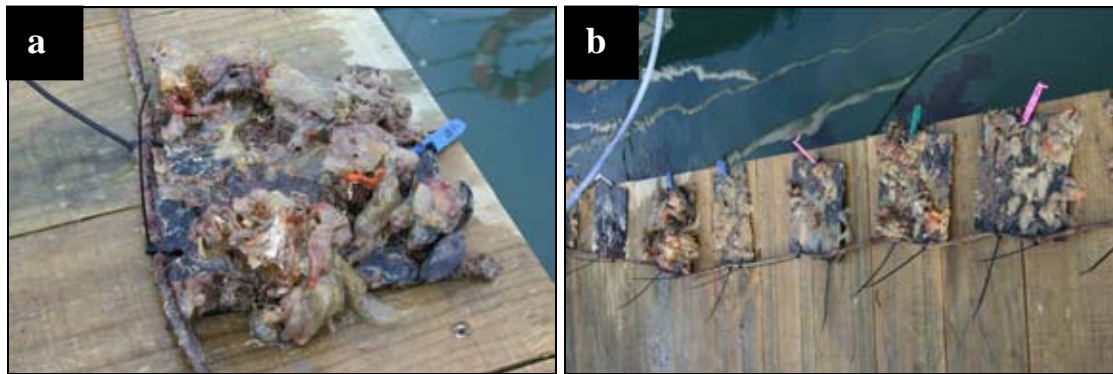


Figure 1. An example of (a) an experimental plate fouled with a variety of invertebrate and algal taxa and labelled with treatment details, and (b) experimental plates attached in random order of treatments onto the backbone ropes.

The efficacy of detergents on *Didemnum* was tested using experimental units comprising 5 x 5 cm Perspex settlement plates fouled with colonies of *Didemnum* (Figure 2a). Fouling was artificially induced by transplanting a fragment of *Didemnum* to each plate by placing rubber bands around the plates and slipping small pieces (~ 2 cm diameter) of *Didemnum* under the rubber bands. Plates were then attached to a backing panel (Figure 2b) that was then suspended beneath a pontoon in Port Nelson and left undisturbed for a period of ~ 4 weeks to allow the colony to attach and grow. This was an effective method of inoculating plates with *Didemnum*, achieving plate coverage of 31 to 99 % after ~ 4 weeks.

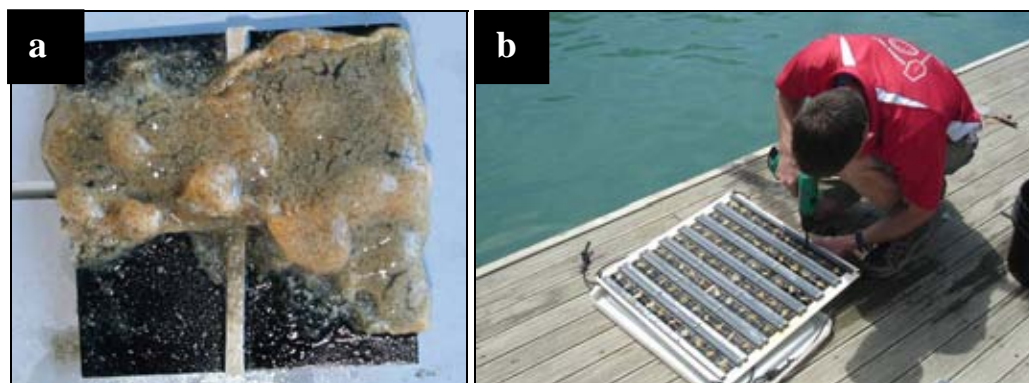


Figure 2. (a) Plate with transplanted *Didemnum* fragment after ~ 4 weeks, and (b) plates attached to the frame.

Experimental units were randomly assigned to each treatment group (Table 1) and labelled using plastic tags (general assemblage plates) or a pre-allocated numbering system (*Didemnum* plates). Immediately prior to treatment, each macroscopic experimental unit was photographed using a Canon EOS 400D digital camera (18-55 mm lens set at wide angle, 10.1 megapixels) to establish the baseline extent of fouling and assemblage diversity. Plastic containers (30 L for general assemblage plates and 2 L for *Didemnum* plates) were filled with treatment solutions used for immersion treatments, while individual 5 L pressure sprayers (one per chemical concentration) were used for applying the spray chemical treatments (Figure 3). Each experimental unit was immersed in, or sprayed with, the appropriate chemical concentration (1 or 5 % Palmolive or Dettol), seawater or freshwater (controls) for the designated exposure period. Control assemblages were similarly sprayed with, or immersed in, untreated seawater or freshwater (0% chemical concentration). Spray treatment experimental units remained out of the water for their assigned exposure times. At the end of each exposure period, experimental units were immediately rinsed (by submerging in seawater).

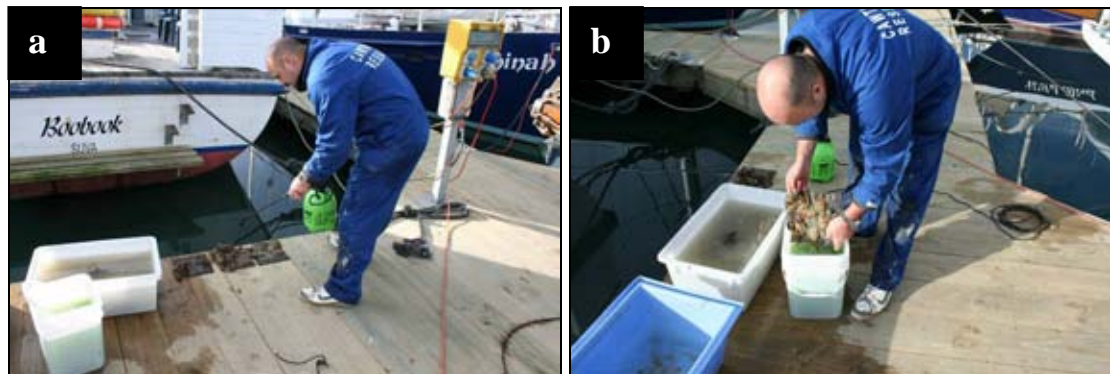


Figure 3. The two delivery methods used for administering chemical treatments to experimental units: (a) spray, and (b) immersion.

Settlement plates with general fouling assemblages were randomly reattached to backbone ropes in the field (Figure 1), and a further three blank plates were also attached to the backbone to assess recruitment over the experimental period (thus allowing discrimination of organisms surviving treatment vs. recolonising treated plates). *Didemnum* plates were randomly attached to backing panels (Figure 2) and suspended beneath marina pontoons. All units were removed from the water and re-photographed 7 and 14 days later to assess treatment effects.

The hot water *Didemnum* experiment was identical to the cold water *Didemnum* experiment described above, except treatment solutions were heated to ~ 40°C prior to treatment and spray delivery was not tested. Hot water treatment baths were kept in insulated containers for the duration of the experiments, but otherwise no attempt was made to try and maintain initial temperatures. A seawater treatment containing ambient temperature seawater was used as a procedural control for handling effects.

2.2.2 Microscopic organisms

The efficacy of detergents on eliminating microscopic organisms was determined using experimental units comprising of 10 ml plastic pots or Petri dishes, settled with either *Undaria* gametophytes, *Ciona* or *Bugula* (see Appendix 3 for culture methods). *Undaria* gametophytes were 2 weeks old at the start of experiments, and invertebrates were 4 days old. Microscopic organisms were counted under a dissecting microscope prior to treatment. Experimental units were treated using the same treatment delivery systems as the macroscopic taxa (see above, 2L plastic containers for immersion and 5 L pressure sprayers for spray delivery). Following treatment, culture pots with microscopic *Undaria*, *Ciona* or *Bugula* were re-filled with seaweed medium (*Undaria*) or seawater (invertebrates), covered and returned to a culture cabinet. *Undaria* abundances were assessed at 1, 4, and 7 days, and invertebrate abundances were assessed at 4 and 7 days.

2.3 ASSEMBLAGE CENSUS AND EVALUATION OF TREATMENT EFFECTS

For the fouling assemblages on settlement plates, digital photographs were rectified using ArcMap 9.2 GIS software (ESRI, Redlands, CA, USA) and analysed using a random dot method (Meese and Tomich 1992). This involved identifying and recording taxa (or bare space) beneath 100 points that were overlaid in a random stratified arrangement. A different overlay pattern was used for each assessment time. A 1 cm perimeter along the edge of each plate was omitted from counting to control for possible edge effects. All point count data were entered into a database and later used to generate summary data. Tube worms (serpulids, polychaete sabellid worms) were often present on the plates. It was not always possible to determine whether worm casings were occupied without dissection, and it was unclear post-treatment whether worms had died due to treatment, or had left their casings. Therefore, due to this difficulty in determining pre- and post-treatment survival, tube worms were not assessed and points were classified as bare space.

The growth of *Didemnum* during the 14 d experiment was determined by measuring the change in surface area (SA) from digital photographs taken before and after treatment (0, 7 and 14 d), using ImageJ image analysis software (Rasband 1997-2008). SA measurements were subsequently converted to percentage cover (% cover). For *Undaria*, counts of live gametophytes were made prior to treatment at a pre-defined point on the culture pot. A single count within an eyepiece grid was made for each pot at 100 x magnification, using an inverted compound microscope. Repeat counts at the same position were made 1, 4 and 7 days after treatment. Gametophytes were considered to be dead if they had a loss of brown pigmentation and/or a necrotic appearance. Gametophytes with slight pigmentation were conservatively considered to be alive. *Ciona* survival was measured by marking 5 live individuals, and assessing them 4 and 7 days after treatment. For *Bugula*, between 5 and 10 individuals were marked and counted pre-treatment, and after 4 and 7 days. For both species, counts were made with the aid of a binocular microscope.

2.4 ANALYSES

For the fouling assemblages experiment, examining the effects of each treatment was complicated by the fact that the plates initially contained a variety of taxa in different abundances. Therefore, proportional changes in % cover were evaluated to avoid underestimating changes due to reduced initial abundances. Proportional changes in % cover of fouling assemblages and survival of *Undaria* gametophytes were analysed using fully factorial analyses of variance (ANOVA) of data at experiment end dates, with the factors of chemical type/concentration, delivery method and exposure duration being fixed. Homogeneity of variances was checked using Cochran's test. Further analysis of fouling

assemblages to species-level was not appropriate due to the large variability in species abundance across the settlement plates, but changes in % cover for taxonomic groups of interest were examined graphically. Data for the other microscopic experiments (*Ciona* and *Bugula*) were not suitable for statistical analysis due to large mortality resulting in no survival.

Changes in *Didemnum* % cover were analysed using a three-way repeated measures analysis of variance (RM-ANOVA), with the factors of chemical type/concentration, delivery method and exposure duration being fixed. Validity of data used in RM-ANOVAs was tested using Mauchly's sphericity test, where a Mauchly's test value of < 0.05 indicates sphericity cannot be assumed. In cases where assumptions of sphericity were violated, we present Greenhouse-Geisser adjusted *p*-values. Where appropriate, Tukey's post-hoc tests were used to identify statistically significant differences between treatments detected in the RM-ANOVA, with significant within-subjects effects tested against the error term of the main test. All data were assessed for homogeneity of variance and normality using residual plots and descriptive statistics (as per Quinn and Keough 2002), and were transformed where necessary to meet the underlying assumptions of the methods used. All ANOVA were conducted using STATISTICA Version 8 (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1 MACROSCOPIC ORGANISMS

3.1.1 General fouling assemblages

Percentage cover (% cover) of fouling assemblages

Larger reductions in proportional % cover were evident following treatment by the two household detergents (Dettol and Palmolive) than that observed following spraying or immersion in the freshwater and seawater controls (Figure 4), and this was reflected in a significant *Chemical x Delivery* interaction ($F_{5,72} = 3.89$, $P = 0.004$; Table 2). The majority of Dettol and Palmolive treatments also had greater reductions in proportional % cover across all exposure periods (relative to controls), resulting in a significant *Chemical x Exposure* interaction ($F_{10,72} = 3.45$, $P = 0.001$). None of the treatments were completely effective against all macroscopic fouling organisms. However, there were noticeable decreases in the % cover of fouling in almost all of the Dettol treatments, with the exception being the 1% 1 min spray treatment where only small (and non-significant) decreases in fouling cover were observed. With the exception of the 1% 1 min immersion and spray treatments (16-32% decreases), the average percentage decrease in fouling cover (as proportions of the original % cover) ranged from 44-93% (Figure 4). Palmolive appeared to be less effective, with generally smaller decreases in % cover. However, Palmolive 10 and 60 min immersion treatments were reasonably effective, with average proportional % cover decreases ranging from 60-77% (Figure 4). For both Dettol and Palmolive, there was a general trend of greater reductions in total cover with increased exposure time, and some immersion treatments were more effective than spray treatments.

Table 2: Summary of analysis of variance (ANOVA) examining the effects of chemical (1 and 5% Dettol and Palmolive, freshwater and seawater), delivery method (immersion or spray) and exposure time (1, 10 and 60 min) on changes in proportional % cover of fouled plates

Source	df	MS	F	P
Chemical	5	6806.5	35.2245	0.000
Delivery	1	1102.6	5.7059	0.020
Exposure	2	4373.3	22.6322	0.000
Chemical x Delivery	5	751.2	3.8875	0.004
Chemical x Exposure	10	667.0	3.4518	0.001
Delivery x Exposure	2	2006.9	10.3860	0.000
Chemical x Delivery x Exposure	10	285.4	1.4770	0.166
Error	72	193.2		

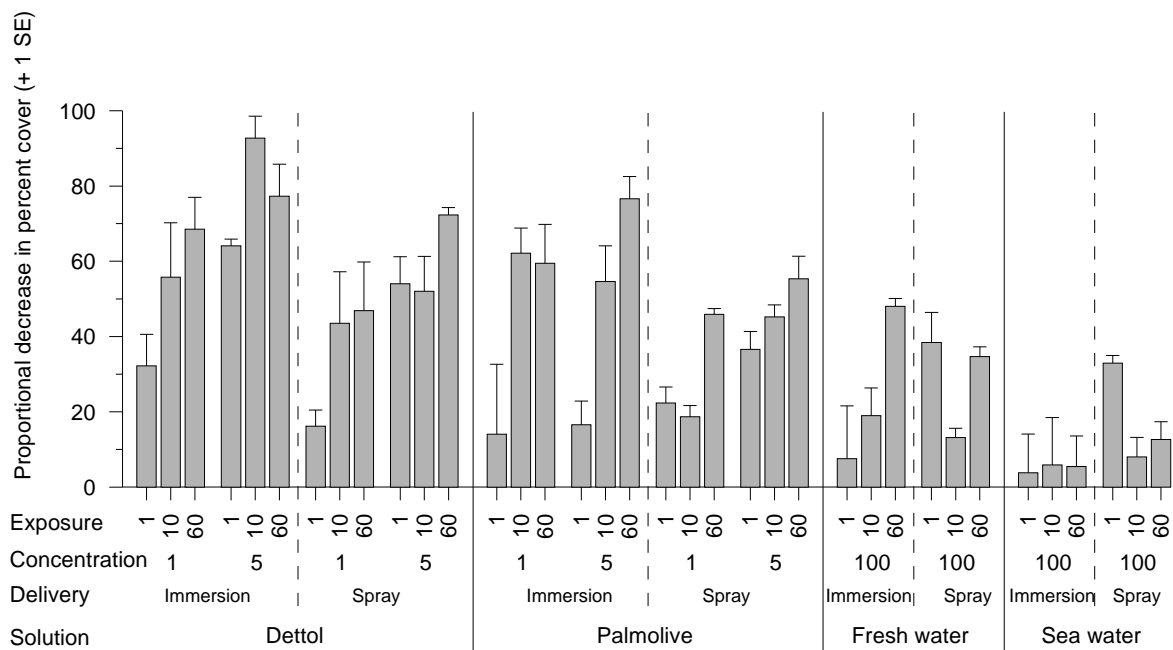


Figure 4. Proportional change in % cover (mean + 1 SE, $n = 3$) of fouling on settlement plates following immersion and spray treatments. All assessments were made two weeks post-treatment.

Solitary ascidians

Solitary ascidians encountered on the settlement plates included *Ciona* spp., *Corella eumyota*, *Asciidiella adspersa*, *Pyura rugata* and unidentified species. Dettol and Palmolive immersion treatments of 10 and 60 min exposure times were effective in reducing abundances (54-96% reductions in proportional cover; Figure 5a, c, e, g), and the Dettol 5% 1 min treatment was also effective (88 % reduction in proportional cover). Spray delivery of 5% solutions of both chemicals with 10 and 60 min exposures also appeared moderately successful (21-75% reductions in proportional cover; Figure 5b, d, f, h).

Colonial ascidians

Didemnum spp., *Botryllus schlosseri*, *Botrylloides leachi*, *Aplidium phortax* and *Diplosoma listerianum* were encountered on the settlement plates. As observed with the solitary ascidians, Dettol and Palmolive immersion treatments of 10 and 60 min resulted in noticeable reductions (e.g. 43-100% proportional reduction) in the abundances of colonial ascidians (Figure 6a, c, e, g). Some spray treatments were also relatively effective (e.g. Dettol 5%: 78-98% proportional reductions), but Palmolive solutions required 60 min exposure times to be effective (51-96% proportional reductions, Figure 6f, h). Immersion and spray freshwater treatments of at least 60 min also appeared relatively effective (69-96% proportional reductions, Figure 6i, j).

Macroalgae

Macroalgae encountered on the plates included filamentous red algae, *Undaria pinnatifida*, *Dictyota* sp., filamentous green algae and other unidentifiable species. Changes in macroalgal cover were characterised by high variability (Figure 7), which is likely due to the high initial variability in cover between replicates. Immersion treatments of all Dettol concentrations and exposure times, and 5% Palmolive solution with 10 and 60 min exposure times were

relatively effective at reducing fouling cover (69-100%, and 70-80 % proportional reductions for Dettol and Palmolive respectively).

Of specific interest to MAFBNZ was the vulnerability of *Undaria* (an introduced species) to treatment effects. Adult *Undaria* plants were only present in 13 of the 36 different treatments options, and as such a full comparison of survival was not possible. However, from observations of plant mortality, it appeared that a number of immersion treatments (1% Dettol 1 and 60 min, 1% Palmolive 10 and 60 min, Palmolive 5 % 60 min, Freshwater 60 min) were effective with 100% mortality, but spray treatments (1% Dettol 10 and 60 min, Palmolive 5 % 60 min, Freshwater 10 and 60 min) were ineffective (100% survival, Appendix 3).

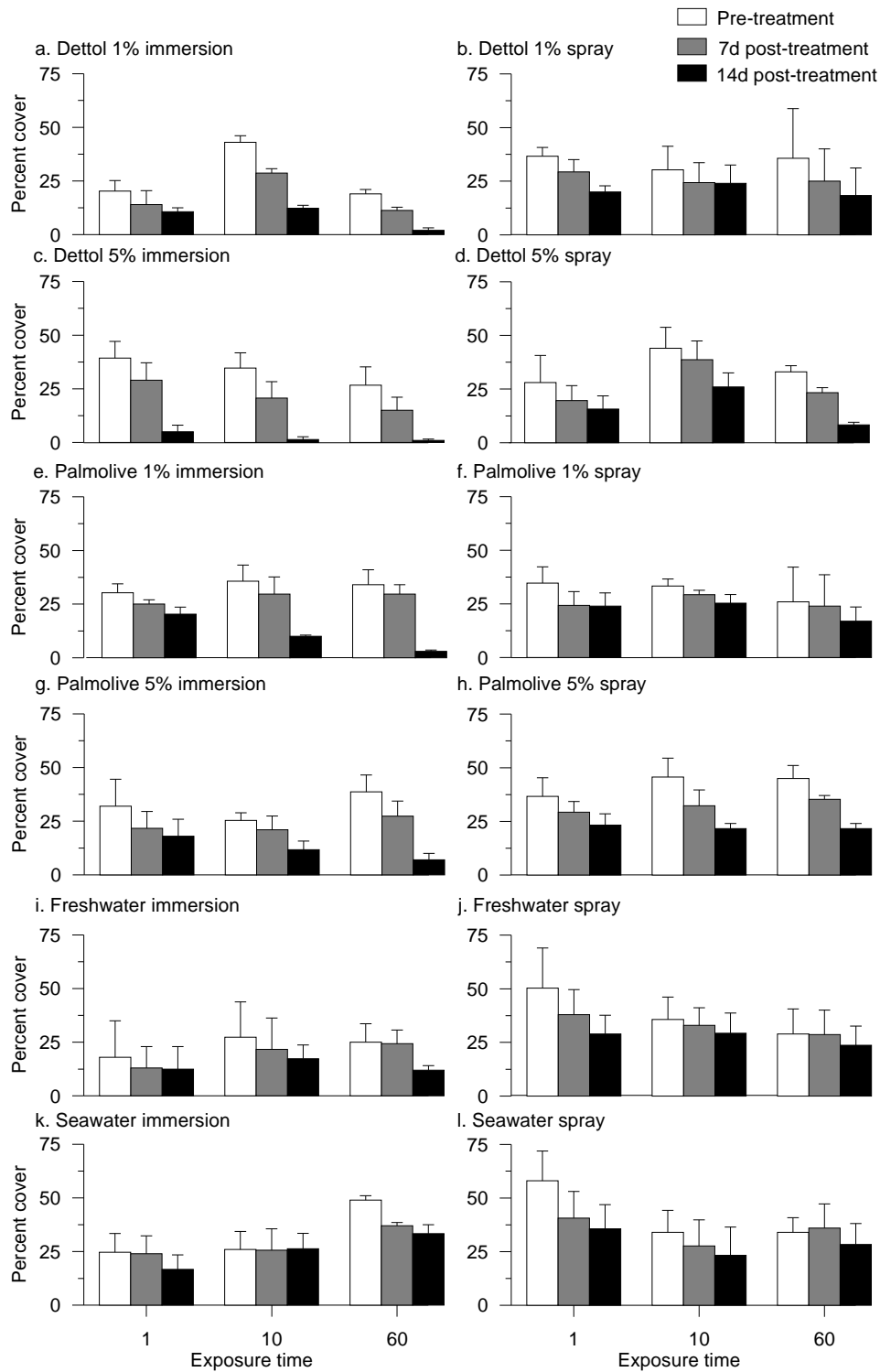


Figure 5. Percentage cover (% cover) of solitary ascidians (mean + 1 SE, $n = 3$) on settlement plates before and after immersion and spray treatments with: (a, b) 1% Dettol solution, (c, d) 5% Dettol solution, (e, f) 1% Palmolive solution, (g, h) 5% Palmolive solution, (i, j) freshwater, and (k, l) seawater.

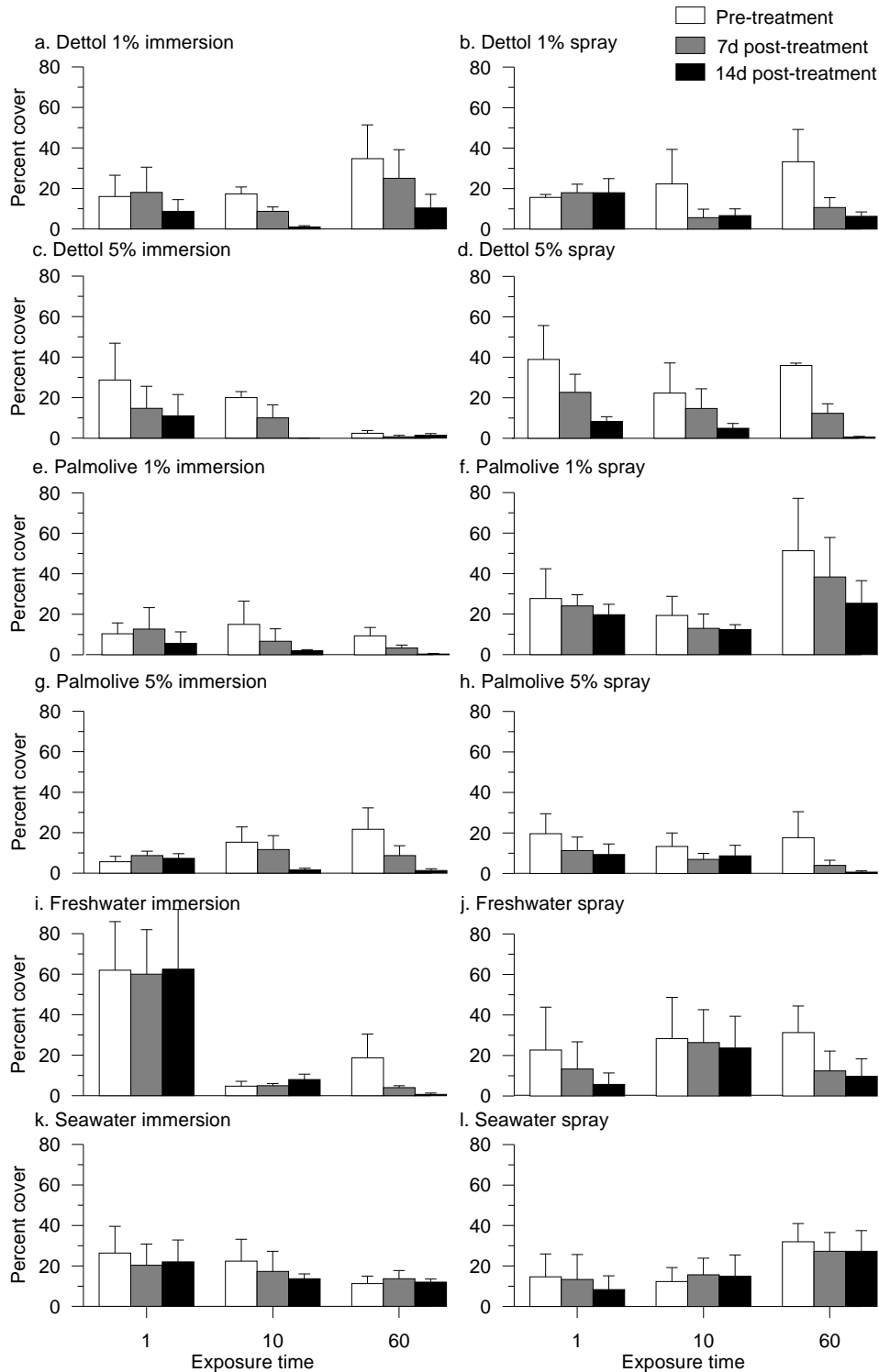


Figure 6. Percentage cover (% cover) of colonial ascidians (mean + 1 SE, $n = 3$) on settlement plates before and after immersion and spray treatments with: (a, b) 1% Dettol solution, (c, d) 5% Dettol solution, (e, f) 1% Palmolive solution, (g, h) 5% Palmolive solution, (i, j) freshwater, and (k, l) seawater.

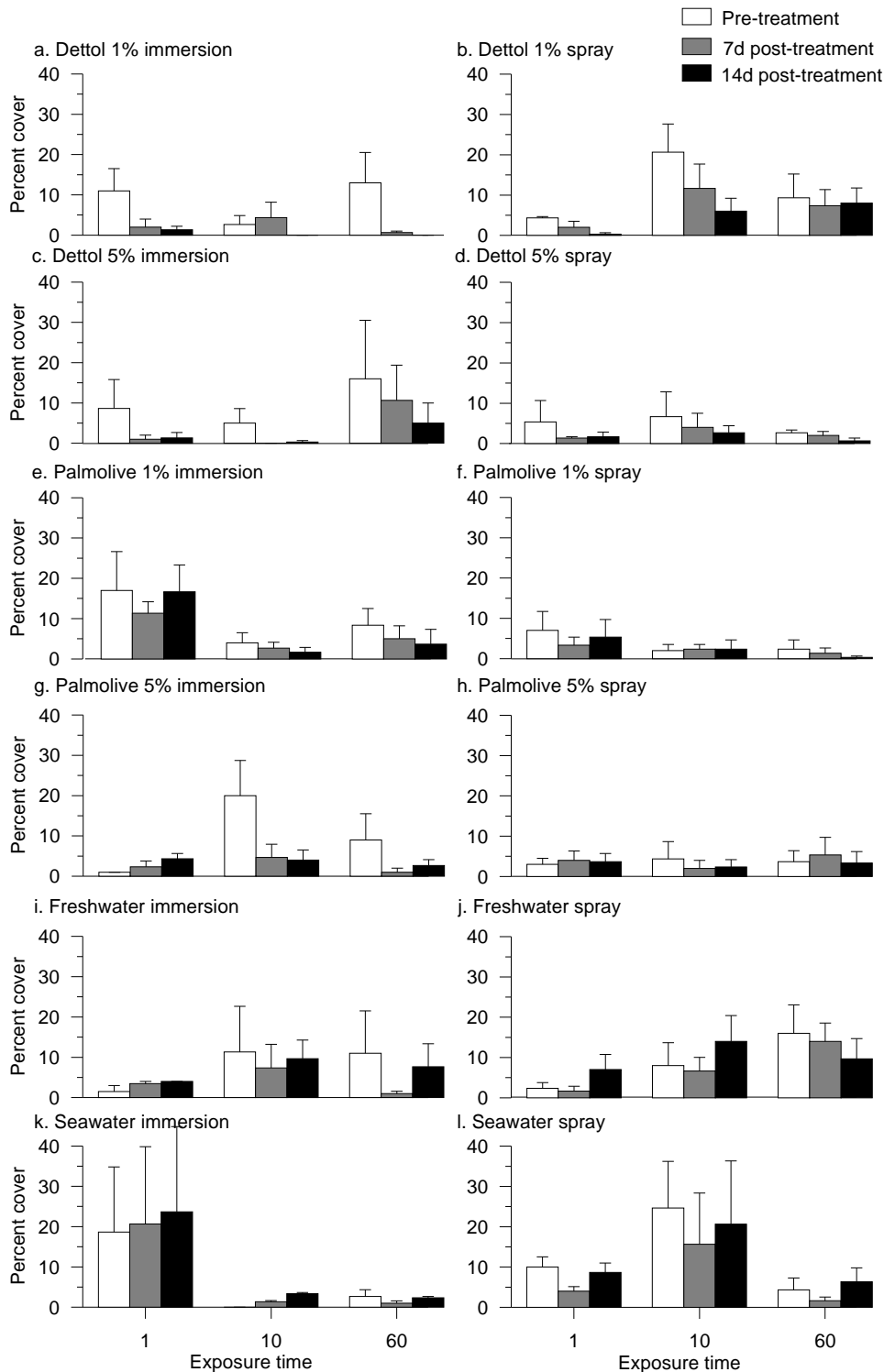


Figure 7. Percentage cover (%) of macroalgae (+ 1 SE, $n = 3$) on settlement plates before and after immersion and spray treatments with: (a, b) 1% Dettol solution, (c, d) 5% Dettol solution, (e, f) 1% Palmolive solution, (g, h) 5% Palmolive solution, (i, j) freshwater, and (k, l) seawater.

3.1.2 *Didemnum vexillum*

Treatments using ambient water temperature

There was high variability in the efficacy of Dettol and Palmolive in reducing the colony size of *Didemnum* (Figure 8). Solutions of 1 and 5% Dettol, and 5% Palmolive were the only treatments able to achieve 100% mortality; however, this was only achieved following a 60

min immersion period (Figure 8a, c, g). Immersion in 1% Palmolive for 60 min also resulted in a large reduction in colony surface area; however, complete removal was not achieved (Figure 8e).

Spray delivery of the treatment chemicals was less effective than immersion. Spraying with 1% Dettol had almost no measurable effect on *Didemnum* colony size when assessed at 7 and 14 d post-treatment (Figure 8b). By contrast, the 5% Dettol spray treatment consistently reduced *Didemnum* cover across all exposure times (1, 10 and 60 min; Figure 8d). Spray delivery of 1% Palmolive had limited effect on *Didemnum* cover (Figure 8f). Spraying with 5% Palmolive solution was effective at reducing colony surface area after 10 and 60 min exposure, but had almost no effect when exposed for only 1 min (Figure 8h). Immersion in freshwater resulted in a reduction in *Didemnum* surface area at exposure times of 10 and 60 min, however very little effect was observed following spray treatment (Figure 8j). Immersion of *Didemnum* in seawater (control conditions) had almost no effect on colony size (Figure 8k); however, some post-treatment variability was observed in treatments sprayed with seawater (Figure 8l).

Results of RM-ANOVA showed that, overall, *Didemnum* colony surface area 7 and 14 d post-treatment was significantly less than that measured pre-treatment (Tukey's post hoc $P < 0.05$). A significant *Time x Chemical* interaction (Table 3, $F_{10,144} = 8.830$, $P < 0.001$) primarily resulted from a greater reduction in the size of Dettol and Palmolive post-treatment colonies relative to all pre-treatment colonies (Tukey's post hoc $P < 0.05$), in addition to freshwater treatments and seawater controls showing no significant post-treatment reduction. A significant *Time x Delivery* interaction ($F_{2,144} = 8.861$, $P = 0.001$) was a result of post-treatment colonies (both 7 and 14 d) being smaller than pre-treatment colonies for both spray and immersion methods; however, immersion was much more effective at reducing *Didemnum* biomass than spraying (Tukey's post hoc $P < 0.05$). An overall *Time x Exposure* effect ($F_{4,144} = 10.868$, $P < 0.001$; Table 1) indicated that over all exposure periods (1, 10 and 60 min), *Didemnum* colony areas were significantly reduced relative to pre-treatment sizes, however, 60 min exposure was more effective than 10 min, which in turn was more effective than 1 min treatments.

Table 3: Summary of repeated measures analysis of variance (RM-ANOVA) examining the effects of chemical (1 and 5% Dettol and Palmolive, freshwater and seawater), delivery method (immersion or spray) and exposure time (1, 10 and 60 min) on % cover of *Didemnum* colonies

Source	df	MS	F	<i>P</i>
Between subjects effects				
Chemical	5	2465	3.101	0.014
Delivery	1	23534	29.600	<0.001
Exposure	2	10044	12.633	<0.001
Chemical x Delivery	5	6507	8.185	<0.001
Chemical x Exposure	10	980	1.232	0.286
Delivery x Exposure	2	2771	3.486	0.036
Chemical x Delivery x Exposure	10	1209	1.520	0.150
Error	72	795		
Within subjects effects				
Time	2	39048	262.225	<0.001*
Time x Chemical	10	1315	8.830	<0.001*
Time x Delivery	2	1293	8.681	0.001*
Time x Exposure	4	1618	10.868	<0.001*
Time x Chemical x Delivery	10	422	2.834	0.009*
Time x Chemical x Exposure	20	129	0.865	0.602*
Time x Delivery x Exposure	4	359	2.414	0.073*
Time x Chemical x Delivery x Exposure	20	162	1.090	0.375*
Error	144	149		

P values in bold indicate significant differences at $\alpha = 0.050$

* denotes use of Greenhouse-Geisser epsilon adjusted *P*-values due to assumption of sphericity violation (when Mauchly's test for sphericity <0.05)

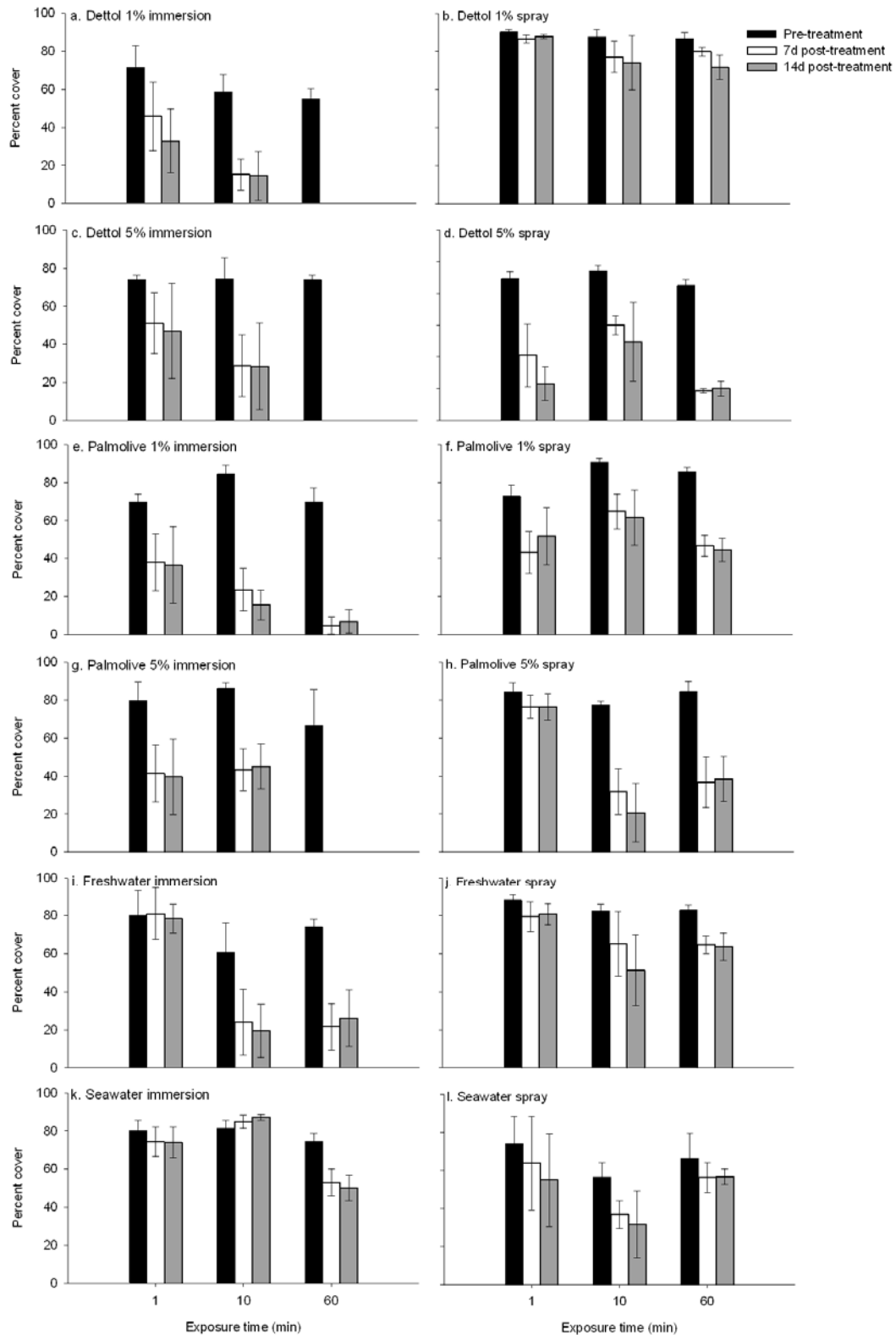


Figure 8. Percentage cover (% cover) of *Didemnum* colonies ($\pm 1SE$, $n = 3$) before and after immersion and spraying treatments with: (a, b) 1% Dettol solution, (c, d) 5% Dettol solution, (e, f) 1% Palmolive solution, (g, h) 5% Palmolive solution, (i, j) freshwater, and (k, l) seawater. The absence of bars indicates 0% cover.

Results of RM-ANOVA also showed a significant *Time x Chemical x Delivery* effect on *Didemnum* colony size pre- and post-treatment (Table 3; Figure 9), and this was evident by differences in initial cover and in the effectiveness of the delivery method between chemicals through time. For example, post treatment colonies immersed in 1% Dettol showed large

reductions in cover compared with colonies that were sprayed using the same chemical. In contrast, colonies immersed or sprayed with 5% Dettol showed similar responses, both with large reductions in cover. Colonies treated with Palmolive, freshwater and seawater had moderate differences between delivery methods.

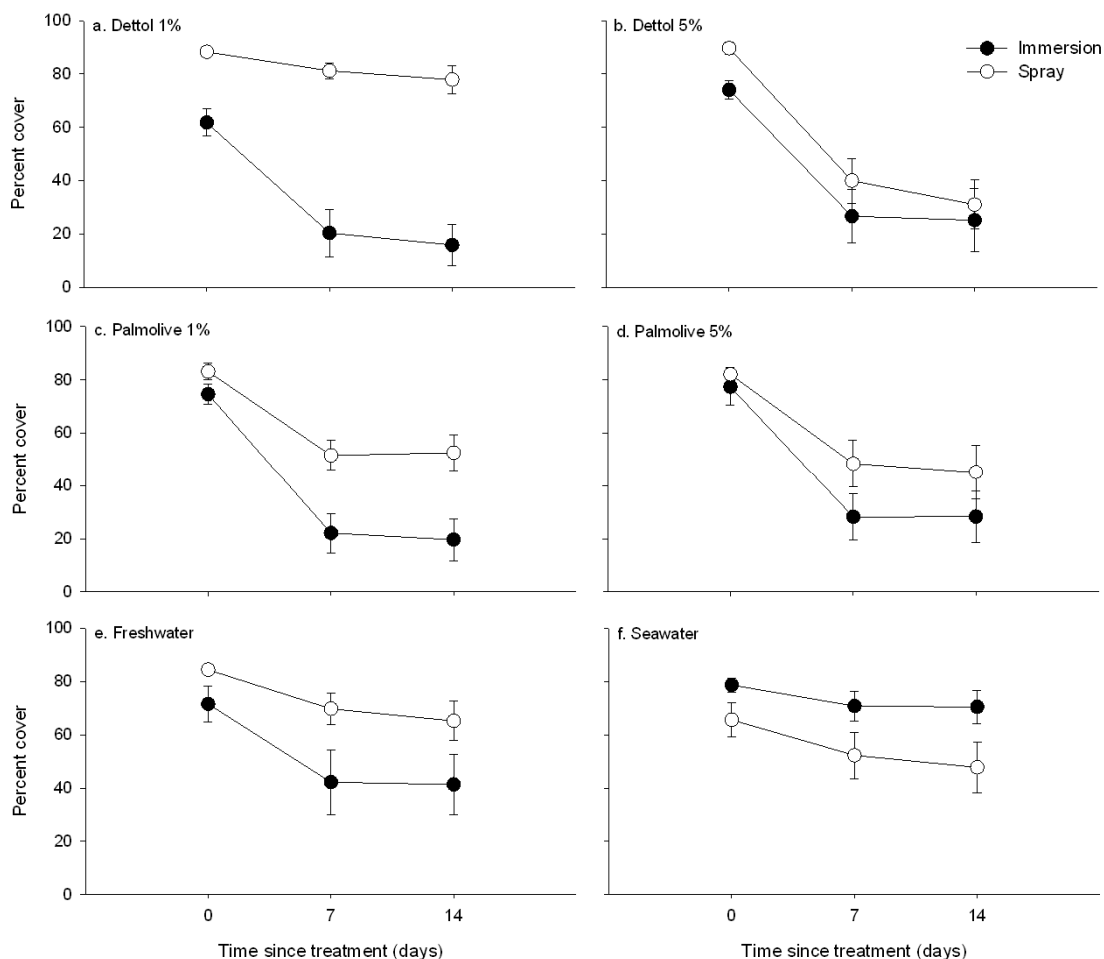


Figure 9. Percentage cover (% cover) of *Didemnum* colonies (pooled data over exposure times, mean \pm 1SE, $n = 3$) following immersion and spray treatments with: (a) 1% Dettol solution, (b) 5% Dettol solution, (c) 1% Palmolive solution, (d) 5% Palmolive solution, (e) freshwater, and (f) seawater.

Hot water immersion combined with chemical treatment

Increasing the temperature of treatment solutions resulted in a dramatic reduction in the size of *Didemnum* treatment colonies. Treatment solutions of 5% Dettol and Palmolive, freshwater and seawater were heated to a temperature of approximately 40°C at the commencement of treatment, and allowed to passively cool (by 6-7°C) during the course of the maximum treatment exposure period (60 min; Figure 10). The ambient temperature seawater control remained at ~ 15°C for the duration of the experiment (Figure 10). Solutions using heated freshwater had no effect on *Didemnum* colony size following 1 min exposure periods, with colony regrowth observed after 14 d (Figure 11). This was surprising given that 1 min immersion in cold water solutions of 5% Dettol and Palmolive led to visible reduction in colony surface area (Figure 11c and g). However, immersion in heated seawater greatly reduced colony surface area (37-82% reductions in cover; Figure 11), but this was potentially due to the higher temperature (by ~ 3°C) of this treatment relative to other heat treatments

(Figure 10). *Didemnum* colonies exposed to heated solutions of Dettol and Palmolive, and heated freshwater and seawater of 10 and 60 min experienced 100% mortality (Figure 11a-d), while colonies immersed in ambient temperature seawater remained largely unaffected (Figure 11e). These patterns were reflected in results of RM-ANOVA analysis that showed a significant *Time x Chemical x Exposure* effect on *Didemnum* colony size pre- and post-treatment (Table 4; Figure 11).

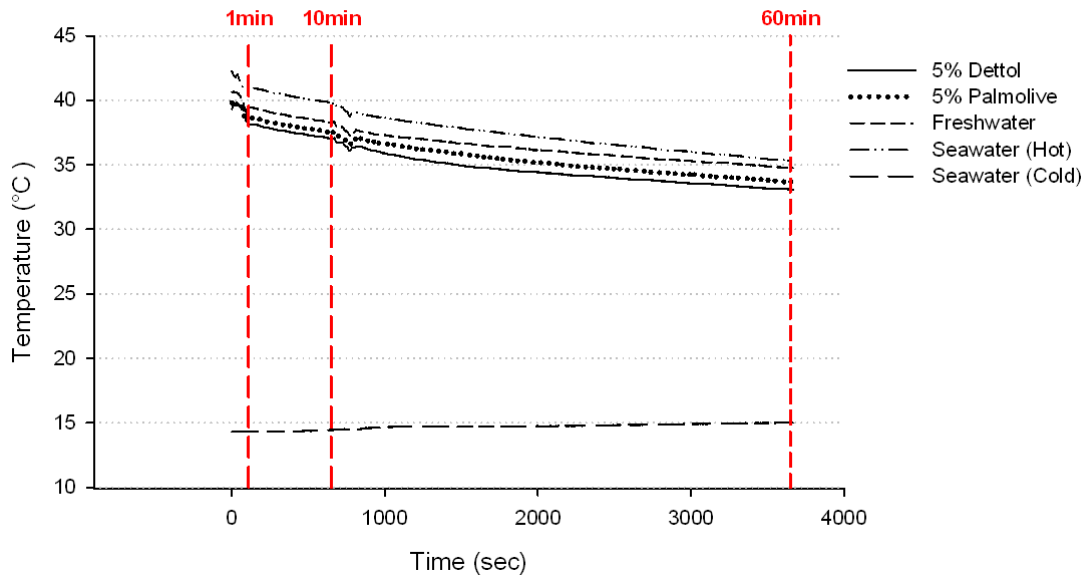


Figure 10. Ambient (control) and water bath temperatures logged during *Didemnum* hot water immersion trials.

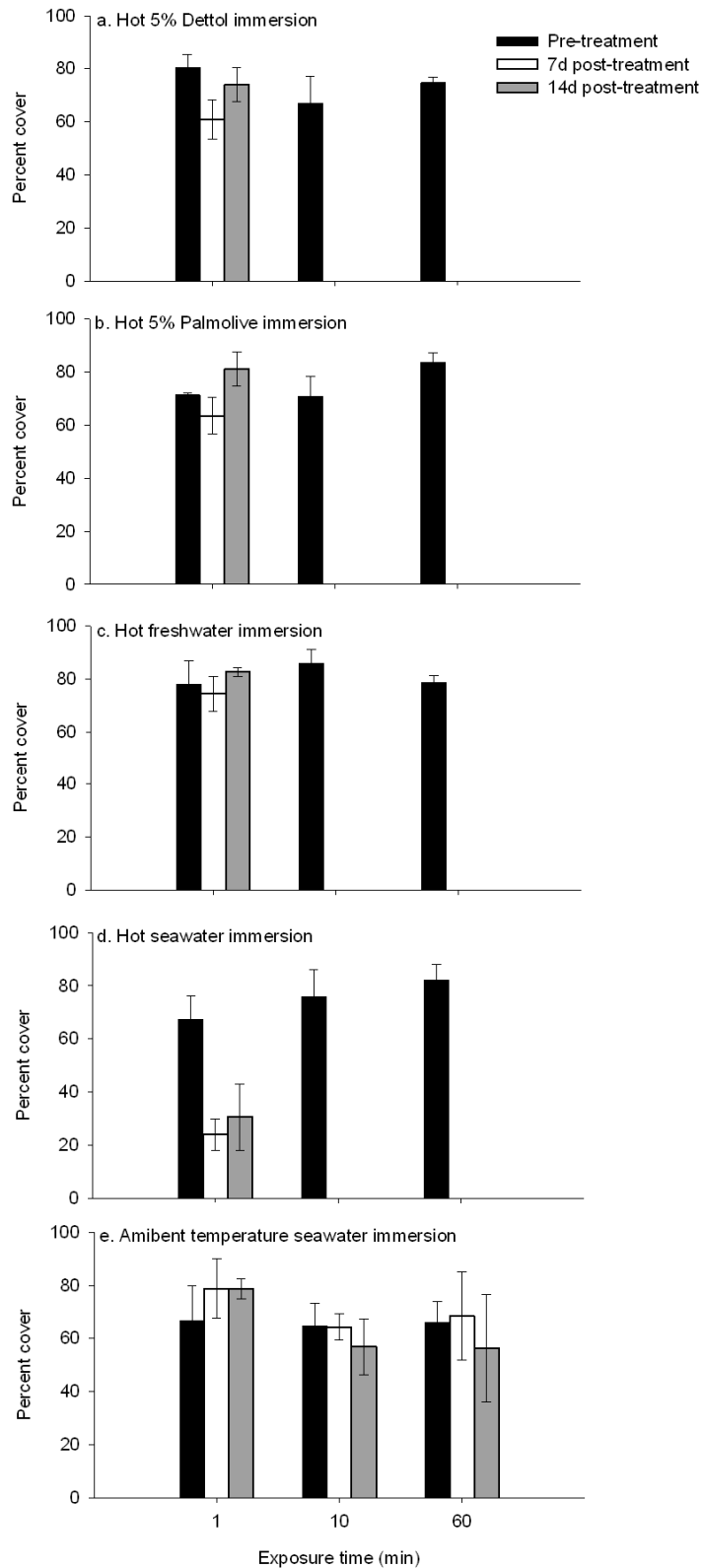


Figure 11. Percentage cover (% cover) of *Didemnum* colonies (mean \pm SE, $n = 3$) before and after treatment with hot water treatments with: (a) 5% Dettol, (b) 5% Palmolive, (c) freshwater, and (d) seawater. Also shown are changes in surface area of control colonies immersed in ambient seawater (e). The absence of bars indicates 0% cover.

Table 4: Summary of repeated measures analysis of variance (RM-ANOVA) examining the effects of heated treatment solutions (1 and 5% Dettol and Palmolive, freshwater and seawater), delivery method (immersion or spray) and exposure time (1, 10 and 60 min) on % cover of *Didemnum* colonies

Source	df	MS	F	<i>P</i>
Between subjects effects				
Chemical	4	4778.6	15.5044	<0.001
Exposure	2	17679.7	57.3632	<0.001
Chemical x Exposure	8	1146.7	3.7205	0.004
Error	30	308.2		
Within subjects effects				
Time	2	29578.3	439.5079	<0.001
Time x Chemical	8	2136.7	31.7488	<0.001
Time x Exposure	4	5156.1	76.6150	<0.001
Time x Chemical x Exposure	16	278.0	4.1310	<0.001
Error	60	67.3		

P values in bold indicate significant differences at $\alpha = 0.050$

* denotes use of Greenhouse-Geisser epsilon adjusted *P*-values due to assumption of sphericity violation (when Mauchly's test for sphericity <0.05)

3.2 MICROSCOPIC ORGANISMS

3.2.1 *Undaria gametophytes*

All Dettol treatments were effective in eliminating gametophytes, with no survival after 7 days (Figure 12). Gametophytes recorded as 'living' at 1 d and 4 d were slightly pigmented, but were potentially dead (*i.e.* the precautionary principle was adopted). The only Palmolive treatment that was 100% effective was the 5% immersion for 60 min, but 1% Palmolive for 60 min was reasonably effective, with only ~ 5% survival after 7 days. Despite differences in survival rates of gametophytes between the Palmolive immersion and spray treatments (Figure 12), delivery was not significantly different (Table 5). There appeared to be dilution medium and handling effects, with decreases in survival in some freshwater and seawater control treatments. However, the exposure effect was not clear, with immersion in freshwater or seawater for 1 min having lower survival than immersion for 10 min (Figure 12).

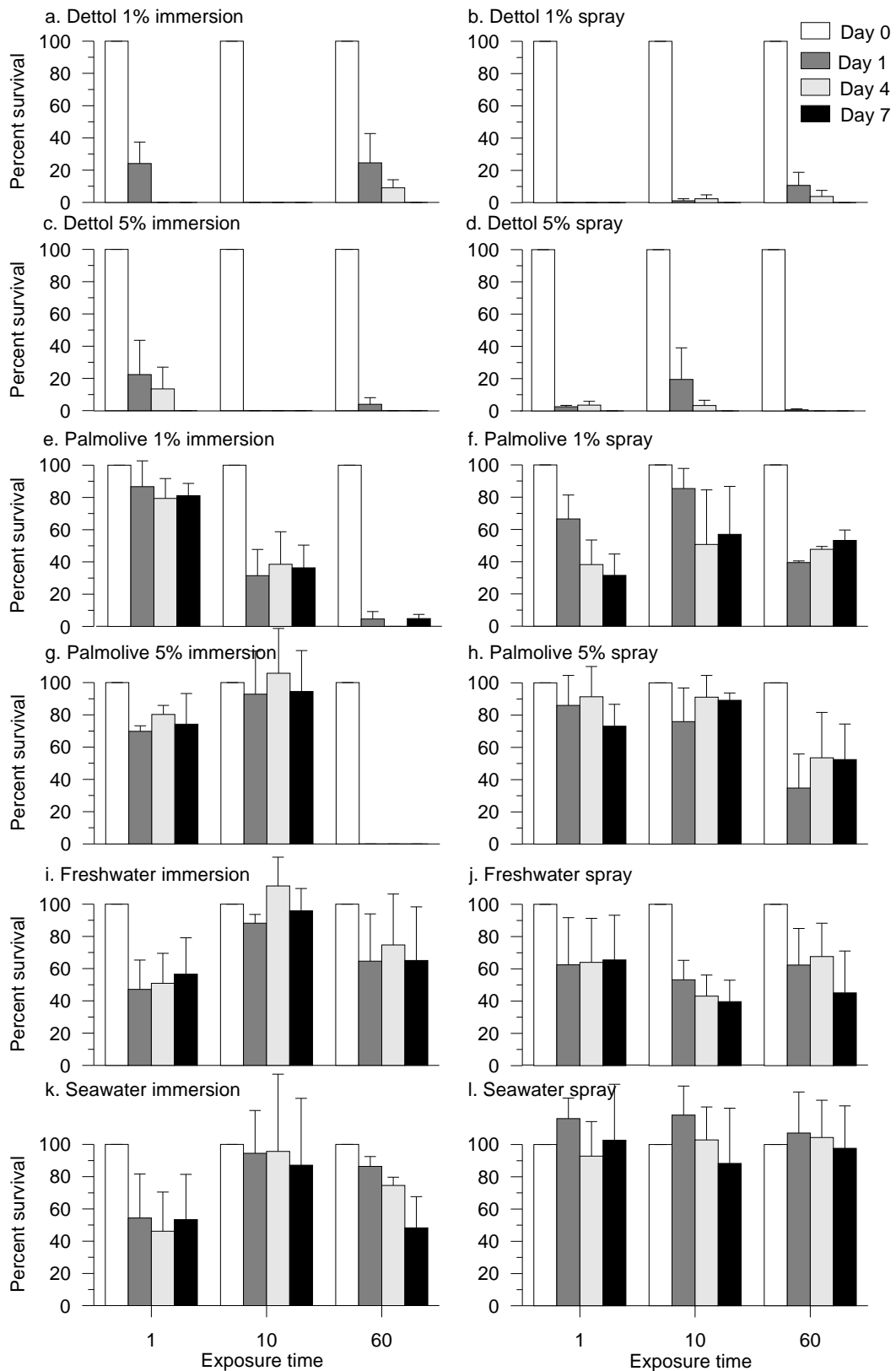


Figure 12. Mean survival (%+ 1SE, $n = 3$) of *Undaria* gametophytes before and after immersion and spray treatments with: (a, b) 1% Dettol solution, (c, d) 5% Dettol solution, (e, f) 1% Palmolive solution, (g, h) 5% Palmolive solution, (i, j) freshwater, and (k, l) seawater.

Table 5: Summary of analysis of variance (ANOVA) examining the effects of chemical (1 and 5% Dettol and Palmolive, freshwater and seawater), delivery method (immersion or spray) and exposure time (1, 10 and 60 min) on the survival of *Undaria* gametophytes

Source	df	MS	F	P
Chemical	5	20863.0	20.1455	0.000
Delivery	1	797.5	0.7701	0.383
Exposure	2	3366.1	3.2503	0.045
Chemical x Delivery	5	1537.7	1.4848	0.205
Chemical x Exposure	10	1049.2	1.0131	0.441
Delivery x Exposure	2	1924.1	1.8580	0.163
Chemical x Delivery x Exposure	10	1241.0	1.1983	0.307
Error	72	1035.6		

3.2.2 *Ciona* spp.

Survival of *Ciona* was the same at day 4 and day 7, so only data from day 4 are presented. Dettol and Palmolive spray and immersion treatments were highly effective, with 100% mortality in all but the 1 min Palmolive treatments where one live individual was observed per spray and immersion treatment (Figure 13). Freshwater was also relatively effective, but there was some survival following the 10 min immersion (40%) and 1 min spray (~13%) treatments. The seawater control 60 min spray treatment had on average only ~13% survival, and this was likely due to desiccation effects.

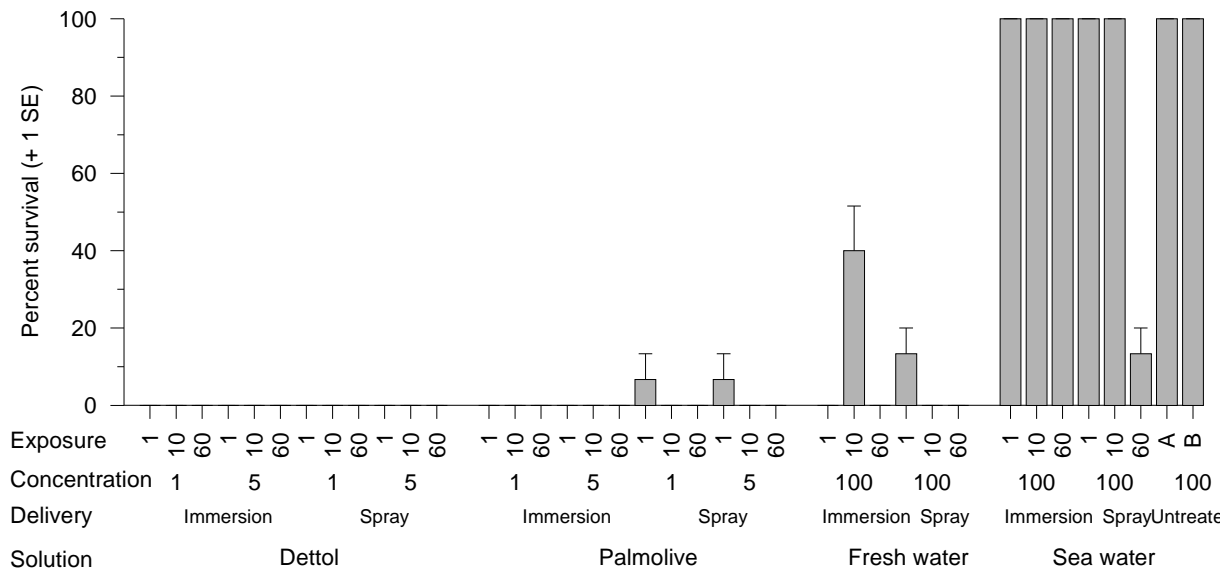


Figure 13. Percentage survival of *Ciona* ($n = 3$) on Day 4 after spray and immersion treatments with 1 and 5% Dettol, 1 and 5% Palmolive, freshwater and seawater.

3.2.3 *Bugula flabellata*

Survival of *Bugula* was the same at day 4 and day 7, so only data from day 4 are presented. None of the 1 min treatments with Dettol and Palmolive were completely effective in killing *Bugula*, with 67-100% of colonises surviving treatment (Figure 14). Palmolive and freshwater 10 min spray treatments were also ineffective, with ~ 95, 82 and 93% survival for 1%, 5% Palmolive and freshwater treatments, respectively. However, Dettol 10 and 60 min immersion and spray treatments, and 10 and 60 min Palmolive immersion treatments were 100% effective. As observed in the *Ciona* experiments, a desiccation effect was apparent, with ~ 30 % survival in the seawater 60 min spray treatment.

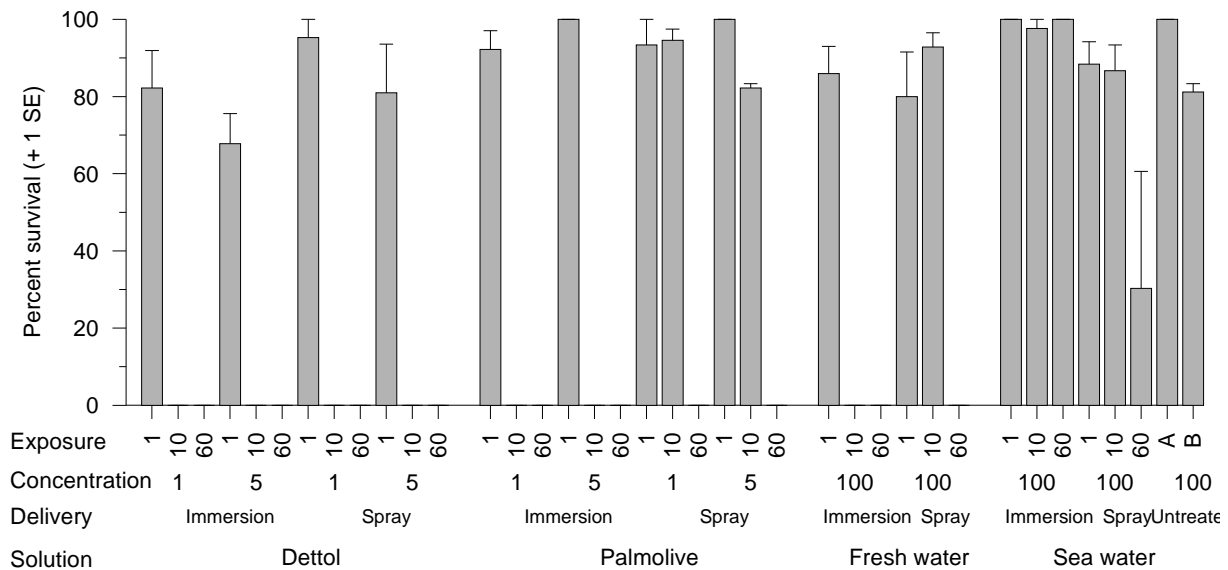


Figure 14. Percentage survival of *Bugula* ($n = 3$) on Day 4 after spray and immersion treatments with 1 and 5% Dettol, 1 and 5% Palmolive, freshwater and seawater.

4. Summary and conclusions

Dettol 1% and 5% and Palmolive 5% immersion treatments were consistently effective across all experiments (Table 6). Although 1 and 10 min exposure times were effective on many organisms, at least 60 min immersion time was required to achieve 100% mortality for adult colonies of the *Didemnum* (both solutions, cold water), and the microscopic gametophyte life-stage of *Undaria* (Palmolive). When hot water solutions were used on *Didemnum* the immersion time to achieve 100% mortality was reduced to 10 min.

Spray delivery was generally not as effective as immersion. However, Dettol spray treatments were successful in most experiments, but were not 100% effective against adult *Didemnum* colonies.

Table 6. Minimum exposure times for each treatment combination (IM = immersion, SP = spray) to achieve 100% mortality of organisms (*Didemnum*, *Undaria*, *Ciona* and *Bugula* experiments), and for decrease in % cover of general fouling organisms. Ineffective treatments are shaded, blank treatments were not tested.

Solution Delivery Concentration Experiment	Dettol				Palmolive				Freshwater		Seawater	
	IM		SP		IM		SP		IM	SP	IM	SP
	1	5	1	5	1	5	1	5	100	100	100	100
<i>Didemnum</i> (cold)	60	60	x	x	x	60	x	x	x	x	x	x
<i>Didemnum</i> (hot)	10	10			10	10			10		10	
<i>Undaria</i>	1	1	1	1	x	60	x	x	x	x	x	x
<i>Ciona</i>	1	1	1	1	1	1	10	10	60	60	x	60
<i>Bugula</i>	10	10	10	10	10	10	60	60	10	60	x	60
Fouling assemblages	10	10	10	10	10	10	60	60	60	60	x	x

The main observations from our trials were as follows:

- Dettol was more effective than Palmolive.
- Immersion treatments were more successful than spray.
- A 60 min exposure time was required to achieve 100% mortality for some organisms (*Didemnum* adults, *Undaria* gametophytes).
- Warmer water temperatures increased effectiveness against *Didemnum*, and this is most likely applicable to other organisms (see Blouin 2002, Forrest & Blakemore 2003).
- Desiccation increased mortality (in some experiments, 60 min. seawater spray treatments incurred mortality).

One of the aims of this project was to use this research and other available information to produce guidelines on treatment options for the mitigation of biosecurity risks posed to the Fiordland Marine Area (FMA) from marine equipment. These guidelines are presented in Appendix 5, and outline options for soaking, spraying and drying equipment over a range of time periods and potential chemicals. An appropriate method should be chosen based on (1) time available (*e.g.* air exposure can take up to 1 month), (2) access to treatment chemicals, (3) size and amenability of the item/s to the treatment methods (*e.g.* a kayak may be too big to soak so spraying or air exposure is likely to be a better approach), and (4) sensitivity of equipment to treatment effects. Treatment options using household chemicals are as follows:

(1) soaking equipment in 5% Palmolive or 1% Dettol solutions for 60 min, or (2) spraying items with a 1% Dettol solution and leaving for 60 min. The effectiveness of these methods can be enhanced by using warm water and by drying gear thoroughly after treatment.

Observations made during the trials indicate that the effectiveness of household chemicals in eliminating organisms is related to the sensitivity of the organism, and this can be related to size, thickness of tissue, protective casings or complex morphologies. Experiments with microscopic organisms were very successful, with 100% mortality, often with short exposure periods, 1% concentrations and spray delivery. However, household chemicals had less of an effect on macroscopic organisms, often requiring longer exposure periods, 5% concentrations and immersion delivery. None of the treatments were 100% effective against macroscopic organisms on the fouled plates (see Section 3.1.1). In addition, the *Didemnum* experiment used relatively 2-dimensional layers of *Didemnum* tissue, and thicker, 3-dimensional organisms may be more resilient to treatment. This reinforces the requirement of removing all macroscopic organisms as part of the treatment process (Appendix 5). The cleaning component of the treatment process using chemical treatments can then target microscopic organisms and macroscopic fragments, which appear to be more susceptible to treatment.

5. Acknowledgements

We thank Mark Heath for his assistance with laboratory and field work, Tim Dodgshun for assistance with culture and aquarium facilities, Patrick Cahill for *Ciona* larval supply, and Paul Barter for Access database setup and advice. We also thank Chris Cornelisen for review of this report, and Barrie Forrest for advice and input during the project and the production of the guidelines. We are grateful to the Port Nelson Harbour Authority, and the managers and residents of Nelson Marina for access to field study sites.

6. References

- Batstone CJ, Elmetri I, Taylor M, Sinner J, Clarke S 2009. Mapping the Values of New Zealand's Coastal Waters. 2. Economic Values. MAF Biosecurity Technical Paper No. 2009/05. 74 pp. <http://www.biosecurity.govt.nz/files/pests/salt-freshwater/economic-value-mapping.pdf>
- Blouin MA 2002. A procedure for the decontamination of SCUBA diving equipment and underwater gear after diving in waters containing zebra mussels (*Dreissena polymorpha*) and other exotic species of Dresseinidae. Standard operating procedure, U.S. Geological Survey.
- Department of Conservation (DOC) 2006. The value of conservation: What does conservation contribute to the economy? <http://www.doc.govt.nz/upload/documents/conservation/value-of-conservation.pdf> [Accessed 21 June 2009].
- Forrest BM, Blakemore K 2003: An evaluation of methods to reduce inter-regional spread of the Asian kelp *Undaria pinnatifida* via marine farming activities. Cawthron Report No. 773. 38 p. plus appendices.
- Forrest BM, Blakemore KA 2006. Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. *Aquaculture* 257: 333-345.

- Forrest BM, Hopkins GA, Dodgshun TJ, Gardner JPA 2007. Efficacy of acetic acid treatments in the management of marine biofouling. *Aquaculture* 262: 319-332.
- Gunthorpe L, Mercer J, Rees C, Theodoropoulos T 2001. Best practices for the sterilisation of aquaculture farming equipment: a case study for mussel ropes. Marine and Freshwater Resources Institute Report 41, Marine and Freshwater Resources Institute, Queenscliff, Australia. 48 p.
- Kilroy C 2005. Tests to determine the effectiveness of methods for decontaminating materials that have been in contact with *Didymosphenia geminata*. NIWA Client Report: CHC2005-004.
- Kuhajek J, Wood S 2009a. Didymo New Zealand Defence Force vector control project: Laboratory studies on viability. Cawthron Report No. 1686. 10 p. plus appendices.
- Kuhajek J, Wood S 2009b. Didymo New Zealand Defence Force vector control project: Field studies on viability. Prepared for Tonkin and Taylor. Cawthron Report No. 1691. 22 p. plus appendices.
- Lewis JA, Dimas J 2007. Treatment of biofouling in internal seawater systems – Phase 2. Maritime Platforms Division, Defence Science and Technology Organisation. 25 p.
- Meese RJ, Tomich PA 1992. Dots on the rocks: a comparison of percent cover estimation methods. *Journal of Experimental Marine Biology and Ecology* 165: 59-73.
- Piola RF, Dunmore RA, Forrest BM 2010. Assessing the efficacy of spray-delivered ‘eco-friendly’ chemicals for the control and eradication of marine fouling pests. *Biofouling* 26: 187-203.
- Rasband, WS 1997-2008. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>.
- Sinner J, Forrest BM, Taylor MD 2000. A Strategy for Managing the Asian Kelp *Undaria*: Final Report. Cawthron Institute, Nelson, New Zealand. Cawthron Report No. 578. 122 p.
- Sinner J, Forrest B, O’Brien M, Piola P, Roberts B 2009. Fiordland Marine Biosecurity Risk Management: Operational Plan Recommendations 2009/10 – 2013/14 Prepared for MAF Biosecurity New Zealand and Fiordland Marine Guardians. Cawthron Report No. 16211621. 32 p plus appendices.
- Tonkin and Taylor 2010. Didymo Vector Control Project. Report prepared for the New Zealand Defence Force.
- Wotton DM, O’Brien C, Stuart MD, Fergus DJ 2004. Eradication success down under: heat treatment of a sunken trawler to kill the invasive seaweed *Undaria pinnatifida*. *Marine Pollution Bulletin* 49: 844-849.

7. Appendices

APPENDIX 1. PRELIMINARY ASSESSMENT OF SUITABLE METHODS FOR THE TREATMENT OF MARINE PESTS: MAFBNZ PROJECT 11815

GENERAL APPROACH

MAFBNZ project 11815 describes the need to identify potential treatment methods for mitigation of biosecurity risks posed to the FMA from marine equipment such as anchors, mooring ropes, fishing nets and pots, kayaks, dive equipment and wetsuits. While previous research and experimentation has identified numerous effective tools (*e.g.* acetic acid, heat treatment) that have been successfully used in managing marine invasive species, other more commonly available treatment options have yet to be extensively trialled (*e.g.* hot soapy water).

Household detergents have been successfully trialled and used as a response tool in the treatment of the freshwater alga *Didymosphenia geminata* (Kilroy 2005, Kilroy *et al.* 2006, Kuhajek and Wood 2009 a,b). They have also been used with some success in trials on a few marine species (Gunthorpe *et al.* 2001, Lewis and Dimas 2007). However, their effectiveness against a wide range of marine species (including both micro- and macroscopic life stages) is yet to be evaluated. We anticipate that household detergent would be more readily accepted as an equipment disinfectant by end-users compared with some other commonly used chemicals (*e.g.* acetic acid, bleach), due to its low cost, ready availability, and relatively low environmental toxicity.

Review of chemicals

There are many brands of detergents and antiseptic cleaners that would likely be suitable for eliminating marine pest species from infected gear and equipment. However, many are unfeasible for everyday use due to high costs, low availability and issues with environmental toxicity. Our recommended approach is to focus on common brands that are readily available to the general public, in the interest of facilitating maximum uptake of treatment measures by relevant end-users. In addition, mild detergents (*e.g.* hand dishwashing liquids) and disinfectants (*e.g.* antiseptic liquids) are more likely to be accepted by end users for use on sensitive gear (such as dive equipment) given they are generally milder than stronger chemicals such as laundry detergents.

A comparison of potential chemicals, their availability, and notes for and against use is presented in Table 1. While the active ingredients in each chemical are listed (when available), we make no assertions that these active constituents are the components ultimately responsible for eliciting the desired treatment response. In fact, the combination of some or all of the ingredients in each solution may be the important factor determining their overall efficacy. For example, Kilroy *et al.* (2006) trialled manufactured detergents and isolated active ingredients, and found that they did not necessarily produce the same results. Trials with Napisan (active ingredient sodium percarbonate) and sodium percarbonate in isolation produced conflicting results. A much higher concentration of sodium percarbonate than that contained in a comparative Napisan solution was required to produce similar mortality of *D. geminata*.

Napisan has been successfully trialled against *D. geminata* (Kilroy *et al.* 2006, Kuhajek and Wood 2009 a,b), and laundry soakers in general are likely to be effective against marine organisms. However, perception of their toxicity and corrosiveness may hinder their ready

adoption by end-users, particularly for use on sensitive equipment such as dive gear. Furthermore, the many different variants that often exist for these products (*e.g.* Napisan, Napisan Plus, Napisan Presoak, Napisan OxyAction) may have different degrees of effectiveness, and lead to confusion among end users as to their individual suitability for use.

In contrast, dishwashing detergents are cost effective, readily available and generally low toxicity options for the disinfection of aquatic equipment. A number of dive websites¹ recommend mild detergents for cleaning dive gear, thus such compounds are already considered acceptable for use on sensitive equipment. Dishwashing detergents have been used with success in trials on *D. geminata*; Palmolive, Sunlight and Down to Earth dishwashing liquids at 5% concentrations required an exposure time of only 1 minute to attain 100% mortality (Kilroy *et al.* 2006).

Palmolive detergent has also been used successfully in trials against the Australian blue mussel *Mytilus galloprovincialis planulatus*, with 1% concentrations killing some mussels after 6 h, and greater mortality evident with increasing concentrations (Lewis and Dimas 2007). It is expected that organisms without the protection of a shell, or other structure allowing them to close off from the environment (*e.g.* the operculum of calcareous tube worms), would be more susceptible to treatment, and that shorter exposure times would be effective. Treatment solutions comprising detergent and freshwater are used by fisherman in Australia as a means of disinfecting equipment to prevent the spread of diseases such as the Abalone Viral Ganglioneuritis (AVG)².

Antiseptic liquids (Dettol and Savlon) have also been effective against *D. geminata* (Kilroy 2005), with 5% solutions for 30 seconds sufficient to cause 100% mortality. These liquids are commonly used in first aid to cleanse wounds, and as general household disinfectants. As such, they would generally be perceived as suitable for use on sensitive gear. They are also cost-effective and readily available from supermarkets and pharmacies.

Disinfectants and other cleaners (*e.g.* moss and lichen remover) often contain the active ingredient benzalkonium chloride, and some of these solutions have been particularly effective against freshwater and marine organisms. Uncle Jack's Surfactant has been trialled against *D. geminata* (Kilroy *et al.* 2006), and disinfectants with benzalkonium chloride as the active ingredient are recommended for treating gear against the spread of *D. geminata*. Lewis and Dimas (2007) trialled a variety of chemicals, including vinegar, detergents, disinfectants, bleach, pipework treatments and descalers against *M. galloprovincialis planulatus*, and found that the two disinfectants containing benzalkonium chloride (Conquest and Quatsam) were the most effective treatments. However, they noted that the toxicity and environmental acceptability of the chemicals would require further examination. The higher toxicity of these chemicals would decrease their acceptability by many end-users.

Commercially available dive cleaners (*i.e.* detergents) are commonly used at dive gear hire shops for the disinfection of wetsuits and equipment. To our knowledge, the efficacy of these detergents have not been trialled in experiments, however, they may be suitable for eliminating organisms since their active ingredients may be similar to detergents or disinfectants. Disadvantages are that these chemicals are only available at specialist shops, and are less cost-effective than other treatments. Salt-away is a corrosion control salt-removing treatment that is used on boats, vehicles, dive gear, etc. However, the active

¹ *E.g.* <http://www.scubaboard.com/forums/new-divers-those-considering-diving/338459-dish-detergent-wetsuits.html>; <http://www.scubadivingsolutions.com/cleangear.html>; <http://en.allexperts.com/q/Scuba-Diving-1649/equipment-storage.htm>

² Tasmanian Department of Primary Industries, Parks, Water and Environment
<http://www.dpiw.tas.gov.au/inter.nsf/Attachments/VWAS-7QLU3X?open>
<http://www.dpiw.tas.gov.au/inter.nsf/WebPages/SCAN-6ZX7S5?open>

ingredients are not provided in the Materials Safety Data Sheet for this compound, and therefore it is unknown whether this solution would be suitable for trials. Other brands of dive equipment cleaners include ProDive and Akona. Pro-Dive marine cleaner is described as an alkaline, multipurpose water-based degreasing detergent, but active ingredients are not listed.

Other products considered were those marketed as 'environmentally friendly' (such as Simple Green and Citrus Based Cleaner). These were not effective at eliminating *D. geminata* except at very high concentrations, which consequently leads to them being very expensive and potentially less environmentally friendly (Kilroy et al. 2006).

Table 1. Comparison of different chemicals for potential use in trials examining their efficacy against marine organisms.

Chemical	Brand	Ingredients (NA = not available)	Irritant to skin/throat etc. ³	Corrosive to metals, rubber etc. ¹	Cost effectiveness ^{1,4}	Availability	Pros	Cons
Dishwashing liquid	Palmolive original	C12-13 alcohol Sodium sulphate Lauramidopropylamine oxide Myristamidopropylamine oxide Magnesium sulphate Free oil Tetrasodium EDTA ⁵	Low*	Low*	\$5.00/L	Supermarket	Well-known, readily available brands, very cost-effective and relatively low environmental toxicity. Proven efficacy against <i>Didymo</i> ; Palmolive proven effective against mussels (Lewis and Dimas 2007).	Possibility of excessive foaming with spray application
	Sunlight	Anionic surfactants Citrus acid	Low*	Low*	\$4.85/L	Supermarket		
	Down to Earth	NA	Low*	Low*	\$4.80/L	Supermarket		
Laundry soaker/Nappy cleaner	Napisan	Active ingredient Sodium percarbonate	Low*	Medium*	\$9.10/kg	Supermarket	Well known brands, relatively cost-effective, Napisan proven efficacy against <i>Didymo</i> (Kilroy 2005, Kilroy <i>et al.</i> 2006, Kuhajek and Wood 2009 a,b).	More corrosive than dishwashing liquid, and usage would not be as acceptable for some gear (<i>e.g.</i> dive gear)
	Sard	Active ingredient Sodium percarbonate	Low	Medium	\$5.90/kg	Supermarket		
Antiseptic/antibacterial	Dettol	Chloroxylenol Pine oil Isopropyl alcohol	Low	?	\$5.60/L (disinfectant) \$18.60/L (antiseptic)	Supermarket	Well known brands, relatively cost-effective. Both effective against <i>Didymo</i> (Kilroy 2005).	Possible dislike of odour by end users.
	Savlon	Chlorhexidine gluconate, cetrimide	Low	?	\$52/L (antiseptic)	Supermarket		
Disinfectant Cleaners	<i>E.g.</i> Spray and away moss killer; Uncle Jack's Surfactant	Active ingredient Benzalkonium chloride	Medium*	Medium*	Spray and away \$9.00/L; Uncle Jack's \$10.00/L*	Specialist	'Uncle Jack's' proven efficacy against <i>Didymo</i> (Kilroy <i>et al.</i> 2006), benzalkonium chloride-based chemicals effective against the Australian blue mussel (Lewis and Dimas 2007).	Usage may not be as acceptable for some gear (<i>e.g.</i> dive gear). Brands less known.

³ Assessments marked with * are from Kilroy *et al.* 2006.

⁴ Costs are approximate only and prices for liquids have been adjusted to 1 L volumes for comparison. Prices are subject to market change and bulk purchase may be cheaper.

⁵ MSDS Palmolive regular dishwashing liquid. Issue date: August 2009. Colgate.

Chemical	Brand	Ingredients (NA = not available)	Irritant to skin/throat etc. ³	Corrosive to metals, rubber etc. ¹	Cost effectiveness ^{1,4}	Availability	Pros	Cons
Dive gear cleaner	Salt-away; Akona dive equipment cleaner; Pro- dive Marine Clean	NA	?	Low	Salt-away \$53/L	Specialist	Safe for use on dive gear, low corrosiveness.	Specialist cleaner that is not as easy to acquire as above chemicals. Expensive. Brands less known. Unknown ingredients and effectiveness not proven.
'Environmentally friendly' cleaners	Simple Green	Citric acids Acetic acids Sulphonic acids Alcohols ¹	Low*	Medium*	\$17.15/L	Supermarket	'Environmentally friendly'	Proven ineffectual on <i>Didymo</i> (Kilroy <i>et al.</i> 2006)
	Citrus Based Cleaner	NA	Low*	Low*	\$70/L	Mail order	'Environmentally friendly'	Proven ineffectual on <i>Didymo</i> (Kilroy <i>et al.</i> 2006)

¹ Assessments marked with * are from Kilroy *et al.* 2006.

² Costs are approximate only and prices for liquids have been adjusted to 1 L volumes for comparison. Prices are subject to market change and bulk purchase may be cheaper.

³ MSDS Palmolive regular dishwashing liquid. Issue date: August 2009. Colgate.

Recommended chemicals and proposed methodology

Subject to agreement by MAFBNZ, and based on the discussion above, we propose to examine the efficacy two common household chemicals; the dishwashing liquid Palmolive Original™, and the liquid disinfectant Dettol™. These two chemicals are readily available from supermarkets and are familiar brands to the general public. Furthermore, their low corrosive properties suggests they would be more acceptable for use on sensitive gear. Both these chemicals have been proven effective treatment options for *D. geminata* (Kilroy 2005).

Using these two compounds, we propose to separately consider treatment criteria for macroscopic (adult) and microscopic (juvenile) life stages of common marine fouling taxa. It is often small or microscopic life-stages (*e.g.* gametophytes of *Undaria*) that are the most significant in terms of pathway risk (Forrest & Blakemore 2003; Forrest *et al.* 2007). Hence, we stress the particular importance of testing treatment efficacy against such life-stages. Our general approaches for each are outlined below.

Field-based trials for assessing treatment options on macro flora and fauna will involve the use of experimental units, such as pre-fouled settlement plates or ropes, containing a range of attached fouling organisms. Our methods (including use of experimental units) will be based on those outlined in Piola *et al.* (2010), which were successfully used in evaluating effectiveness of environmentally friendly chemicals (*e.g.* acetic acid) on a variety of invasive taxa. Use of these units allows flexibility in experimental manipulations and consistency across treatments.

To test the efficacy of each of the two compounds, experimental units will be exposed to a range of concentrations across several exposure times (Table 2). We propose to test two concentrations (1 and 5%) with freshwater used as the dilution medium. These concentrations have been selected based on information from trials on *D. geminata* and *M. galloprovincialis planulatus*. Organisms will be exposed to the treatments for three exposure periods (1, 10 and 60 minutes). Also included will be two controls treatments comprising freshwater only and seawater only, to control for the dilution medium and exposure/drying time, respectively. Chemical delivery options will include full immersion and spray/wash treatments techniques. While immersion-based treatments are adequate for treating some types of gear, circumstances exist where immersion treatment is not practical or possible (*e.g.* treatment of large pieces of equipment such as kayaks or commercial fishing gear). The efficacy of spray/wash-delivered treatments will be investigated for these circumstances. An added benefit of spray/wash treatment over immersion baths and dips, is that effective chemical concentrations do not become reduced during successive treatment events through dilution (*e.g.* Forrest *et al.* 2007).

Table 2. Proposed experimental design

Chemicals	Delivery method	Concentration	Exposure time (minutes)	Replicates	Experimental units
2	2 (spray, immersion)	4 (diluted in freshwater: 1%, 5%; controls: 100% freshwater, 100% seawater)	3 (1, 10, 60)	3	144

To test the efficacy of treatments on microscopic organisms, we propose to conduct experiments using fouling species that are regarded as pests in their own right, but can also be considered as surrogates for the microscopic life-stages of other marine species. In the interests of time and budget, we propose to use species for which Cawthron has already established experimental protocols. These would be the spore/gametophyte life-stage of *Undaria* and the larvae of sea squirt(s) *Didemnum vexillum* and/or *Ciona intestinalis*. Microscopic life-stages of these organisms obtained and cultured under lab conditions and tested according to methods described by Mountfort *et al.* (1999; *Undaria* spores), Forrest & Blakemore (2003, 2006; *Undaria* gametophytes), Fletcher *et al.* (2010; *Didemnum* larvae and recruits), and Cawthron (unpubl. data; *Ciona* larvae). Treatment regimes will be the same as those used during macro-organism treatment experiments (outlined above). The efficacy of different treatment options will be determined based on the mortality rates of the target organisms.

Prior to the commencement of full-scale experiments, pilot studies will be employed to initially gauge the feasibility of the two recommended chemicals, or other compounds preferred by MAFBNZ. In the event that the chosen chemicals are found to be ineffectual, MAFBNZ will be notified and alternative treatment options will be discussed.

While heat treatment (*i.e.* hot water immersion, steam application) has been trialled extensively for the control and treatment of marine pest species, we have chosen not to include it as an option in the present study due to several operational issues associated with its use. For example, access to hot water and difficulties in achieving high water temperatures preclude its use in many situations (Piola *et al.* 2009). Additionally, the highly controlled conditions under which experimental heat treatment studies are undertaken (*e.g.* ready availability of thermometers, ability to maintain exact water temperatures) are unlikely to be present in most real world treatment situations. As such, any heat treatment parameters recommended as a result of this study (*e.g.* desired water temperatures for optimal treatment) are unlikely to be readily interpreted and/or achievable by most end users in the real world. For this reason, we recommend focusing research on the efficacy of different concentrations of chemicals and exposure times, rather than including heat as a treatment. Past research has shown temperature would almost certainly have a magnifying effect on the efficacy of any toxicant, thus the scenarios presented here represent a worse case scenario, and increasing temperature would almost certainly increase effectiveness.

REFERENCES

- Blakemore KA, Forrest BM 2007. Heat treatment of marine fouling organisms. Prepared for Golder Associates/Biosecurity New Zealand Cawthron Report No. 1300. 64p.
- Fletcher LM, Forrest BM, Bell JJ. 2010. Spawning and culture techniques for the invasive ascidian *Didemnum vexillum*. Third International Invasive Sea Squirt Conference, Woods Hole, Massachusetts, April 2010.
- Forrest BM, Hopkins GA, Dodgshun TJ, Gardner, JPA, 2007. Efficacy of acetic acid treatments in the management of marine biofouling. *Aquaculture* 262: 319-332.
- Forrest BM, Blakemore, KA 2006. Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. *Aquaculture* 257: 333-345.
- Forrest BM, Blakemore K 2003: An evaluation of methods to reduce inter-regional spread of the Asian kelp *Undaria pinnatifida* via marine farming activities. Cawthron Report No. 773. 38 p. plus appendices.
- Gunthorpe L, Mercer J, Rees C, Theodoropoulos T 2001. Best practices for the sterilisation of aquaculture farming equipment: a case study for mussel ropes. Marine and Freshwater Resources Institute Report 41, Marine and Freshwater Resources Institute, Queenscliff, Australia. 48 p.
- Kilroy C 2005. Tests to determine the effectiveness of methods for decontaminating materials that have been in contact with *Didymosphenia geminata*. NIWA Client Report: CHC2005-004.
- Kilroy C, Lagerstedt A, Davey A, Robinson K 2006. Studies on the survivability of the invasive diatom *Didymosphenia geminata* under a range of environmental and chemical conditions. NIWA client report: CHC2006-116.110 p.
- Kuhajek J, Wood S 2009a. Didymo New Zealand Defence Force vector control project: Laboratory studies on viability. Cawthron Report No. 1686. 10 p. plus appendices.
- Kuhajek J, Wood S 2009b. Didymo New Zealand Defence Force vector control project: Field studies on viability. Prepared for Tonkin and Taylor. Cawthron Report No. 1691. 22 p. plus appendices.
- Lewis JA, Dimas J 2007. Treatment of biofouling in internal seawater systems – Phase 2. Maritime Platforms Division, Defence Science and Technology Organisation. 25 p.
- Mountfort DO, Hay C, Taylor M, Buchanan S, Gibbs W 1999. Heat treatment of ships' ballast water: development and application of a model based on laboratory studies. *J. Mar. Env. Eng.* 5, 193-206.
- Piola RF, Denny CM, Forrest BM, Taylor MD 2009. Marine biosecurity: management options & response tools. Chapter 14 In: Clout M, Williams P (eds) *Invasive Species Management: A Handbook of Principles and Techniques*. Oxford University Press, Oxford.
- Piola RF, Dunmore RA, Forrest BM 2010. Assessing the efficacy of spray-delivered 'eco-friendly' chemicals for the control and eradication of marine fouling pests. *Biofouling* 26: 187-203

1. Background

MAFBNZ project 11815 describes the need to identify potential treatment methods for mitigation of biosecurity risks posed to the Fiordland Marine Area (FMA) from marine equipment such as anchors, mooring ropes, fishing nets and pots, kayaks, dive equipment and wetsuits. While previous research and experimentation has identified numerous effective tools (*e.g.* acetic acid, heat treatment) that have been successfully used in managing marine invasive species, other more commonly available treatment options have yet to be extensively trialed (*e.g.* hot soapy water). Household detergents have been successfully trialed and used as a response tool in the treatment of the freshwater alga *Didymosphenia geminata* (Kilroy 2005, Kilroy *et al.* 2006, Kuhajek and Wood 2009 a,b). They have also been used with some success in trials on a few marine species (Gunthorpe *et al.* 2001, Lewis and Dimas 2007). However, their effectiveness against a wide range of marine species (including both micro- and macroscopic life stages) is yet to be evaluated. This report details the findings of initial pilot trials examining the efficacy of two common household chemicals, the dishwashing liquid Palmolive Original™ and the liquid disinfectant Dettol™, for the eradication of common marine fouling species.

2. Methods

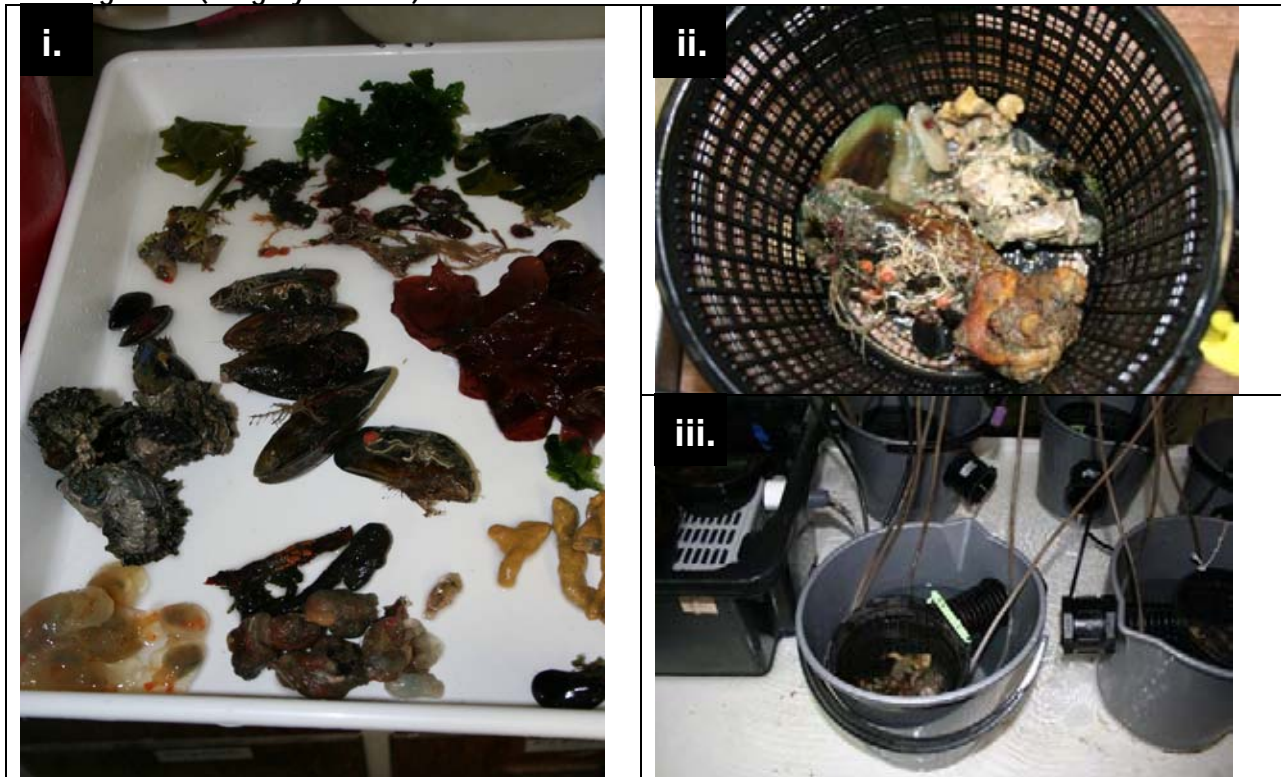
2.1 GENERAL APPROACH

On 7 July 2010, the tolerance of fouling organisms to two different treatment chemical solutions (Dettol, Palmolive) was tested, at a single concentration of 5%. The pilot study was conducted on commonly occurring fouling taxa that may conceivably be subject to treatment with detergent solutions. These taxa included species of mussels, oysters, ascidians (colonial and solitary), bryozoans, hydroids and algae (Table A; Figure A(i)). Test organisms were collected from Nelson marina and transferred to the Cawthron laboratories, where they were sorted and arranged into 2L plastic mesh cages. Each cage contained multiple individuals from each taxonomic group, and was then immersed in a solution of either 5% Palmolive, 5% Dettol or ambient seawater (control) for an exposure period of 60 min. Following treatment, test organisms were rinsed thoroughly in fresh seawater and all invertebrates were placed within replicate plastic baskets ($n = 3$) for regular assessment of post-treatment effects (Figure A(ii)). Plastic baskets (with holes to allow water exchange) were placed in individual holding tanks within a recirculating seawater system (Figure A(iii)). Due to inadequate light conditions in the laboratory, treated algae were transferred to a separate series of replicate baskets, which were then suspended from ropes at the Port Nelson marina at a depth ~ 0.5m.

Table A. Invertebrate and algal species tested during pilot trials examining the efficacy of Dettol and Palmolive as chemical treatments for fouling biota.

Phylum, Class	Order	Family	Genus and species
Bryozoa			
Gymnolaemata	Cheilostomata	Bugulidae	<i>Bugula flabellata</i>
Gymnolaemata	Cheilostomata	Bugulidae	<i>Bugula neritina</i>
Cnidaria			
Anthozoa	Actiniaria	Sagartiidae	<i>Actinothoe albocincta</i>
Hydrozoa	Hydroida	Bougainvilliidae	<i>Bougainvillia muscus</i>
Mollusca			
Bivalvia	Mytiloidea	Mytilidae	<i>Mytilus galloprovincialis</i>
Bivalvia	Mytiloidea	Mytilidae	<i>Perna canaliculus</i>
Phycophyta			
Phaeophyceae	Dictyotales	Dictyotaceae	<i>Dictyota</i> sp.
Phaeophyceae	Laminariales	Alariaceae	<i>Undaria pinnatifida</i>
Rhodophyceae	Ceramiales	Ceramiaceae	<i>Ceramium</i> sp.
Rhodophyceae			Unidentified red
Ulvophyceae	Ulvales	Ulvaceae	<i>Ulva</i> sp.
Urochordata			
Asciacea	Aplousobranchia	Cionidae	<i>Ciona</i> spp.
Asciacea	Aplousobranchia	Didemnidae	<i>Didemnum vexillum</i>
Asciacea	Stolidobranchia	Botryllinae	<i>Botrylloides leachii</i>
Asciacea	Stolidobranchia	Styelidae	<i>Botryllus schlosseri</i>
Asciacea	Stolidobranchia	Styelidae	<i>Cnemidocarpa bicornuata</i>
Asciacea	Stolidobranchia	Styelidae	<i>Styella plicata</i>

Figure A. Photographs showing (i) a selection of invertebrate and algal species used for pilot studies examining the efficacy of Dettol and Palmolive as chemical treatments for fouling biota; (ii) allocation of test organisms in replicate plastic baskets for post-treatment monitoring and assessment; and (iii) replicate post-treatment baskets arranged in individual seawater system holding tanks (*i.e.* grey buckets).



2.2 ASSEMBLAGE CENSUS AND COMPARISON

Condition and mortality of invertebrate test organisms was assessed after 1, 2, 3 and 5 days. Invertebrates in replicate plastic baskets were visually assessed for viability based on the following taxa-specific criteria:

- Bryozoans: visible lophophore activity and muscle movement within zooid cavities (during examination under a dissecting microscope).
- Hydroids: moving tentacles visible on hydranths, and response to touch stimuli (during examination under a dissecting microscope).
- Anenomes: visible tentacle movement and response to touch stimuli.
- Mussels and oysters: shell being open when submerged (indicating active water filtering) and subsequent closure in response to touch stimuli.
- Colonial ascidians: discolouration, tissue and no signs of tissue necrosis (*i.e.* colonies appear firm and healthy looking).
- Solitary ascidians: visibly open siphons (indicating active water filtering) and subsequent siphon closure in response to touch stimuli. There was also a tendency for dead solitary ascidians to expel putrefied internal contents when light pressure was applied to the outer test.

Condition and mortality of algae was assessed after 1, 2 and 5 days. Algae were visually assessed and assigned a qualitative rank score ranging from 1 to 3, with the criteria of each score being: (1) healthy plant with no signs of discolouration or decomposition, (2) visible

discolouration of plant blades and/or stipe, but tissue integrity intact (*i.e.* no decomposition), and (3) severe discolouration of plant blades and/or stipe and visible decomposition and/or sloughing of tissue.

3. Results

Immersion for 60 min in solutions of both 5% Dettol and 5% Palmolive detergent were effective in killing colonies of colonial ascidians, arborescent bryozoans and hydroids within 24h post-exposure (Figure Bi,iii,iv). Dettol was also effective at killing 100% of anenomes 24h post-exposure; however, no mortality was recorded following immersion in Palmolive solution (Figure Bv). Substantial mortality was recorded after 24 h for solitary ascidians exposed to Dettol and Palmolive (67 and 81%, respectively), with mortality continuing to occur up to 5d post-treatment (Figure Bii). Mussels immersed in Dettol resulted in ~ 50% mortality 24h post-exposure, with only 25% of individuals still alive after 5 days (Figure Bvi). In contrast, mortality in mussels immersed in Palmolive was more measured, with gradual and consistent declines in survival recorded over the 5d post-treatment period (with c. 10 – 15% mortality recorded per day; Figure Bvi). No mortality was recorded in oysters up to 5d post-treatment (Figure Bvii) Mortality in seawater controls was minimal, ranging from 0 – 20% after 5 days (Figure B).

Solutions of 5% Dettol and Palmolive were 100% effective at killing *Undaria pinnatifida* plants, after only 24 h post-exposure (Table C). In fact, visible discolouration was evident immediately following the 60 min immersion period. Dettol and/or Palmolive also resulted in severe discolouration and decomposition of plant structure in some *Dictyota* and *Ceramium* plants 1 – 5d post-treatment (Table C). Most of the remaining plants displayed discolouration within 24h of exposure, with the exception of *Ulva*, which appeared unaffected following immersion in Palmolive (Table C). Seawater controls exhibited no ill-effects of treatment, with the exception of some slight discolouration in one replicate *Dictyota* plant (Table C). Interestingly, when algal experimental units (*i.e.* plastic baskets) were retrieved from the field after one month, no Dettol treatment plants and only Palmolive *Ulva* plants remained alive, while all control plants appeared healthy and growing.

Figure B. Plots showing percent survival of organisms from a range of taxonomic groups following 60 min immersion in 5% Dettol solution (▲), 5% Palmolive solution (○) and ambient seawater (■).

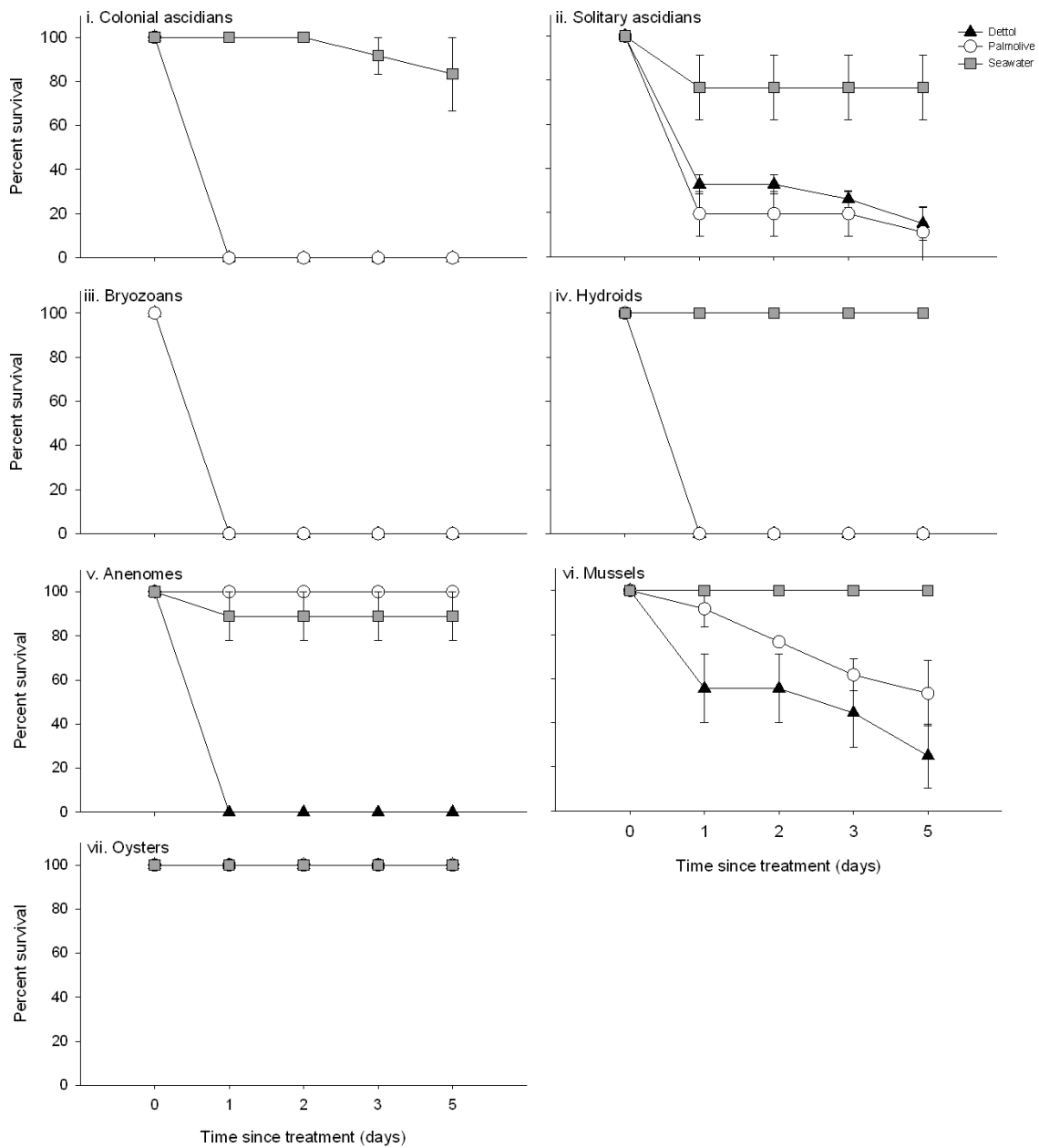





Table C. Qualitative assessment of replicate (1 – 3) algae following 60 min immersion in either 5% Dettol solution, 5% Palmolive solution or ambient seawater.

Chemical	<i>Undaria</i>			<i>Ulva sp.</i>			<i>Dictyota sp.</i>			<i>Ceramium sp.</i>			Unident. Red		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Palmolive															
Day 1	3	3	3	1	1	1	2	2	2	2	2	2	2	2	1
Day 2	3	3	3	1	1	1	2	2	2	2	2	2	2	2	1
Day 5	3	3	3	1	1	1	3	3	2	2	2	2	2	2	2
Dettol															
Day 1	3	3	3	1	2	2	3	2	2	2	2	1	2	2	2
Day 2	3	3	3	1	2	2	3	3	2	3	2	2	2	2	2
Day 5	3	3	3	2	2	2	3	3	2	3	2	2	2	2	2
Seawater															
Day 1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Day 2	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1
Day 5	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1
Key:															
 Healthy plant			 Discolouration to blades			 Discolouration and decomposition of blades									

4. Recommendations

Results from this pilot study suggest that solutions of Dettol and Palmolive may be effective treatment agents for cleaning gear and equipment infected with macro- and microscopic fouling. Solutions of 5% Dettol and Palmolive killed the majority of soft-bodied fouling that are commonly associated with gear and equipment (*e.g.* colonial and solitary ascidians, bryozoans, hydroids). Given that microscopic and/or larval life stages of most marine organisms are generally considered to be more sensitive than adults to toxicant exposure (Connor 1972, Calabrese *et al.* 1977), we hypothesise that the use of weaker chemical concentrations (*e.g.* 1% solutions) for shorter durations may be equally effective in killing these early life-stages. In fact, weaker treatment solutions may even be more effective in treating hard-bodied foulers such as mussels or oysters, since the organisms may not sense the biocide in the water at low concentrations and continue to actively feed; whereas at higher concentrations the organism likely responds to the chemical stimulus and closes its shell (Lewis and Dimas 2007). Finally, it would be beneficial to examine the efficacy of several treatment methods for applying chemical solutions to gear and equipments, such as immersion baths (as tested in this pilot study) and spray delivery.

Our specific recommendations for the continued trial of these products for disinfection of gear and equipment include:

1. A full-scale trial of solutions of Dettol and Palmolive on marine macrofouling assemblages, over a several chemical concentrations (1 and 5%), durations of exposure (1, 10 and 60 min) and chemical delivery methods (immersion baths, spraying)
2. A repeat full-scale trial of the above-listed chemical concentration and exposure durations (see Point 1) on the colonial ascidian *Didemnum vexillum*, a well-recognised marine pest species

3. A repeat full-scale trial of the above-listed chemical concentration and exposure durations (see Point 1) on early life-stages (*e.g.* larvae, algal gametophytes) of common marine fouling taxa, including invertebrate (*e.g.* ascidians) and algal species.
4. Supplementary small-scale trials to determine if the added parameter of temperature (*e.g.* hot water solutions of Dettol and Palmolive) further increases the effectiveness of this treatment method.

6. References

- Calabrese A, Collier R, Nelson DA, MacInnes J 1973. The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. *Marine Biology* 18: 162-166
- Connor PM 1972. Acute toxicity of heavy metals to some marine larvae. *Marine Pollution Bulletin* 3: 190-192
- Gunthorpe L, Mercer J, Rees C, Theodoropoulos T, 2001. Best practices for the sterilisation of aquaculture farming equipment: a case study for mussel ropes. *Marine and Freshwater Resources Institute Report 41*, Marine and Freshwater Resources Institute, Queenscliff, Australia. 48 p.
- Kilroy C 2005. Tests to determine the effectiveness of methods for decontaminating materials that have been in contact with *Didymosphenia geminata*. NIWA Client Report: CHC2005-004.
- Kilroy C, Lagerstedt A, Davey A, Robinson K 2006. Studies on the survivability of the invasive diatom *Didymosphenia geminata* under a range of environmental and chemical conditions. NIWA client report: CHC2006-116. 110 p.
- Kuhajek J, Wood S 2009a. Didymo New Zealand Defence Force vector control project: Laboratory studies on viability. Cawthron Report No. 1686. 10 p. plus appendices.
- Kuhajek J, Wood S 2009b. Didymo New Zealand Defence Force vector control project: Field studies on viability. Prepared for Tonkin and Taylor. Cawthron Report No. 1691. 22 p. plus appendices.
- Lewis JA, Dimas J 2007. Treatment of biofouling in internal seawater systems - Phase 2. Maritime Platforms Division, DSTO Defence Science and Technology Organisation

APPENDIX 3. CULTURE AND MONITORING METHODOLOGY FOR MICROSCOPIC ORGANISMS

Undaria gametophytes

Undaria gametophytes were cultured using the same methodology as described by Forrest and Blakemore (2003). Spores were obtained from 6 sporophylls which were cut into segments and cleaned by scrubbing with a small brush and immersing in 0.5% bleach solution for 1 minute. After rinsing, the sporophyll tissue was wrapped in paper towels and held overnight at approximately 15°C. The sporophyll tissue was immersed in seawater (UV-sterilised and filtered to 35µm) for 10 minutes to induce spore release. The spore solution was filtered through a 20 µm filter, and 3 ml aliquots were placed in 10 ml plastic pots. Pots were left for 1 hour to allow for spore settlement, and then the spore solution was replaced with seaweed medium. Lids were placed on pots, and the units were placed in a constant temperature cabinet (22°C) under a 12:12 light:dark regime. Medium was changed once a week, and cultures were maintained for 2 weeks before treatment, and 2 weeks after treatment.

For survival of *Undaria*, counts of live gametophytes were made prior to treatment at a pre-defined point on the culture pot. A single count within an eyepiece grid was made for each pot at 100 x magnification, using an inverted compound microscope. Repeat counts at the same position were made 1, 4 and 7 days after treatment. Gametophytes were considered to be dead if they had a loss of brown pigmentation and/or a necrotic appearance (Figure 0.1.). Gametophytes with slight pigmentation were conservatively considered to be alive.

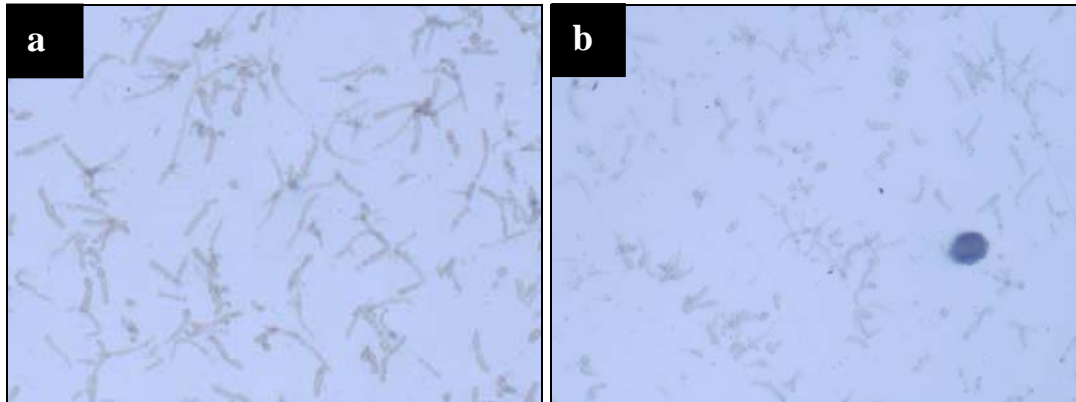


Figure 0.1. (a) Live *Undaria* gametophytes pre-treatment, and (b) Dead *Undaria* gametophytes 1 day post-treatment (immersion for 60 minutes in 5% Dettol).

Ciona larvae

Ciona eggs and sperm were dissected from four *Ciona* adults, and left for approximately 18 hours to fertilise. After this, swimming larvae were clearly visible and 15-20 larvae were pipetted into 10 ml plastic Petri dishes, and left in a constant temperature cabinet (22°C) under a 12:12 light:dark regime for 3 days for settlement to occur. At this time, initial counts were obtained and experimental units were treated. Seawater (UV-sterilised and filtered to 35µm) was changed every 2-3 days. Survival was measured by marking 5 live individuals, and assessing them 4 and 7 days after treatment. Counts were made with the aid of a binocular microscope, and dead individuals were easily identified by their loss of internal structures, discolouration or change in transparency and/or decomposition of tissue (Figure 0.2.)

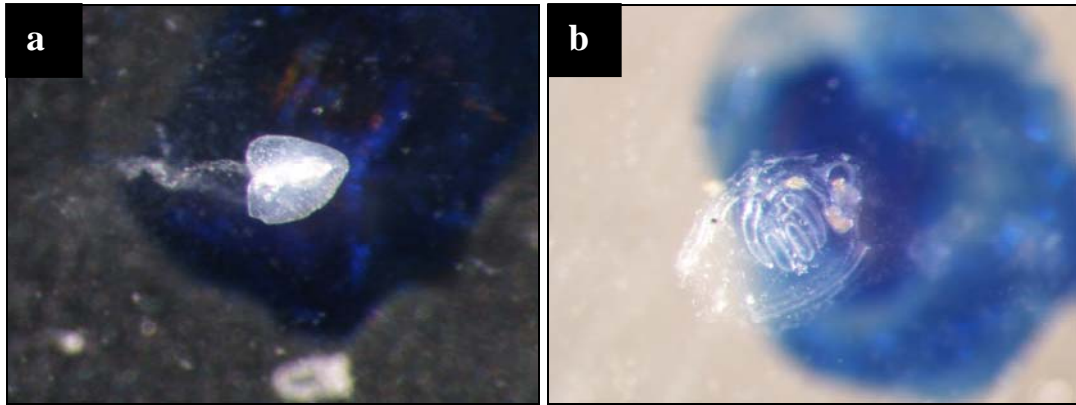


Figure 0.2. (a) Dead *Ciona* 4h after immersion in 5% Dettol for 60 min, and (b) live *Ciona* after 7 d.

Bugula larvae

Bugula adult colonies were collected from the field and kept in a darkened aquarium tank overnight. The following day they were induced to release larvae by direct exposure to sunlight. Swimming larvae were clearly visible and 15-20 larvae were pipetted into 10 ml plastic pots, and left in a constant temperature cabinet (22°C) under a 12:12 light:dark regime for 3 days for settlement to occur. At this time, initial counts were obtained and experimental units were treated. Seawater (UV-sterilised and filtered to 35µm) containing microalgae (predominantly *Isochrysis* sp.) was changed every 2-3 days. Survival was assessed by marking the pot adjacent to ~ 5 - 10 individuals with the aid of a binocular microscope, and following their survival 4 and 7 days after treatment. Mortality was easily recognised by a loss of internal and feeding structures (Figure 0.3.).

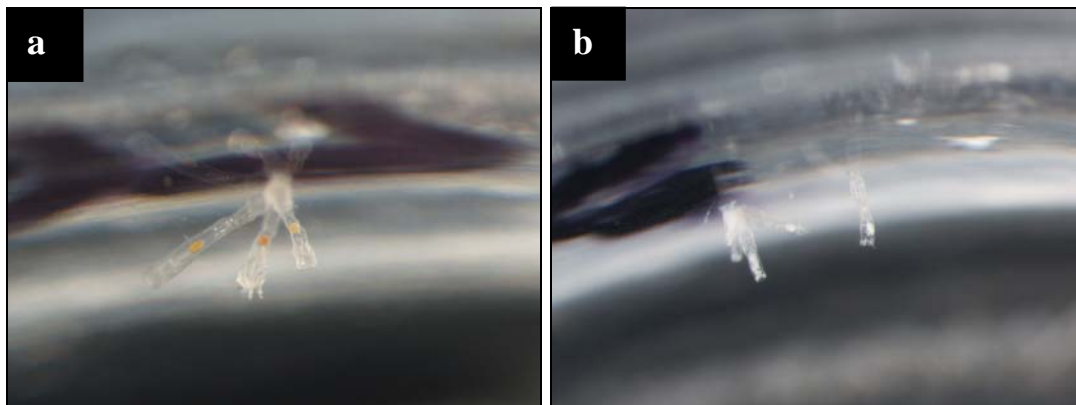


Figure 0.3. *Bugula* 1 d post-treatment: (a) live individuals after immersion in seawater for 60 min, and (b) dead individuals from immersion in 5% Dettol for 60 min.

APPENDIX 4. SURVIVAL OF ADULT UNDARIA PLANTS ON FOULED PLATES

Chemical	Delivery (immersion or spray)	Exposure time (min)	No. plants pre- treatment	No. plants 2 weeks post- treatment
Dettol 1%	I	1	1	0
		10		
		60		
	S	1	4	0
		10		
		60		
Dettol 5%	I	1		
		10		
		60		
	S	1		
		10		
		60		
Palmolive 1%	I	1	1	1
		10		
		60		
	S	1	3	0
		10		
		60		
Palmolive 5%	I	1		
		10		
		60		
	S	1	3	0
		10		
		60		
Freshwater	I	1		
		10		
		60		
	S	1	3	0
		10		
		60		
Seawater	I	1		
		10		
		60		
	S	1	1	1
		10		
		60		

APPENDIX 5. GUIDELINES FOR THE TREATMENT OF MARINE GEAR TO PREVENT THE INTRODUCTION OF MARINE PESTS INTO FIORDLAND

Protect Fiordland's exceptional marine biodiversity and valuable marine resources

The introduction of unwanted marine pest species to pristine environments such as Fiordland has the potential to cause irreparable harm to biodiversity and the beauty of these regions. It is everyone's responsibility to reduce the risk of introducing marine pests into this iconic natural environment. When taking marine gear and equipment (*e.g.* fishing, diving gear, nets, pots, ropes, anchors) and non-moored craft (*e.g.* kayaks) and other equipment into Fiordland, remember to follow these simple steps:

Check – Check for and remove any living or dead marine growth from equipment prior to arriving in Fiordland.

Clean – Clean canoes/kayaks, snorkelling/diving gear, fishing/boat equipment prior to arriving in Fiordland (see table for guidance on treatment methods).

When cleaning equipment (as per guidelines overleaf), we recommend that you:

- Remove any visible marine organisms and dispose of them appropriately on land.
- Remember to also clean equipment having no visible marine organisms present – microscopic life stages of organisms can be on equipment, in seawater trapped inside kayaks, boats, or within ropes or nets and dive equipment.
- Follow correct handling precautions when diluting cleaning chemicals from concentrated solutions. Ensure there is adequate ventilation and, where possible, use protective gloves and appropriate eye wear.
- Where possible, use hot water ($\geq 40^{\circ}\text{C}$) to make up a treatment solution, as this dramatically increases its effectiveness. A good rule of thumb is to use water that is hot enough to submerge your hand in without significant discomfort.
- Dispose of cleaning solutions well above the high tide mark and away from streams and rivers.
- Where possible, completely dry equipment following cleaning. Some marine organisms can survive days exposed to air, so the longer equipment is dried the more effective any cleaning measures will be.



Contact

For further information on marine pests contact your local Regional Council, or visit the Biosecurity New Zealand website at www.biosecurity.govt.nz

CLEANING GUIDANCE

Provided below are a range of cleaning options to minimise the risk of transferring marine pests associated with canoes/kayaks, snorkelling/diving gear and fishing/boat equipment (*e.g.* nets, pots, anchors, ropes). Choose the best treatment option for your item/s, taking into consideration: (1) time available (*e.g.* air exposure can take up to 1 month), (2) access to treatment chemicals, (3) size and

amenability of the item/s to the treatment methods (e.g. a kayak may be too big to soak so spraying or air exposure is likely to be a better approach), (4) sensitivity of equipment.

SOAK	SPRAY/WASH	DRY
<p>Soak the item/s as per one of the methods below:</p> <ul style="list-style-type: none"> • Freshwater for at least 72 hours. If soaking ropes, freshwater should be replaced after 12 hours.^{6,7,8,9} • Hot water ($\geq 40^{\circ}\text{C}$) for 20 minutes^{3,10}. Temperatures exceeding 48°C should not be used on dive equipment as certain temperature-sensitive gear may be damaged¹¹. • 5% Palmolive dishwashing detergent/freshwater solution for 60 minutes. (5% solution = 500 mls of detergent into 10 litres of freshwater).⁵ • 1% Dettol antiseptic/ freshwater solution for 60 minutes. (1% solution = 100 mls of dettol into 10 litres of freshwater).⁵ • 2% bleach/freshwater solution for 30 minutes * (2% solution = 200 mls of bleach into 10 litres of freshwater).^{1,4} • 2% Decon 90TM/freshwater solution for 30 minutes.^{1,4} • 5% acetic acid/ freshwater solution OR undiluted household vinegar for 10 minutes * (5% solution = 500 mls of acetic acid into 10 litres of freshwater).^{1,2} <p>Palmolive dishwashing detergent, Dettol, bleach and vinegar can be readily purchased from most supermarkets and service stations.</p> <p>* Not recommended for dive gear as it may compromise the integrity of some plastics¹¹.</p>	<p>For items too large or difficult to soak, spray the item/s as per one of the methods below:</p> <ul style="list-style-type: none"> • 1% Dettol antiseptic/ freshwater solution and leave for 60 minutes.⁵ • 5% acetic acid/ freshwater solution OR undiluted household vinegar and leave for 10 minutes.¹² <p>When spraying an item, ensure you generously cover all surfaces.</p> <p>Handheld sprayers can be readily purchased at a hardware store, or in the gardening department of supermarkets and other department stores.</p>	<p>For an item where chemical/ freshwater treatment is not feasible, remove from water and thoroughly air dry for 1 month.^{1,2,3,4}</p> <p>Care is needed to ensure that the item is laid out in a manner that ensures all surfaces are completely dried.</p> <p>Prolonged air exposure is also an ideal complementary treatment for any item/s that has been soaked or sprayed.</p>

Sources of information

⁶ Clean boats – living seas. MAFBNZ.

⁷ Coutts A Forrest B 2005. Evaluation of eradication tools for the clubbed tunicate *Styela clava*. Prepared for Biosecurity New Zealand. Cawthron report No. 1110

⁸ Forrest B, Blakemore K 2003. An Evaluation of Methods to Reduce Inter-regional Spread of the Asian Kelp *Undaria pinnatifida* via Marine Farming Activities. Prepared for the Ministry of Fisheries. Cawthron Report No. 773.

⁹ Gunthorpe L, Mercer J, Rees C, Theodoropoulos T 2001. best practices for the sterilisation of aquaculture farming .equipment: A case study for mussel ropes. Marine and Freshwater Resources Institute Report No. 41. (Marine and Freshwater Resources Institute: Queenscliff).

¹⁰ Dunmore RA, Piola RF, Hopkins GA 2010. Assessment Of The Effects Of Household Cleaners For The Treatment Of Marine Pests: MAFBNZ Project 11815.

¹¹ Blouin MA 2002. A procedure for the decontamination of SCUBA diving equipment and underwater gear after diving in waters containing zebra mussels (*Dreissena polymorpha*) and other exotic species of Dressenidae. Standard operating procedure, U.S. Geological Survey.

¹² Piola RF, Dunmore RA , Forrest BM 2008. Evaluation of spray treatments for the management of marine pests. Prepared for MAF Biosecurity New Zealand.