Determining the efficacy of incursion response tools: Rotating brush technology (coupled with suction capability)

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Executive Summary

The present study tested two diver-operated rotating brush systems, coupled with suction and collection capabilities, on flat and curved surfaces with varying marine fouling levels, to determine their efficacy as incursion response tools for marine biosecurity. Both rotating brush systems proved effective (>>80%) in removing low-to-moderate levels of fouling from the experimental surfaces, however performance was generally poorer at removing more advanced levels of fouling. In particular, mature calcareous organisms were relatively resistant to the rotating brushes, with a high proportion remaining on plates following treatment. On average, > 95% of defouled material was collected and retained by both systems, with less retention on surfaces that were curved or had more advanced levels of fouling present. The majority (typically >80%) of fouling not captured by the systems was crushed by the brushes (i.e. non-viable); however a wide range in types of viable organisms (*e.g.* barnacles, hydroids, etc) were lost to the environment during the defouling trials.

A trial on a fouled vessel revealed that, while the devices were capable of removing 100% of biofouling from the areas treated, unintentional detachment of fouling organisms through physical disturbance by divers operating the devices and by equipment associated with the rotating brush was reasonably high. Furthermore, residual biosecurity risks were also likely to remain due to diver error (i.e. missed patches), persistent fouling remaining on treated surfaces (including microscopic life-stages) and the inaccessibility of niche areas to the brush systems.

As such, the rotating brush systems tested in the present study are not considered appropriate to treat vessels known to be fouled with non-indigenous marine species (NIMS) or pest species, particularly taxa that can survive fragmentation. Several risks relating to this method of treatment of fouled vessels need to be investigated if this method is to be used as a method for border control of NIMS or for removing pest species in an uncontaminated area. These include: (i) determining the survivorship of defouled material lost to the environment, (ii) the potential for gamete release (spawning and/or stripping) following physical disturbance by in-water hull cleaning devices, (iii) factors influencing enhanced colonisation of defouled surfaces, and (iv) understanding invasion risks posed by fragmented colonial organisms. The efficacy of other in-water treatment methods (e.g. encapsulation) also needs to be determined and alternative strategies to treat niche areas of a vessel (e.g. sea chests) developed.

Keywords: in-water cleaning, rotating brushes, incursion response tool, biosecurity

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1 Introduction

1.1 BACKGROUND

Vessel traffic is an important pathway for the transfer of non-indigenous marine species (NIMS) at a range of spatial scales in the marine environment (Hewitt et al. 1999; Gollasch 2002; Ruiz & Carlton 2003). The main vessel-related transport mechanisms are ballast water (Carlton 1985; Olenin et al. 2000; Taylor et al. 2007); hull fouling (Lewis et al. 2003; Coutts & Taylor 2004); and fouling of niche areas such as sea chests, intake pipes and gratings (Carlton et al. 1995; Coutts et al. 2003; Minchin & Gollasch 2003; Coutts & Dodgshun 2007). There has been considerable research globally into treatment solutions for ballast water (e.g. Mountfort et al. 1999; Oemcke et al. 2004), with a range of potential methods proposed; such as heat, use of chemicals and ballast water exchange. By contrast, treatment solutions for hull fouling and fouling of niche areas have proven difficult to achieve, with current strategies relying on the application of anti-fouling paints and regular maintenance within recommended timeframes (Floerl & Inglis 2005).

There are several well-documented examples of NIMS being discovered on a vessels' hull upon arrival in a recipient port (e.g. Apte et al. 2000; Coutts et al. 2003); and recent research has demonstrated that this occurs over a broad range of vessel types (Floerl et al. 2008; G. Hopkins, unpublished data). In many instances, the discovery of NIMS is made by chance, due to an absence of routine vessel fouling inspections prior to departure or upon arrival in the recipient port. As such, the number of NIMS being transported around the globe by vessel traffic is likely to be grossly under-reported. Furthermore, it is possible that bioinvasions from hull fouling will increase due to the absence of an effective alternative to TBT-based paints (Nehring 2001), which are due to be phased out globally by September 2008.

Hopkins & Forrest (2008) identify several management options for high risk vessels (i.e. those vessels fouled with NIMS or pest organisms). Refusing entry (i.e. risk avoidance) upon arrival at a recipient port is arguably the most desirable approach. In New Zealand this option is available under relevant biosecurity legislation, but in reality is rarely enforced. An alternative option is to treat the vessel in-water. In fact, for large vessels fouled with high risk species or heavily fouled due to being overdue for their scheduled dry-docking, in-water cleaning may be the only available option as it is not always practical to remove such vessels to land.

Traditionally, in-water cleaning methods involve the mechanical removal of fouling using a range of devices depending on the vessel size, composition (e.g. wood, steel, fibreglass) and the type of paint coating used. For example, a small recreational yacht is typically defouled by divers using plastic or metal handheld scrapers or brushes (which may take several hours), while a large merchant ship is more likely to be defouled over 1-2 days using diver-operated devices such as rotating brushes. In most cases, defouled material is not collected and retained by in-water cleaning devices and may settle on natural seabed habitats or artificial structures adjacent to the vessel, or be more widely dispersed by currents (Hopkins & Forrest 2008). In part because of the perceived ecological risk from the release of this material and paint fines containing toxic substances, a number of countries have placed restrictions on this approach or are considering doing so (ANZECC 1996, Hopkins & Forrest 2008).

1.2 PROJECT OBJECTIVES AND SCOPE OF THIS REPORT

Development of in-water cleaning technologies is in its infancy. Coutts (2002) described a vacuum system designed to remove the ascidian *Didemnum vexillum* from the hull of a barge moored near Picton, New Zealand. A post-treatment re-survey of the barge showed that the system was 80% effective in removing fouling biomass. Although the majority of defouled material was captured by the suction device, the amount of loss and its ecological risk was not quantified. Two New Zealand commercial diving companies have also independently developed diver-operated rotating brush systems that are designed to clean vessel hulls (to improve fuel efficiency) and collect the defouled material. Both systems have been in commercial operation for several years now, and their efficacy was evaluated in the present study. To our knowledge, there are presently no other commercially available systems that have been designed to collect fouling material immediately during removal.

The application of any in-water cleaning method as a potential incursion response tool requires knowledge in two key areas: (1) efficacy of the method in eliminating biosecurity risks posed by vessel fouling, and (2) ecological risks associated with the application of such methods (for example, the release of viable organisms to the environment). In January 2006, MAF Biosecurity New Zealand (MAFBNZ) commissioned the Cawthron Institute to determine the efficacy of the two rotating brush systems as incursion response tools for marine biosecurity. It is envisaged that their application will be largely associated with vessel hulls that have, or are suspected to have, pest species present; however alterative applications using similar methods are also conceivable (e.g. defouling of wharf piles). Specific objectives of the project, as specified in the contract, were to:

(i) In consultation with MAFBNZ incursion response and surveillance, design an efficacy trial(s) for the two rotating brush technologies.
(ii) Implement the trial methods(s) developed in Specific Objective 1, ensuring that a seasonal analysis can occur.

There was *a priori* knowledge that rotating brush devices were incapable of treating all areas of a vessel hull. In particular, niche areas would not be accessible and would require alternative treatment approaches. As such, the present study focused on determining the efficacy of the rotating brush systems in removing fouling biomass from flat and slightly-curved experimental surfaces over a range of fouling levels and seasons. The amount of defouled material lost to the environment during defouling trials was quantified, as this represents one of the greatest treatment risks posed by in-water methods (Hopkins & Forrest 2008). The report discusses environmental risks associated with the application of these methods in coastal marine environments, practical considerations for the application of these tools in incursion response (e.g. costs, mobilisation time), and alternative in-water methods (e.g. encapsulation; Denny 2007; Coutts & Forrest 2007).

2 Methods

2.1 DESCRIPTION OF SYSTEMS TRIALLED

The project trialled two independent rotating brush systems: System A was developed by Diver Services Ltd, and consisted of a Phosmarine™ brush unit (Figure 1) and hydraulic pump; System B was developed by New Zealand Diving & Salvage Ltd and consisted of a Charlyn™ hydraulic motor running a commercial road-sweeping brush head (Figure 1). Refer to Appendix 1 for system specifications. Both brush units were fitted with a purpose-built shroud that was designed to enhance the retention of defouled material. During normal operation on a fouled vessel, System A pumps the defouled material directly into a collection bag that floats on the surface adjacent to the vessel being cleaned. System B pumps the material through filters connected in series (from large to small mesh sizes), which are housed in a unit that either remains on the wharf or is positioned on a vessel.

Figure 1: Rotating brush devices used in the trials. Note: Suction/collection systems are not pictured; System A (left) and System B (right).

2.2 EFFICACY TRIALS ON FOULED SETTLEMENT PLATES

2.2.1 Experimental design

The performance of the rotating brush systems was assessed by their ability to remove and collect fouling material from pre-fouled settlement plates over a range of fouling levels and during different seasons. Systems were trialled on flat and curved perspex settlement plates (350 x 350 x 4.5 mm) to mimic the main surfaces encountered on a vessel hull. Plates were coated with a non-toxic paint (plasti-kote® T-19 Red Oxide Primer) to mimic the colour/texture of a painted vessel surface.

In April 2006, 224 settlement plates (112 flat, 112 curved) were suspended vertically beneath a commercial wharf in Wellington Harbour (Figure 2) at a depth of 3-5 m. Efficacy trials were undertaken every three months commencing in July 2006 (i.e. July and October 2006, January and April 2007). During the first efficacy trial, both systems were trialled on settlement plates that had three months of fouling present (i.e. fouling growth from April to July 2006). During subsequent trials, the systems were applied to plates that had been deployed since April 2006, thus providing an opportunity to trial plates with more advanced fouling communities under the assumption that the fouling biomass and diversity would increase over time.

The effect of season on brush performance was investigated by trialling the systems at four different times of the year (i.e. every three months) on experimental plates that had been deployed three months prior to treatment. The rationale behind the seasonal trials was that plates deployed at different times of the year were likely to develop distinct fouling communities due to seasonal changes in recruitment. Such changes could alter the efficacy of fouling removal and collection by the brush systems, especially where species occur with broad morphological differences that could affect susceptibility to treatment effects (e.g. soft versus hard-bodied organisms).

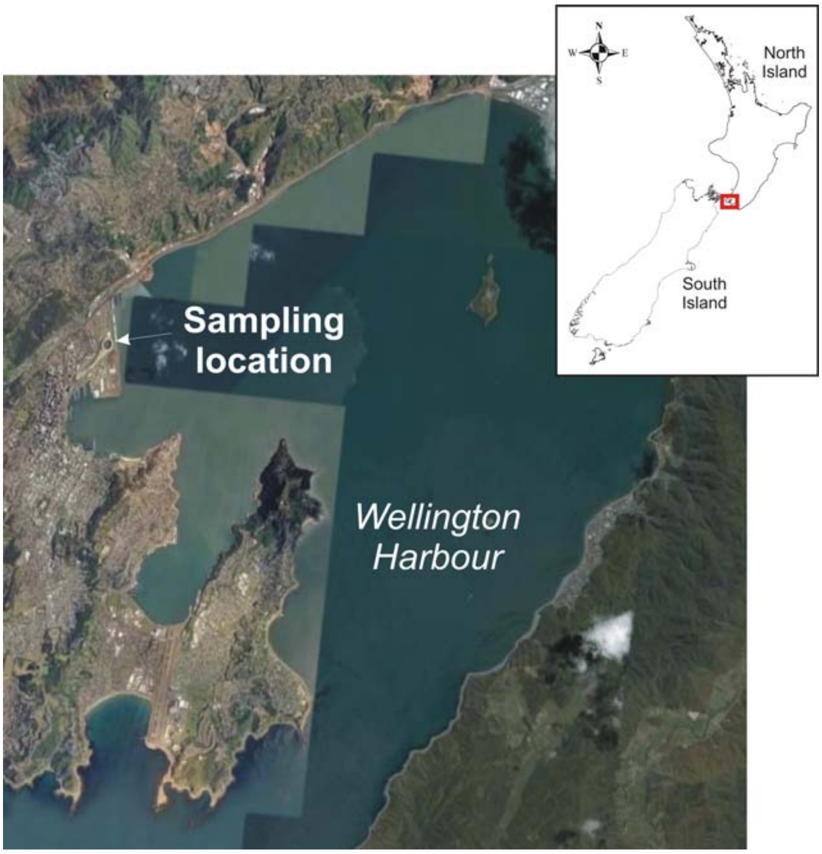

Figure 2: Sampling location (-41° 16.368’, 174° 47.291’), Wellington Harbour, New Zealand. Note: Base map downloaded from Google Earth.

2.2.2 Sampling procedure

Prior to the defouling trials, plates retrieved by divers were photographed, drained (standardised to two minutes) and weighed. Plates were then returned to the water and attached to a metal backing plate (1.8 m x 0.8 m, Figure 3) using screws. The metal backing plate was rigidly fixed to a wharf pile approximately 3-5 m below the surface. The plate was then subjected to the defouling trial (n = 3 per combination of treatment factors).

Figure 3: The metal backing plate used to replicate curved areas of a vessel hull. Note: System B is in the foreground.

For each defouling treatment, the operator (a commercial diver) started the rotating brush in motion on the metal backing plate and then slowly moved the brush system across the settlement plate, continuing to the opposite side of the backing plate. Both systems used the same brush grade/type throughout the project. Defouled material not captured by the brush systems that would have been lost to the environment was collected using large hoop-shaped nets (60 µm mesh size) manoeuvred by divers during the trial (Figure 4). All collected material was weighed, inspected for physical damage and preserved for later analyses.

Collected organisms or fragments were determined as being viable or non-viable following a microscopic examination and assessment, according to criteria developed by Woods et al. (2007). For species that can establish and reproduce from fragments (e.g. colonial ascidians, hydroids, bryozoans), the precautionary principle was adopted (i.e. fragments were considered viable) where the extent of damage was difficult to determine.

The brush systems were cleaned and flushed between treatments. After treatment, each plate was again photographed, drained and weighed. Plates were then re-suspended beneath the wharf for a further two weeks to determine the chronic effects of treatment (compared with immediate mechanical effects), after which they were re-photographed and preserved to assist with image analyses. Chronic images of plates were used as the endpoint against which the efficacy of the brush systems in removing fouling biomass was measured.

Figure 4: Diver-operated collection bags (60 µm mesh size) used during the defouling trials to capture defouled material not collected by the rotating brush systems.

2.2.3 Assessing performance

Changes in species richness and percent cover were estimated from differences in pre-treatment (before) versus chronic (two weeks post-treatment) photos of the settlement plates. Images were rectified in ArcMap 9.2 GIS software (ESRI, Redlands, CA, USA) and a systematic 49-point grid (7 x 7) was superimposed (Figure 5). Taxa present beneath each point were identified and entered into a database. A 1 cm perimeter along the edge of each plate was omitted from counting to control for possible edge effects.

Due to the inherent difficulties of identifying some organisms to species level from photographs (e.g. colonial ascidians, bryozoans and hydroids), some were grouped to a coarser taxonomic resolution (e.g. Genus or Family), as necessary (Table 1). Aggregation was considered appropriate in this study, given that we were testing devices whose mechanical effects were expected to be dependent on gross morphological characteristics shared by species within broad taxonomic groups. Control plates were used to assist with the identification of taxa and post-treatment plates were inspected to confirm whether an organism was fully intact. The number of taxa present on the entire plate was also counted to give total richness. Tubeworm remnants were included in the % cover tally, however they were not included in species richness tallies as the organisms were no longer alive.

Divers occasionally missed patches on the settlement plates. Because this was due to operator error (rather than actual system performance), these data were not included in our analyses, however the frequency of this occurring was recorded.

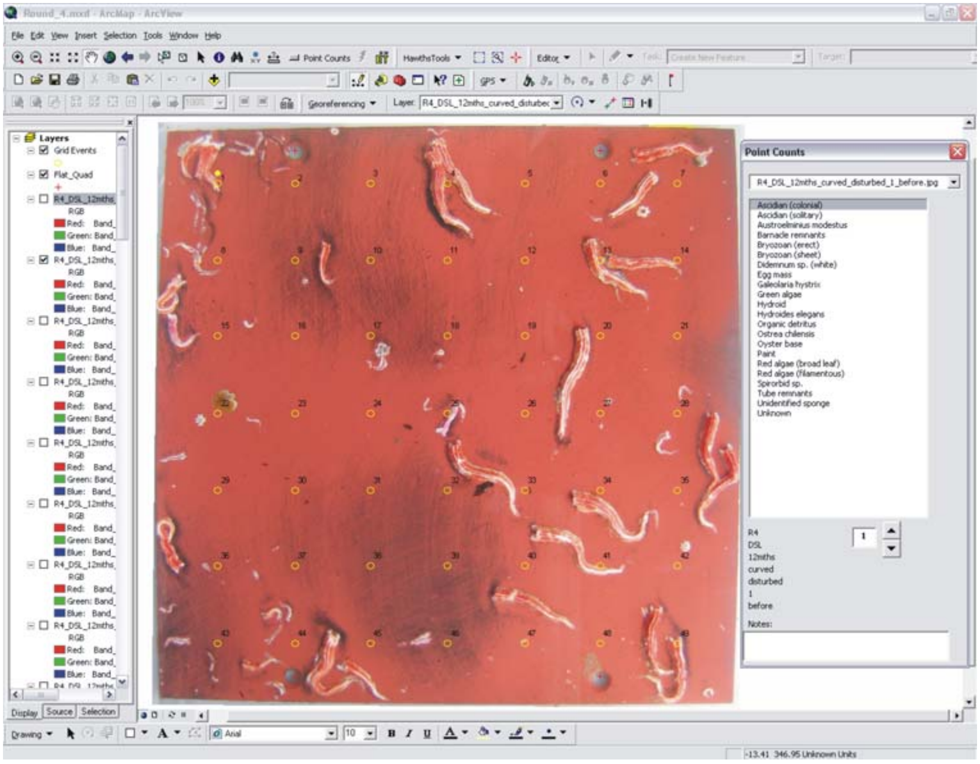

Figure 5: A systematic 49-point grid overlaid upon a defouled settlement plate within Arcmap™. Taxa beneath each point were identified and entered into a Microsoft Access™ database via a custom-designed interface.

Table 1: Taxonomic groups used for assessing taxa richness.

Group/taxa	**Description**
Bryozoan (erect)	Erect bryozoans were dominated by *Bugula neritina* during the present study. However, bryozoans require microscopic examination for reliable identification to species level.
Bryozoan (encrusting)	Encrusting bryozoans are very difficult to identify unless viewed under a microscope. One of the more easily distinguished species is *Watersipora subtorquata*, which has a characteristic brick-red appearance.
Austrominius modestus	Only one species of barnacle colonised the settlement plates during this study.
Galeolaria hystrix	This species was the largest of the calcareous tubeworms that colonised the plates during the project. *G. hystrix* is easily recognised by the reddish colour and the shape of its tube.
Hydroides elegans	*H. elegans* is a small calcareous tubeworm commonly found in coastal and estuarine fouling communities and was found on plates throughout the project.
Spirorbidae	Spirobid tubeworms are much smaller than the other calcareous tubeworms and were easily recognised by their spiral-shape.
Colonial ascidian	*Diplosoma listerianum* and *Didemnum* sp. were the dominant colonial ascidians on the settlement plates.
Hydroid	Thecate (sheathed) and athecate (unsheathed) hydroids were commonly observed on the settlement plates throughout the project.
Sponge	Various species of sponge were encountered on the settlement plates. Microscopic examination of spicules is required for a reliable identification to species level.
Foliose algae	Many species of algae are difficult to identify from photographs (particularly juveniles).
Filamentous algae	As for foliose algae.
Ostrea chilensis	Oysters became more dominant on the settlement plates after 9-12 months deployment.
Egg mass	Various eggs presumed to be from small fish and gastropods were present on the plates (although not easily visible).

2.3 EFFICACY TRIALS ON A FOULED VESSEL

The settlement plate defouling trials provided a robust method for comparing the efficacy of the two rotating brush systems on plates (flat and curved) that were designed to mimic vessel hulls. As a comparison with the plate trials, we also evaluated the efficacy of the two systems on a fouled vessel. The vessel trial was undertaken in April 2007 on a 47 m squid fishing boat *Pacific Wind* (Figure 6). Both systems were trialled on six 1 m x 1 m curved regions of the hull toward the bow of the vessel, with photographic images taken before and after the trials. During the ground-truthing trials, fouling levels on areas of the hull treated were less than that observed on settlement plates that had been deployed for three months during the plate trials. Scrapings were taken from several regions of the hull to identify the fouling taxa present and assist with photo IDs. During the trials, material not captured by the brush systems was collected by divers using the same methods as in the plate trials.

Figure 6: The vessel *Pacific Wind* used for the vessel trial exercise undertaken in Wellington Harbour, April 2007.

2.4 STATISTICAL ANALYSES

Control plates used to test for handling and other effects were not significantly different ($P>>0.05$); therefore control data were removed from all subsequent analyses of treatment effects. Data were explored for homogeneity and normality using STATISTICA Version 7 (StatSoft Inc., Tulsa, OK, USA), and dependent variables were log(x+1) transformed where necessary. Untransformed data were used in the analyses if a transformation did not remove the heterogeneity of variances (Underwood 1997). Data were analysed using multiple linear regression and generalised additive modelling (using Gaussian and Poisson distributions). Although Poisson analyses are more appropriate for these types of data, statistical outputs based on Gaussian were comparable and are presented here for ease of interpretation. Akaike Information Criteria (AIC) were used for model selection, and the model of best fit for each analysis was validated by plotting residuals. Statistical outputs for all analyses are provided in Appendix 2.

A non-metric MDS ordination procedure, based on the Bray-Curtis similarity measure, was used to describe changes in taxa composition and dominance patterns on plates following treatment by the rotating brush devices in relation to season and deployment time using PRIMER Version 5.2.2 (PRIMER-E Ltd, Lutton, Ivybridge, UK). Data were fourth-root transformed to down-weigh the influence of the most dominant taxa (Clarke & Warwick 1994). Non-metric MDS plots were also used to describe changes in taxa composition (presence/absence) of material lost to the environment during defouling trials. Similarity Percentage analyses (SIMPER) in PRIMER were then used to explore trends evident in the nMDS plots (Clarke & Warwick 1994).

3 Results

3.1 GENERAL DESCRIPTION OF FOULING COMMUNITIES

3.1.1 Changes with deployment time

Fouling percent cover was extensive on flat (mean = 98.3%, SE = 0.3%) and curved (mean = 95.6%, SE = 0.6%) settlement plates within the first three months of deployment (Figure 7). For flat plates, cover remained high (mean = 99.3%, SE = 0.4%) throughout the deployment period. Similarly, fouling cover on curved plates reached 100% after 6 months, and remained high (> 98%) throughout the remainder of deployment. Taxa richness on both flat and curved plates ranged between approximately 8 and 11 taxa, with highest mean richness values recorded on plates deployed for 12 months (Figure 7).

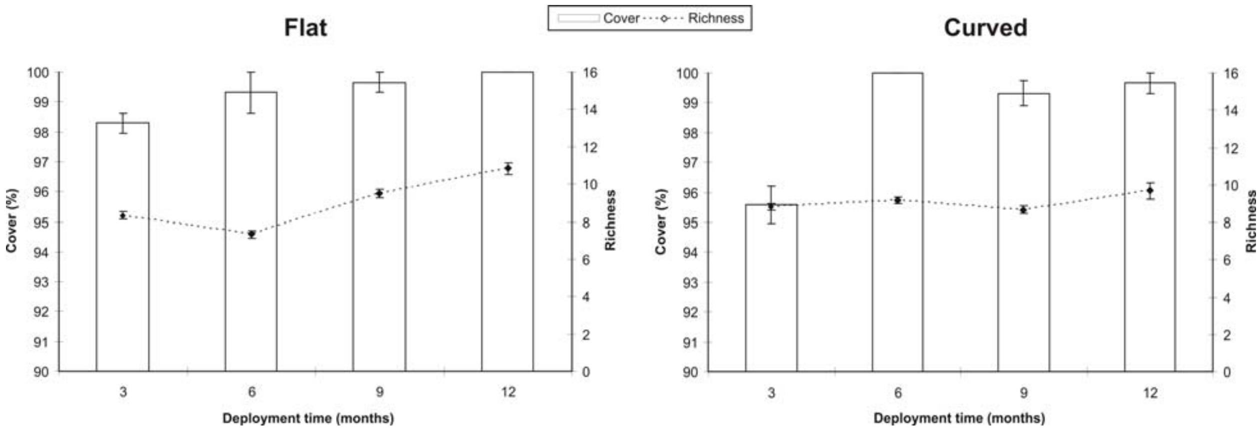

Figure 7: Changes in pre-treatment cover (%, ± 1SE) and richness on flat and curved settlement plates with increasing deployment time.

During the first three months of deployment, barnacles, hydroids, bryozoans (encrusting and branched), ascidians (colonial and solitary) and serpulid polychaetes colonised the plates, with barnacles being the most numerically dominant (Appendix 3a,b). After six months, erect bryozoans and hydroids increased in biomass and calcareous tubeworms (in particular *Galeolaria hystrix*) became more prominent (Figure 8). Fouling community biomass peaked after 9 months (Figure 9), with calcareous tubeworms and colonial ascidians (e.g. *Didemnum* sp.) becoming well established on plates. Fouling biomass on settlement plates decreased between 9 and 12 months (Figures 8 & 9). This effect was most pronounced with curved plates (16.6% reduction in biomass, Tukey's HSD $P < 0.001$, Table A2- Appendix 2), whereas the reduction on flat plates was not significant (6.5% reduction, Tukey's HSD $P = 0.280$, Table A2). Inspection of control plates revealed that the reduced biomass was largely due to a reduction in branched bryozoans (particularly *Bugula neritina*). Over the same time period, juvenile red algal taxa became more conspicuous (Figure 8).

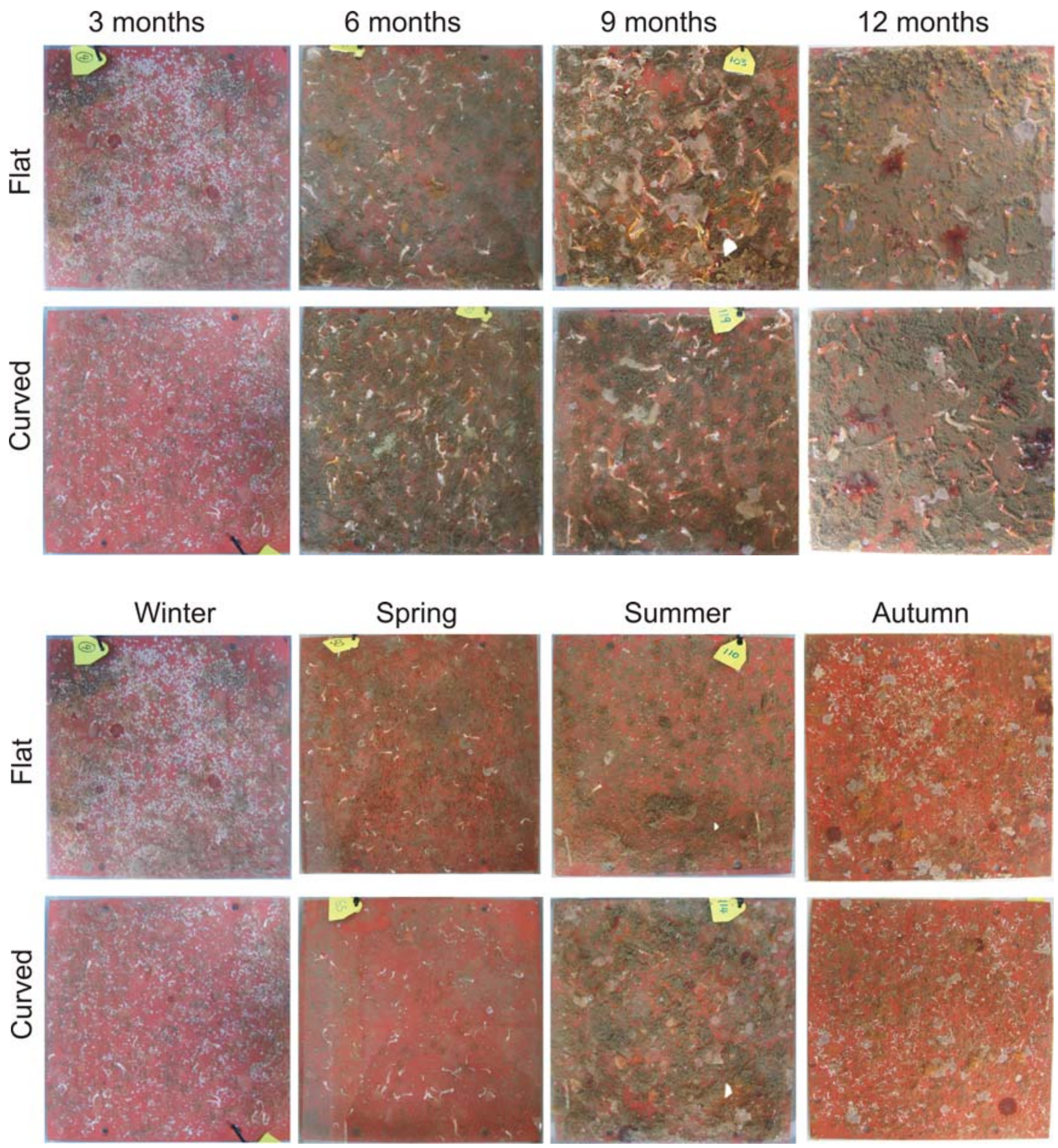

Figure 8: Representative flat and curved settlement plates showing the progression of fouling over 12 months, and over four seasons of the study.

3.1.2 Changes with season

Fouling cover was consistently high (mean > 95%) on plates deployed for three months over all four seasons (Figure 10). Taxa richness was relatively constant for both flat (mean = 7.9; SE = 0.3) and curved plates (mean = 8.3; SE = 0.4), with a slight reduction in richness evident on plates deployed three months prior to the spring efficacy trials. Changes in taxa composition were evident with season. For example, barnacles (*Austrominius modestus*) were more abundant on settlement plates during efficacy trials undertaken in winter and autumn, while erect bryozoans were larger and more abundant during trials undertaken in summer (see Figure 8). Refer to Appendix 3a,b for a list of taxa present on plates during the 12 month trial period.

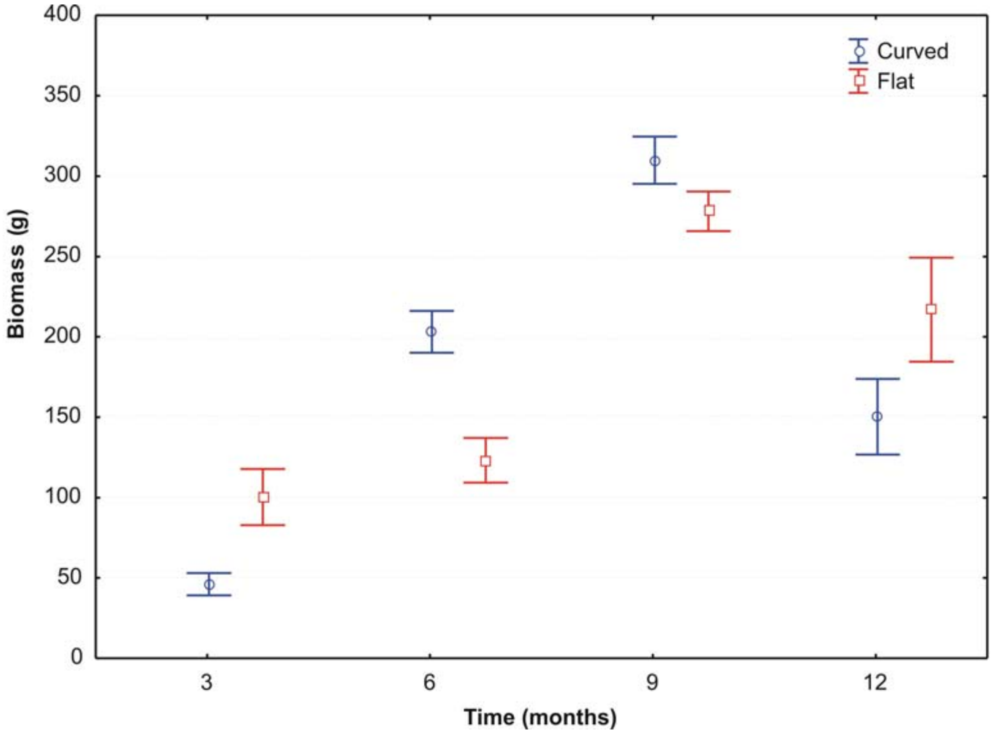

Figure 9: Average fouling biomass (± 1SE) on flat and curved settlement plates with increasing deployment time (months).

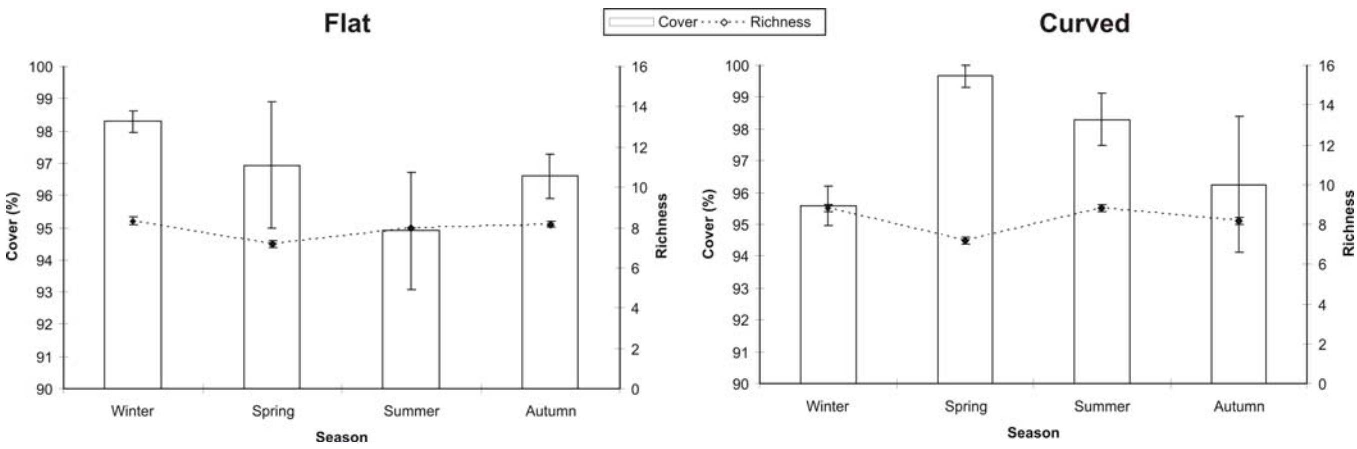

Figure 10: Changes in pre-treatment cover (%, ± 1SE) and richness on flat and curved settlement plates with season.

3.2 EFFICACY OF FOULING REMOVAL WITH INCREASING DEPLOYMENT TIME

3.2.1 Reduction in fouling cover

During the first efficacy trial (deployment time = 3 months), System A removed on average approximately 90% (SE = 2.1%) of fouling cover from flat and 93% (SE = 1.5%) from curved plates (Figure 11). The performance of System B was comparable, with a mean reduction of fouling percent cover of 88% (SE = 3.2%) and 89% (SE = 5.0%) for flat and curved plates, respectively. Performance on plates with 6 months of fouling was comparable to 3 months (Tukey's HSD $P >> 0.05$, Table A3); however the efficacy of both systems generally decreased with increasing deployment time (Figure 11). In particular, fully intact and/or partially damaged calcareous tubeworms (e.g. *Galeolaria hystrix*, *Hydroides elegans* and spirorbids) remained on plates that had been deployed for a period of 9 months or more (Figures 12 & 13). As fouling became more advanced (i.e. deployment time > 6 months), System A, and to a lesser extent System B, performed significantly better on curved settlement plates than on flat plates ($F_{3,32} = 6.44$, $P = 0.002$, Table A4).

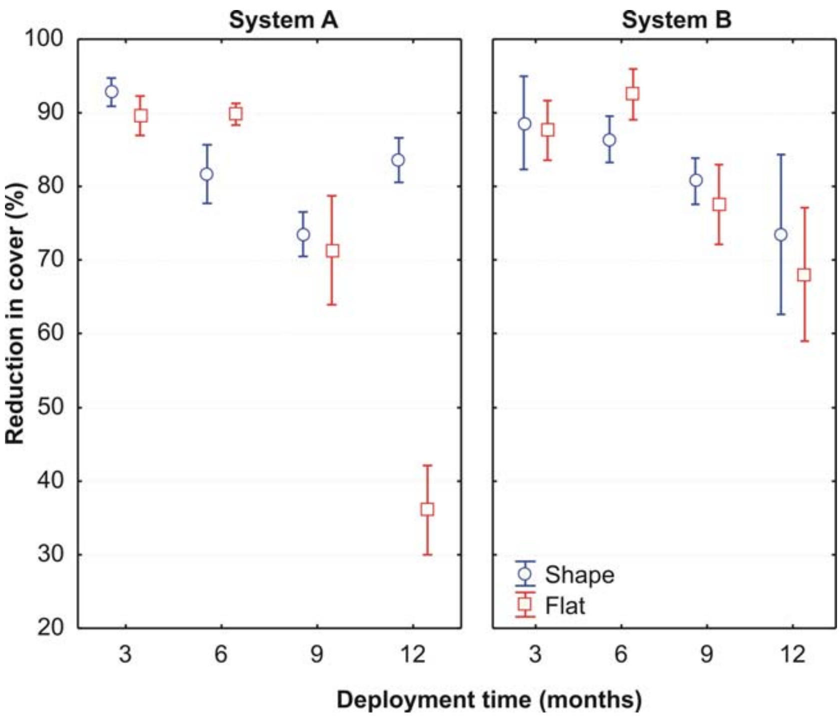

Figure 11: Reduction in cover (%, ± 1SE) of fouling on settlement plates following defouling by two independent rotating brush systems with increasing deployment time.

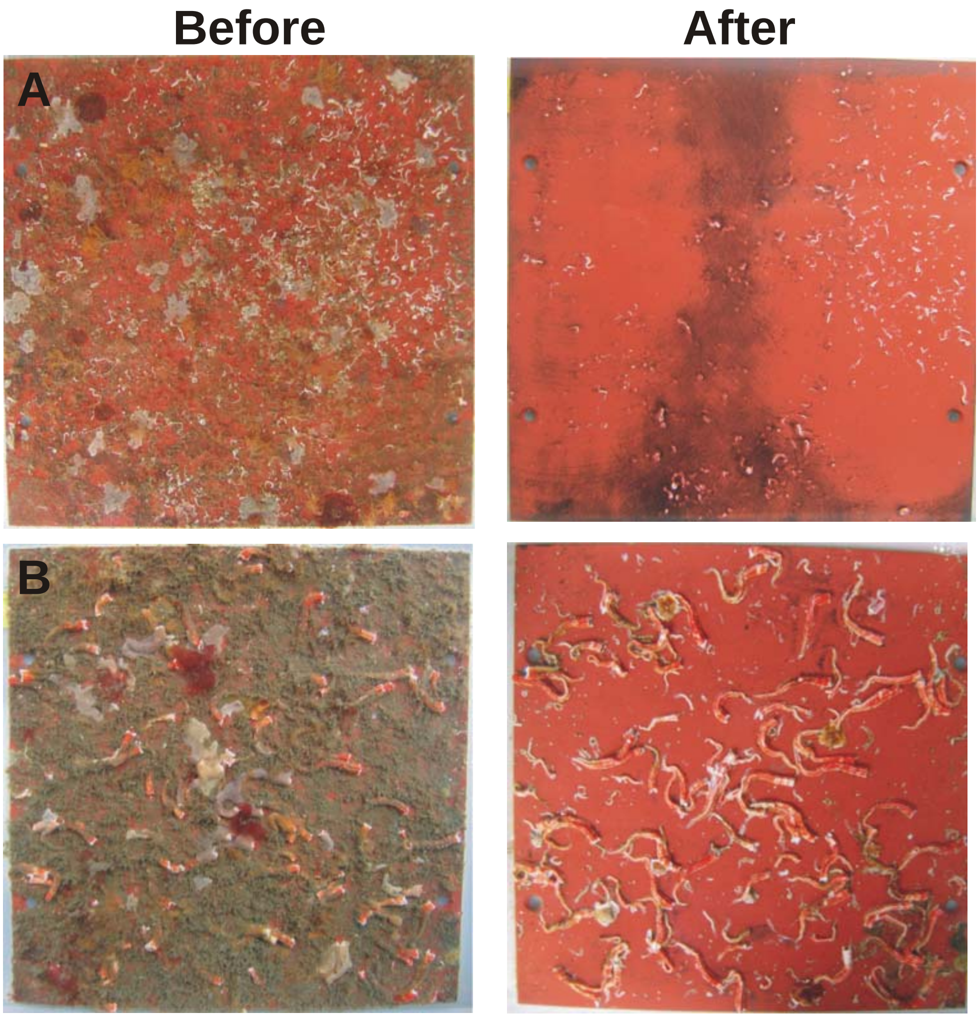

Figure 12: Photographs of settlement plates (350 x 350 mm) before and after treatment by rotating brushes for (A) 3 months and (B) 12 months of fouling.

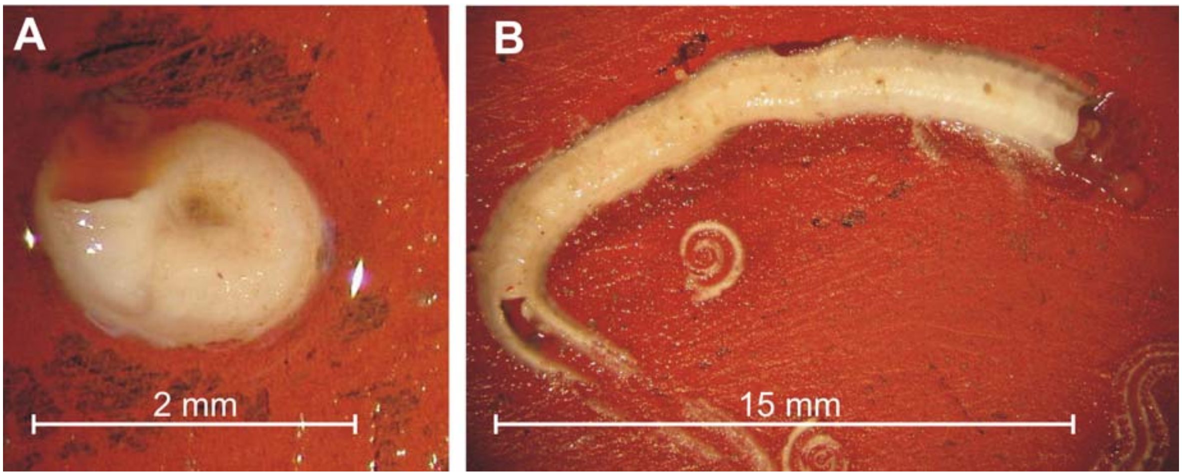

Figure 13: Microphotographs of fouling organisms remaining on settlement plates following defouling treatments by rotating brushes: (A) a viable spirorbid (Spirorbidae) and (B) a damaged serpulid polychaete (*Pomatoceros* sp.).

With increasing deployment time, the cover of soft and/or erect (herein referred to as soft/erect) fouling organisms generally increased on plates, while the cover of hard and/or encrusting (hard/encrusting) organisms either varied (flat plates) or decreased (curved plates) (Figure 14). Both systems were highly effective (> 95% reduction) in removing soft/erect fouling from flat and curved settlement plates that had been deployed for 9 months or less (Figure 14). However, on average approximately 20% (SE = 7.2%) of soft/erect organisms remained on flat plates treated by System A that had been deployed for 12 months, compared with 100% removal achieved by System B. Both systems removed 100% of hard/encrusting fouling from plates that had been deployed for 3 months. However, efficacy decreased as fouling became more advanced, with up to 61% (mean = 40.1%, SE = 10.9%) remaining on flat plates that were defouled by System A following 12 months (System B averaged 21.8%, SE = 4.8%).

There was no strong pattern evident in the percent cover of tubeworm remnants remaining on flat settlement plates over the four deployment times tested (range = 3.4 and 19.7%), with an average of 8.2% (SE = 1.6) and 12.4% (SE = 2.6) for System A and B, respectively (Figure 15). Tubeworm remnant cover was within a comparable range following treatments on curved plates (0.7 to 16.3%), with higher levels present on plates deployed for longer times. An exception to this trend (for both systems) was plates that had been deployed for 12 months, which had levels generally less than plates that had been deployed for 3 months. This corresponded with an increase in the density of fully intact tubeworms remaining on settlement plates (i.e. impervious to treatment) following treatment (refer Figure 15).

3.2.2 Reduction in taxa richness

System A removed on average 96% (SE = 3.7%) of taxa from flat plates and 89% (SE=6.4%) from curved plates during the three month efficacy trial (Figure 16). System B performance was comparable, removing 100% (SE = 0%) of taxa from flat plates and 80% (SE = 15.2%) from curved. For flat plates, performance of both systems steadily decreased as fouling became more advanced (i.e. with increasing deployment time). By contrast, performance of both systems in reducing taxa richness on curved plates declined in the period of 3-6 months deployment, but was relatively constant thereafter ($F_{3,32}$ =8.872, P = 0.002, Table A5).

Taxa composition on settlement plates altered with increasing deployment time (refer to Section 3.1), which is reflected by a gradient in pre-treatment samples for both flat and curved plates in MDS plots (Figure 17). There was also a pronounced change in fouling composition evident at all fouling levels following treatment, revealed in Figure 17 by the marked separation between before- and after-treatment samples. An exception to this trend was 12 month flat plates treated by System B, which were compositionally more similar to untreated than treated plates. SIMPER analyses revealed that this was due to higher densities of calcareous tubeworms (e.g. *Galeolaria hystrix*), hydroids and colonial ascidians remaining on the plates following this treatment. The MDS plots also highlighted a greater ‘before’ versus ‘after’ effect for plates deployed for 3 to 6 months (revealed by a more marked separation in the plot).

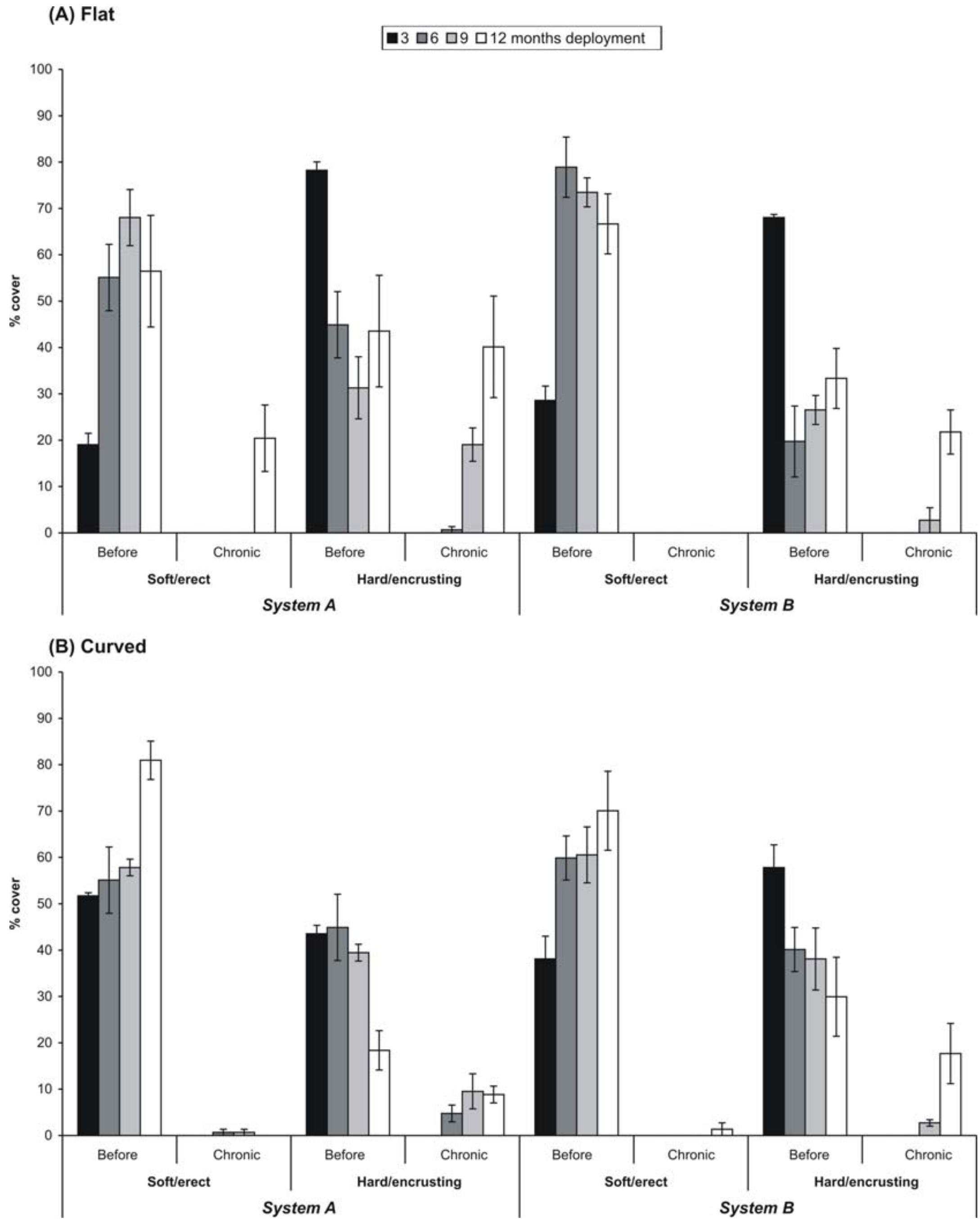

Figure 14: Percentage (± 1SE) of soft/erect and hard/encrusting organisms on settlement plates pre- and post-defouling treatments with increasing deployment time.

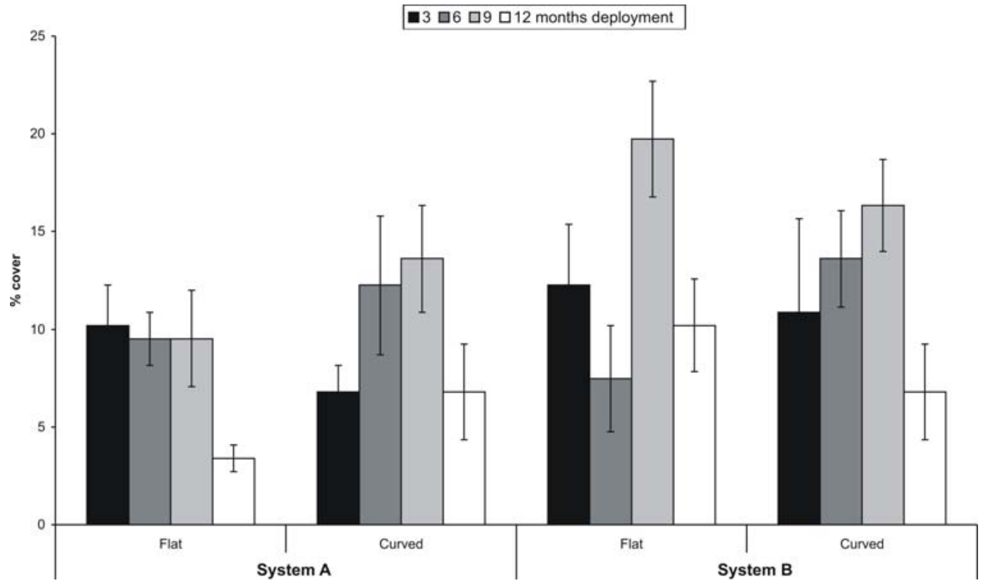

Figure 15: Percent cover (± 1SE) of tubeworm remnants remaining following defouling treatments on flat and curved settlement plates over a range of deployment times.

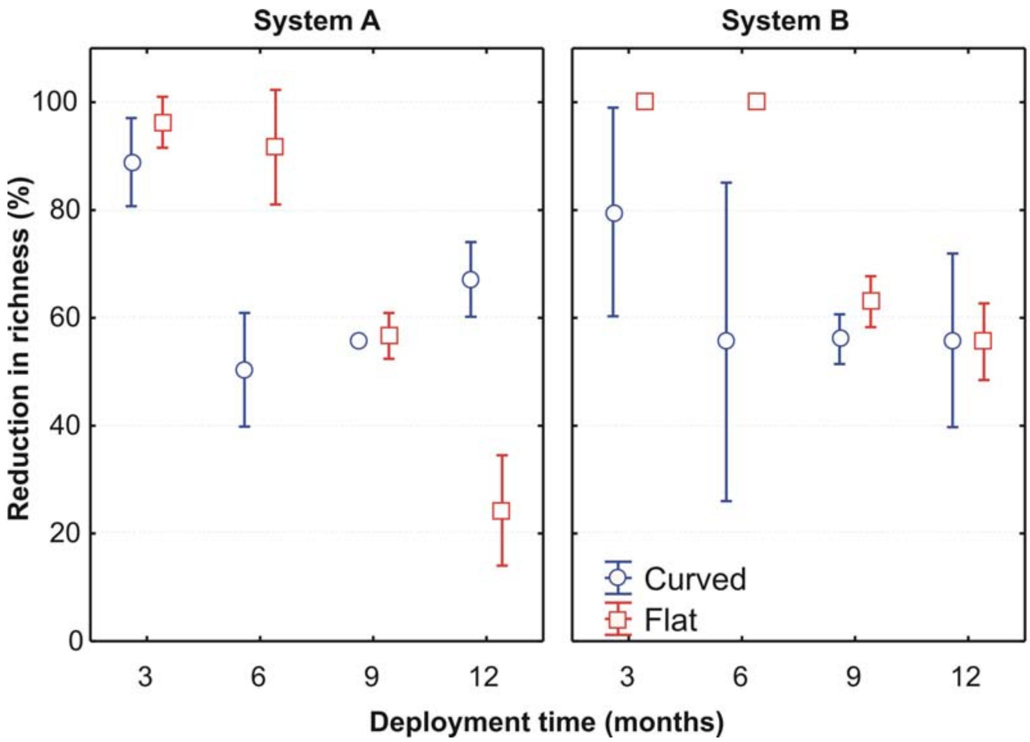

Figure 16: Change in richness (%, ± 1SE) on settlement plates following defouling by two independent rotating brush systems in relation to plate deployment time.

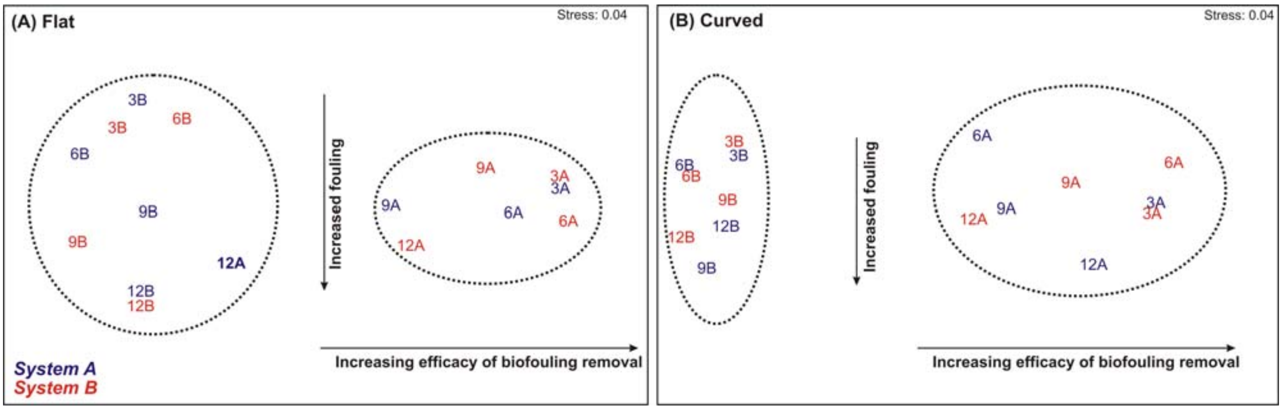

Figure 17: MDS ordination showing changes in averaged (n=3) fouling composition following treatment by two rotating brush systems on (A) flat and (B) curved plates with increasing levels of fouling. B = Before, A = After treatment. Note: Data were fourth-root transformed (2D stress for both ordinations was 0.04, indicating little or no prospect of a misleading plot; Clarke & Warwick 1994). Clusters (identified by dotted lines) indicate treatments having a within-group Bray-Curtis similarity of >60%.

3.3 EFFICACY OF FOULING REMOVAL WITH SEASON

3.3.1 Reduction in fouling cover

The performance of both systems in removing fouling cover was significantly affected by season ($F_{3,32}$ =15.680, $P < 0.001$, Table A6), with both systems more effective during the spring and summer trials compared with winter and autumn (Figure 18). This was mainly due to higher levels of tubeworm remnants remaining on plates defouled in winter and autumn months (Figure 19). This corresponded well with pre-treatment % cover data, which identified that higher levels of hard/encrusting taxa were present on plates sampled during the winter and autumn efficacy trials (Figure 20).

System A achieved 100% removal of soft/erect organisms from plates throughout the four seasonal trials (Figure 20). System B performance was comparable; however 3.5% (SE = 1.8%) of soft/erect material remained on curved plates defouled in spring. Both systems removed on average > 99% of hard/encrusting organisms, with marginally higher levels remaining on plates during the summer (overall mean for both systems= 0.2%, SE =0.2%) and autumn trials (1.9%, SE =1.2%).

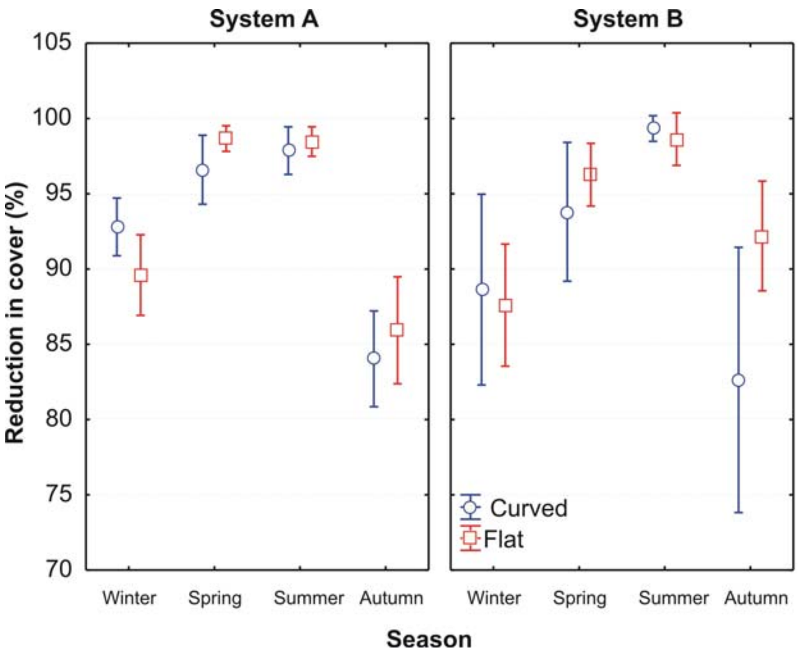

Figure 18: Change in cover (%, ± 1SE) of fouling organisms on settlement plates following defouling by two independent rotating brush systems. Deployment time = 3 months for all four seasons.

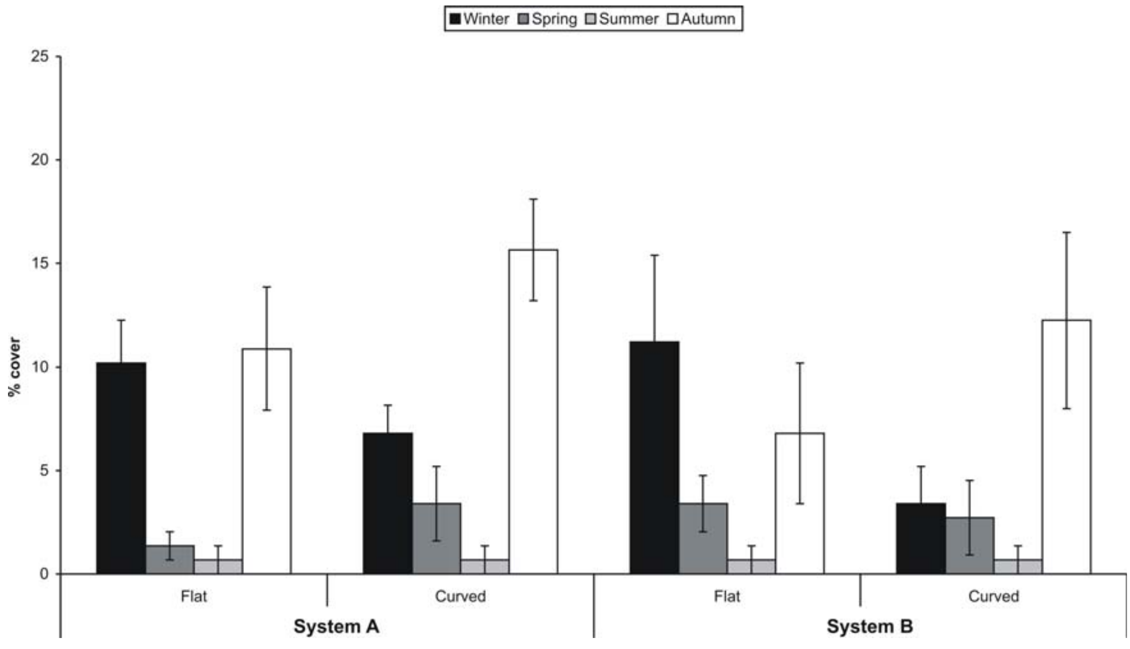

Figure 19: Percent cover (± 1SE) of tubeworm remnants remaining on flat and curved settlement plates following defouling treatments. Deployment time = 3 months.

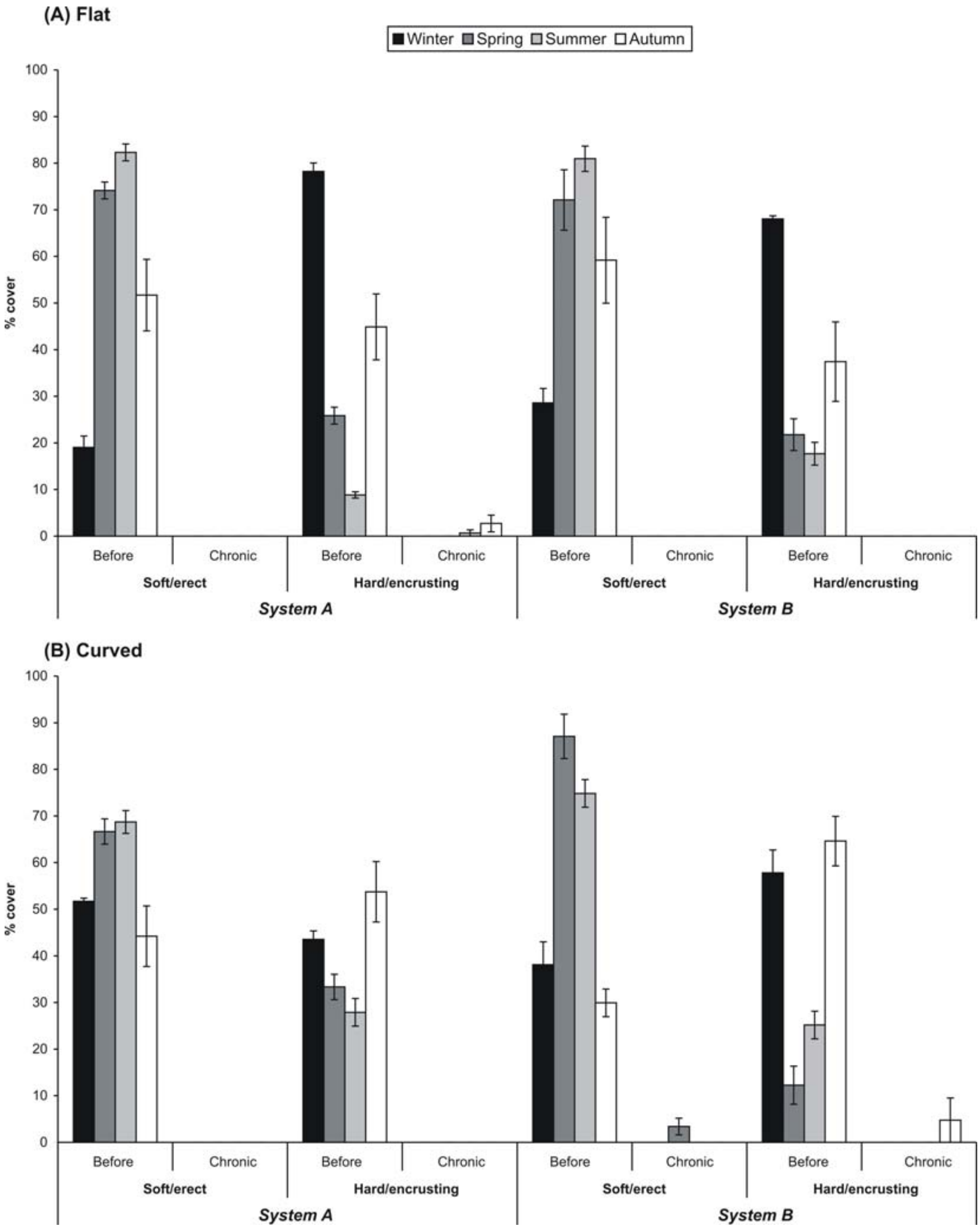

Figure 20: Percentage (± 1SE) of soft/erect and hard/encrusting organisms on settlement plates pre- and post-defouling treatments over season. Deployment time = 3 months.

3.3.2 Reduction in taxa richness

Both systems were highly effective in reducing taxon richness (Figure 21), with significantly better performance on flat (average = 94.4%, SE = 3.0%) versus curved plates (average = 88.2%, SE=1.8) ($F_{1,32} = 4.738$, $P = 0.037$, Table A7). There was no significant difference between the systems in their efficacy in reducing species richness ($F_{1,32} = 0.975$, $P = 0.331$, Table A7).

MDS ordinations (Figure 22) showed a marked effect of brush treatment on community composition in all seasons on both flat and curved settlement plates, with before and after communities forming very distinct clusters. This result is consistent with the greater reduction in % cover and taxa richness described above. Fouling remaining on curved plates defouled in autumn by System B were distinct from the main clusters (< 60% Bray-Curtis similarity). SIMPER analyses revealed that this was attributable to a small number of calcareous tubeworms (*Hydroides elegans* and spirorbid polychaetes) remaining on the settlement plates following treatment.

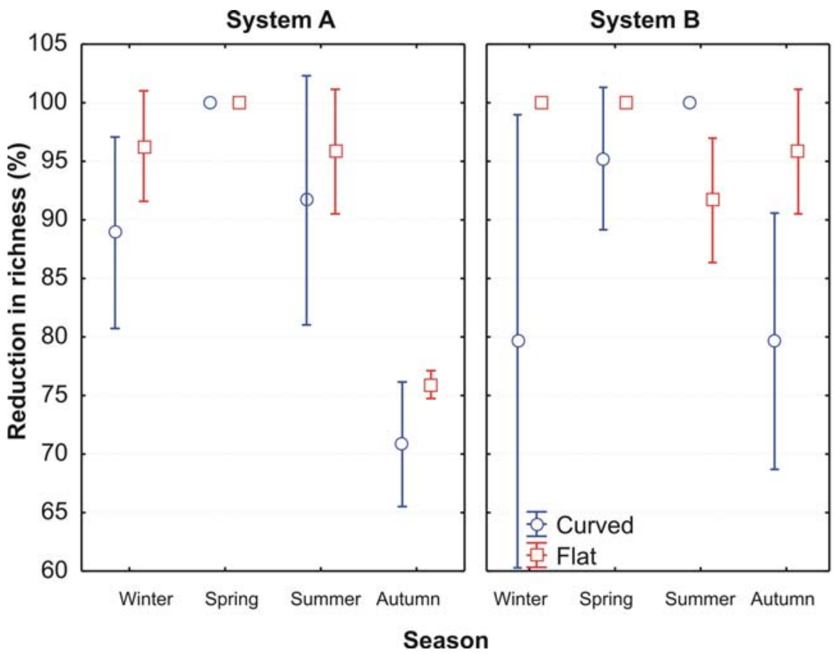

Figure 21: Change in taxa richness (%, ± 1SE) on settlement plates following defouling by two independent rotating brush systems. Deployment time = 3 months for all four seasons.

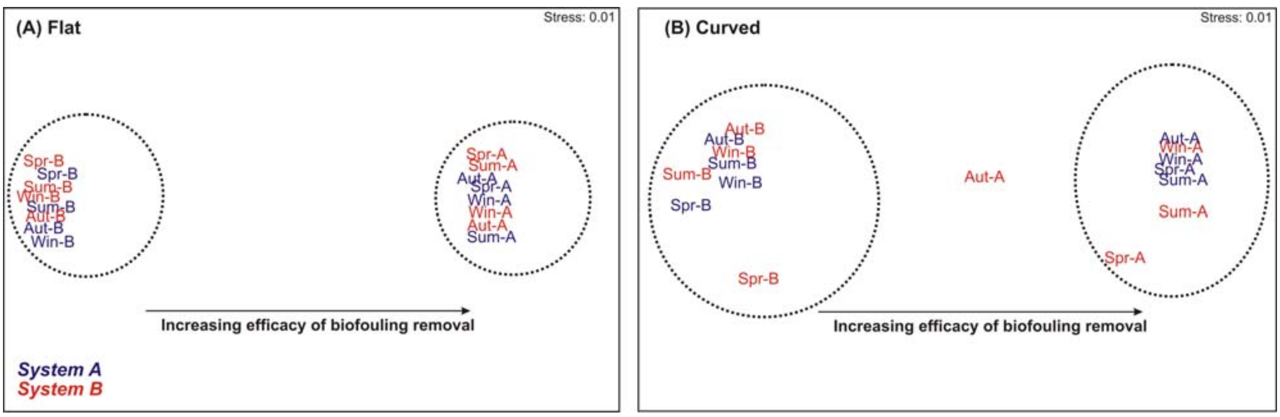

Figure 22: MDS ordination showing changes in averaged (n = 3) fouling composition following treatment by rotating brush devices on (A) flat and (B) curved plates at four different times of the year. B = Before, A = After treatment. Note: Data were fourth-root transformed (2D stress for both ordinations was 0.01, indicating little or no prospect of a misleading plot; Clarke & Warwick 1994). Clusters (identified by dotted lines) indicate treatments having a within-group Bray-Curtis similarity of >70%.

3.4 EFFICACY OF FOULING COLLECTION WITH INCREASING DEPLOYMENT TIME

3.4.1 Total material lost to the environment

The mass of defouled material (viable and non-viable) that would have been lost to the environment but collected in nets by divers during efficacy trials was low and represented a relatively small proportion (mean = 3.8%, SE = 0.8%) of total material removed from the settlement plates (Figure 23). Fouling levels, and their interactions with plate shape ($F_{3,32}$ =11.70, $P < 0.001$, Table A8) and system ($F_{3,32} = 4.07$, $P = 0.015$, Table A8) affected the mass of defouled material not collected by the brush systems, with highest levels lost (by both systems) from curved plates that had the greatest fouling biomass present (Figure 24). The total amount lost from flat plates remained relatively constant throughout the four trials (mean = 0.5 g, SE = 0.1 g), with System B losing on average slightly higher amounts than System A.

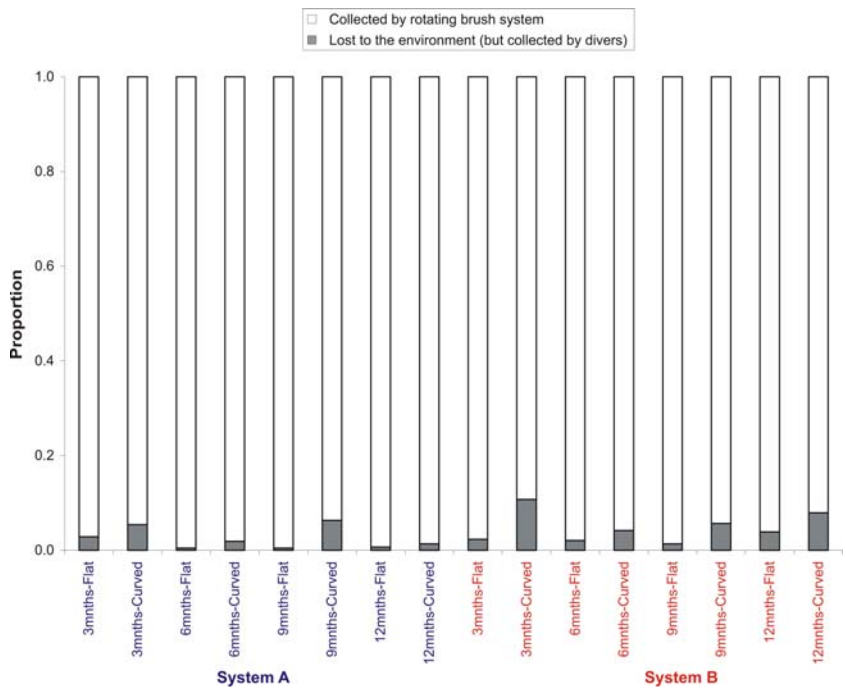

Figure 23: Average (n=3) proportion of material collected (g, wet wt) and 'lost' (i.e. not retained by systems but collected by divers) by the two rotating brush systems during efficacy trials on flat and curved settlement plates with increasing deployment time.

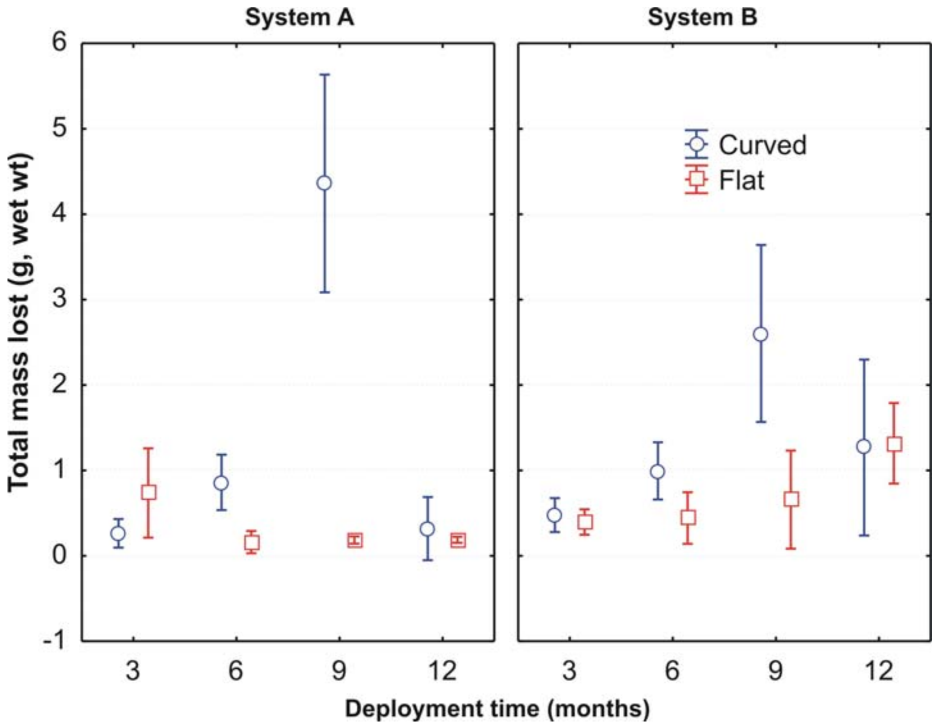

Figure 24: Average total mass (± 1SE) of defouled material not collected by the brush systems following defouling of settlement plates with increasing deployment time.

3.4.2 Viable material lost to the environment

The mass of viable material not captured by the brush systems (Figure 25) was very low (mean = 0.09 g, SE = 0.02 g) and represented a relatively small proportion (8%; SE =10.6%) of the total mass of material 'lost' to the environment (but captured by divers during the trial) (Figure 26). The amount of viable material lost varied significantly with plate deployment time ($F_{3,32}$ = 4.13, P = 0.014, Table A9) and plate shape ($F_{1,32}$ = 10.24, P = 0.003). The amount of viable material lost from flat plates was comparable across all levels of fouling (Figure 20); however the amount of viable material lost from curved plates generally increased with increasing plate deployment time. A reversal of this trend was evident when curved plates deployed for 12 months were defouled, where the amount of viable material lost was comparable to trials undertaken on plates deployed for 3 to 6 months. Overall, a higher biomass of viable material was lost from curved plates (average=0.122 g, SE= 0.023 g) compared with flat plates (average=0.050 g, SE= 0.012 g), which supported our field observations. On average, System A retained more viable material than System B, however this was not statistically significant ($F_{1,32}$ = 3.86, P = 0.058, Table A9). Finally, given the very low mass of viable material 'lost' (but subsequently collected by divers) during the trials, the trends identified above should be treated with appropriate caution given the potential for large relative changes that could result from the collection or loss of a single individual (e.g. barnacles) or colony (e.g. ascidians) during the efficacy trials.

Viable material 'lost' to the environment (but collected by divers) included a wide range of fully intact organisms (e.g. juvenile mussels, barnacles, calcareous and non-

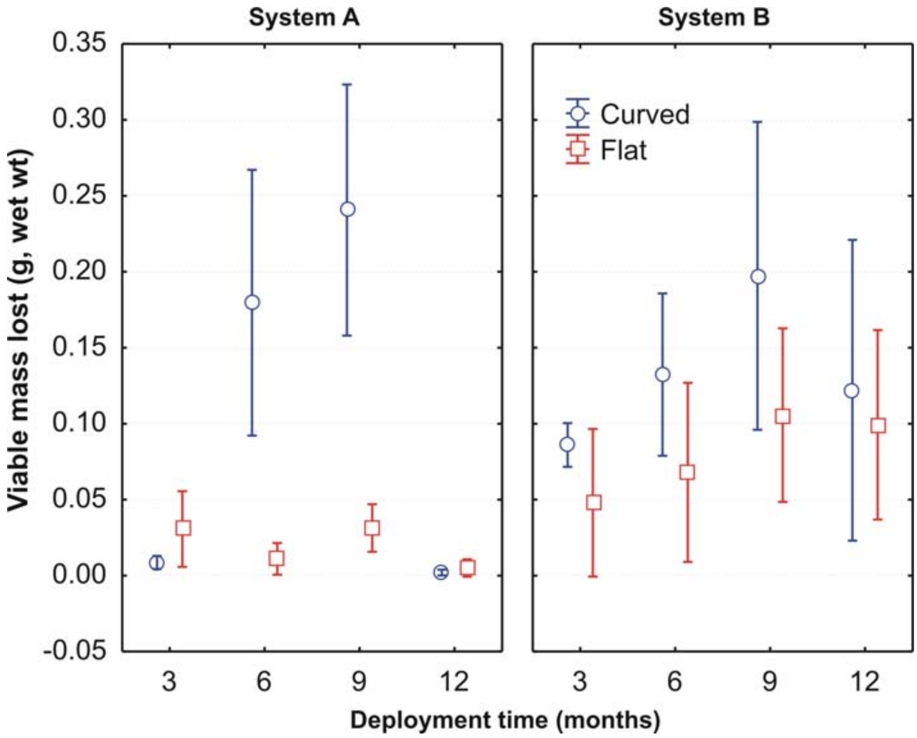

Figure 25: Average mass (± 1SE) of viable defouled material not collected by the brush systems following defouling of settlement plates with increasing deployment time.

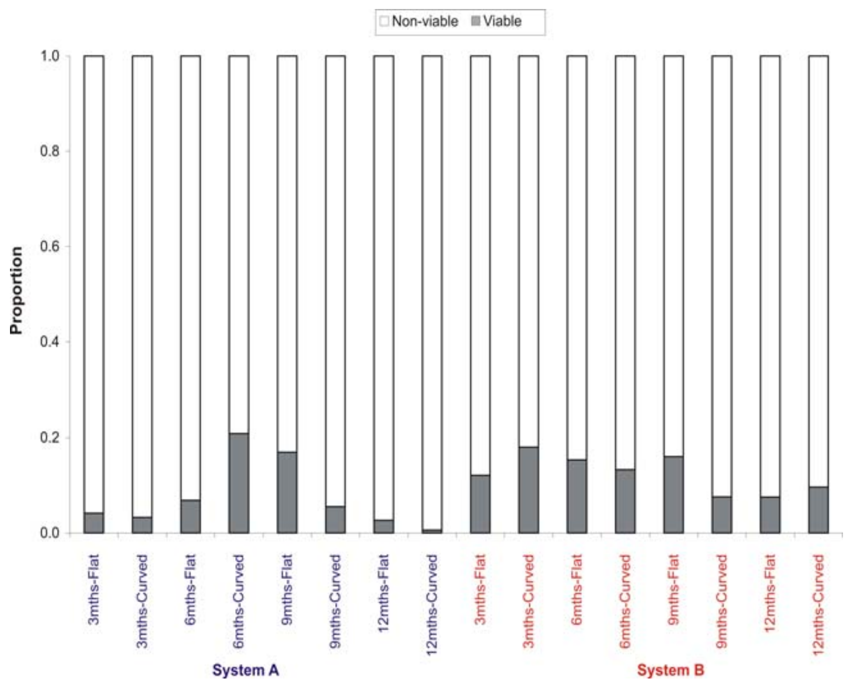

Figure 26: Average (n=3) proportion of viable and non-viable material (g, wet wt) 'lost' (but subsequently collected by divers) by the two rotating brush systems during efficacy trials on flat and curved settlement plates with increasing deployment time.

calcareous polychaete worms), as well as fragmented bryozoans, hydroids and colonial ascidians (Figure 27). Collectively, the composition of viable samples lost from both flat and curved plates was relatively dissimilar (Figure 28). Several samples were distinct from the main cluster (formed based on relatively low similarity values), which was due to relatively few, or in some cases, no viable material present (Figure 28).

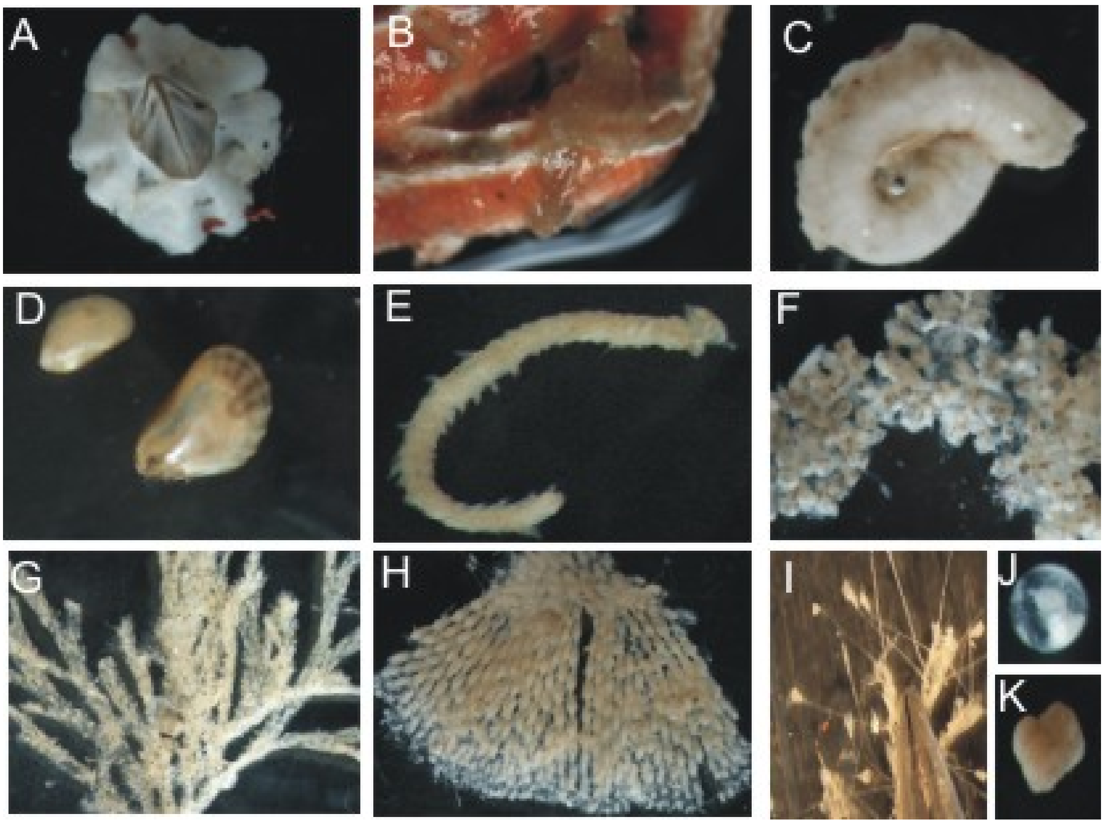

Figure 27: Microphotographs of viable fouling material 'lost' to the environment during defouling trials. A) *Austrominius modestus*; B) *Galeolaria hystrix* with an unidentified juvenile algae species attached; C) Spirorbid (Spirorbidae); D) unidentified juvenile mussels; E) Spionid polychaete (Spionidae); F) *Diplosoma listerianum*; G) *Bugula neritina*; H) encrusting bryozoan; I) thecate hydroid; J) unidentified fish egg; and K) unidentified egg mass.

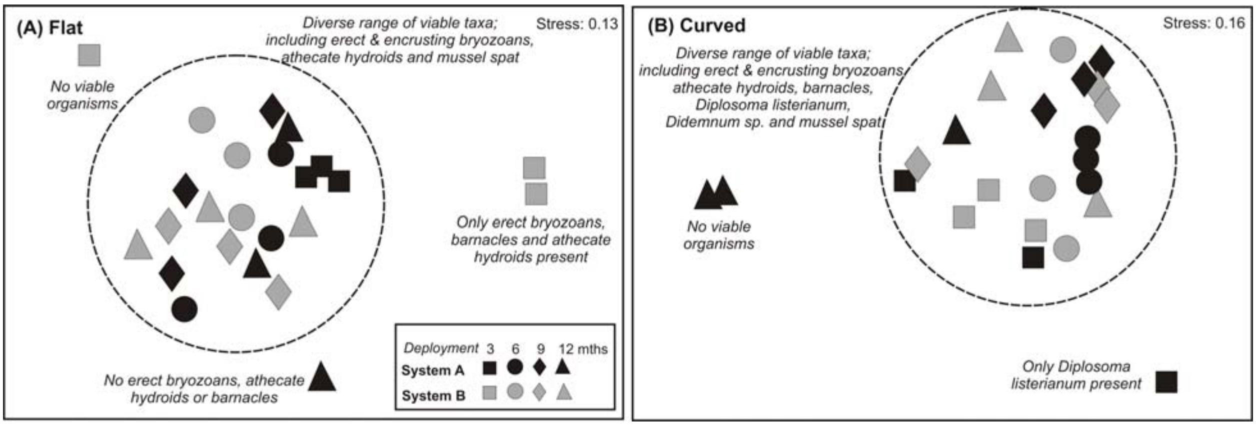

Figure 28: MDS ordination of viable material lost to the environment during defouling trials on flat and curved settlement plates over a range of deployment times. Note: Presence/ absence data used. Average similarities of the main clusters were based on >36 and 38% Bray-Curtis similarity for flat and curved plates, respectively.

3.5 EFFICACY OF FOULING COLLECTION WITH SEASON

During seasonal trials, a small proportion (average = 2.4%; SE = 0.8%) of defouled material was 'lost' to the environment (but collected by divers) (Figure 29), of which 34.4% (SE = 10.4%) was viable (Figure 30). Season significantly affected the mass of material not collected by the systems ($F_{3,32}$ = 7.26, *P* = 0.001, Table A10), with highest levels lost during the. winter defouling trial (Figure 31). This corresponded well with field observations, where small barnacles (*Austrominius modestus*) were observed being dislodged by the brush systems without subsequent collection. Both systems performed comparably throughout the seasonal trials ($F_{,32}$ = 0.441, *P* = 0.4414, Table A10), with plate shape having no detectable affect on system performance ($F_{1,32}$ = 0.827, *P* = 0.3699).

The amount of viable fouling material lost to the environment was not affected by season ($F_{3,32}$ = 1.38, *P* = 0.265, Table A11) (Figure 32). However, there was a significant difference between systems ($F_{1,32}$ = 5.54, *P* = 0.0249, Table A11), with System A retaining on average a higher proportion of viable defouled material than System B. On average, higher amounts of viable material were lost from curved plates (mean = 0.05 g, SE = 0.01) compared with flat plates (mean = 0.02 g, SE = 0.01), however this small difference was not statistically significant ($F_{1,32}$ = 3.71, *P* = 0.06, Table A11). As mentioned previously, trends identified above should be treated with appropriate caution given the potential for large relative changes that could result from the collection or loss of a single individual or colony.

Viable material 'lost' to the environment during defouling trials was dominated by bryozoans, hydroids and barnacles, reflecting the dominant taxa present on the plates at the different times of the year (refer Appendix 4).

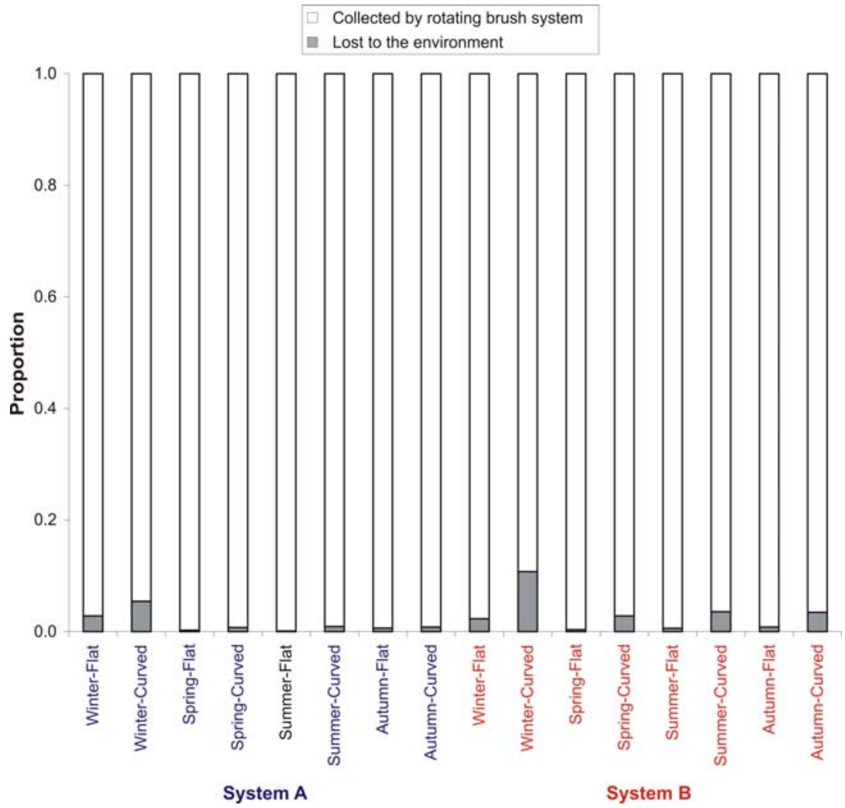

Figure 29: Average (n=3) proportion of material collected and 'lost' to the environment (g, wet wt) by the two rotating brush systems during efficacy trials on flat and curved settlement plates at different times of the year. Deployment time = 3 months.

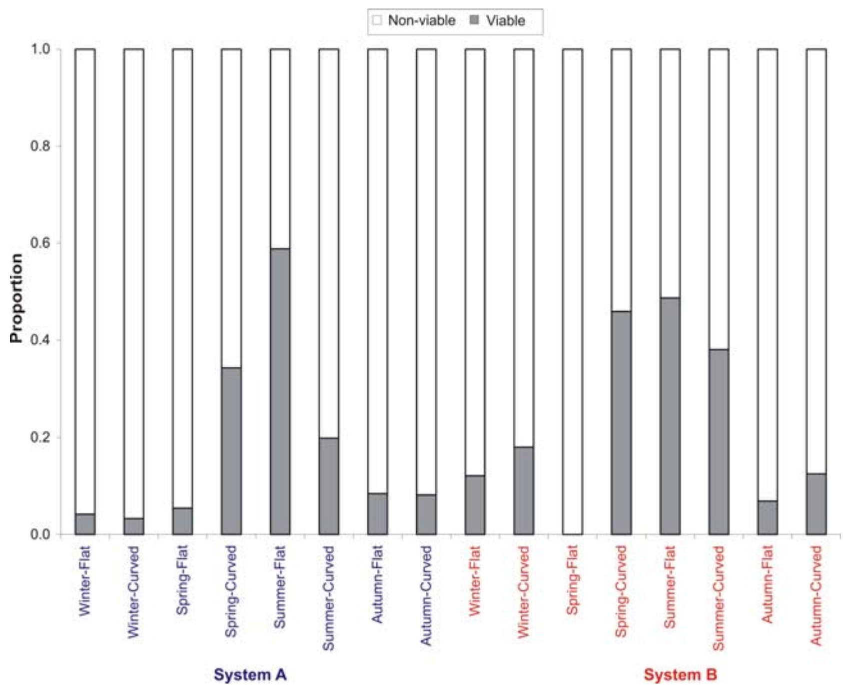

Figure 30: Average (n=3) proportion of viable and non-viable material 'lost' to the environment by the two rotating brush systems during efficacy trials on flat and curved settlement plates at different times of the year Deployment time =3 months.

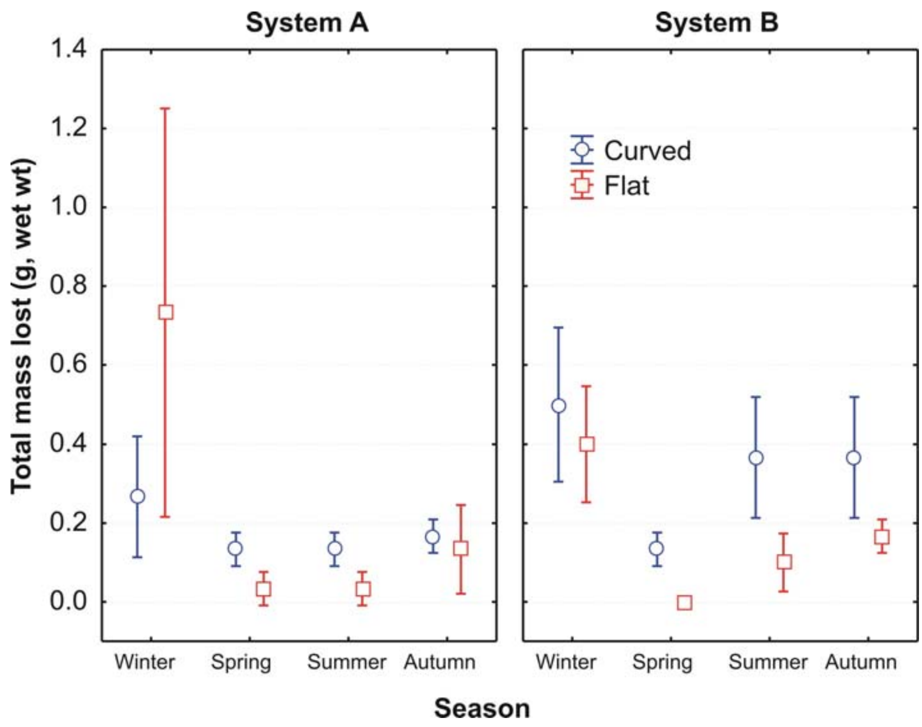

Figure 31: Average total mass (± 1SE) of defouled material (viable and non-viable) 'lost' to the environment following defouling of settlement plates. Deployment time =3 months.

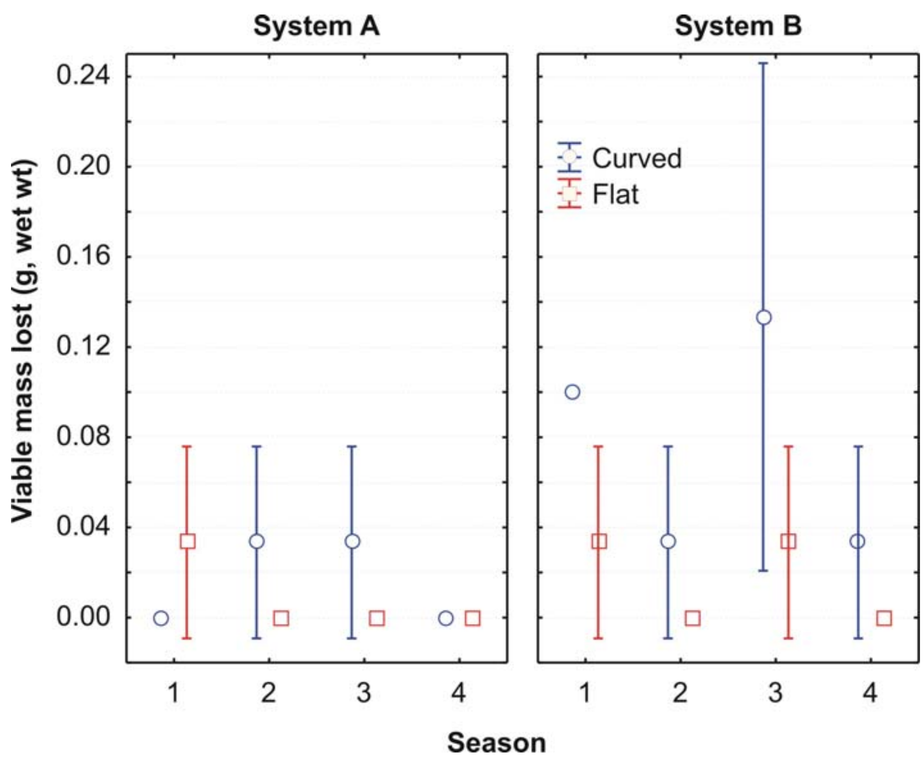

Figure 32: Average mass (± 1SE) of viable defouled material 'lost' to the environment by the brush systems following defouling of settlement plates. Deployment time =3 months.

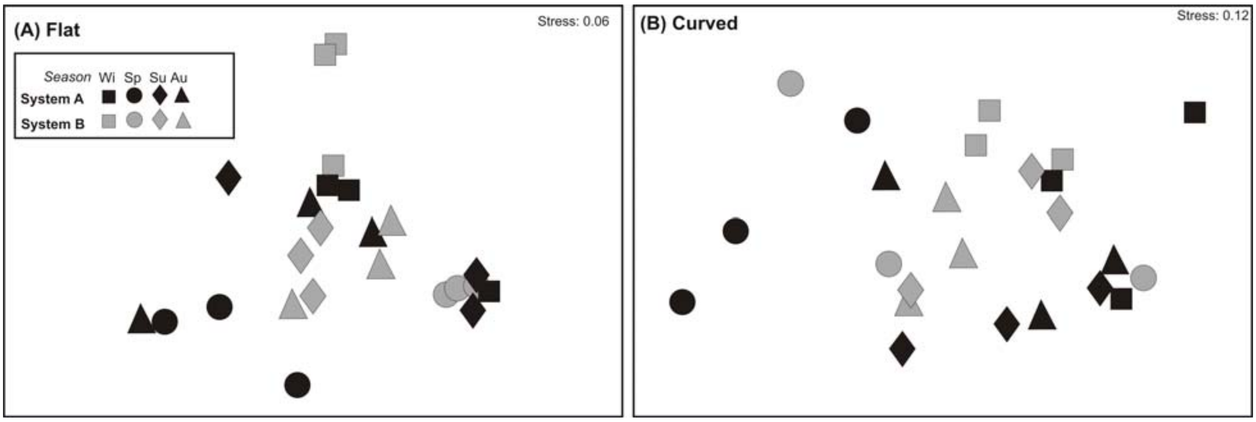

Figure 33: MDS ordination of viable material 'lost' to the environment (i.e. not collected by the rotating brush devices) during defouling trials undertaken during different seasons. Note: Presence/absence data were used. Deployment time = 3 months.

3.6 MISSED AREAS ON SETTLEMENT PLATES

Divers operating System A unintentionally missed a small section on 1 plate out of the 42 treated during the entire project, while divers operating System B incompletely defouled 11 of the 42 plates treated (i.e. 26%). In most cases, areas missed were small (< 10% of the total plate area); however larger areas (approximately 15-20%) were missed on two occasions (Figure 34). As mentioned in Section 2.2.3, data generated from missed patches were not included in statistical analyses because it represented operator error rather than system performance.

Figure 34: A photograph of a flat settlement plate following a defouling treatment. Deployment time = 3 months. The bottom-right section of the plate was 'missed' by the divers during the treatment (i.e. diver error).

3.7 EFFICACY TRIALS ON A FOULED VESSEL

Fouling levels on *Pacific Wind* were low-to-moderate, and dominated by the erect bryozoan *Bugula neritina* and hydroids. Taxa that were relatively resilient to treatment effects in experimental trials on plates, such as calcareous taxa, were not present. Mobile taxa, such as crustaceans (e.g. amphipods and copepods), nematodes and polychaete worms were also observed living amongst the fouling assemblage. More advanced levels of fouling were observed on niche areas of the vessel (e.g. gratings, propeller shaft and keel), however these regions of the vessel were not targeted during the efficacy trials. Both systems removed 100% of fouling from areas treated by the brushes.

Several patches (up to 5% of the 1 m x 1 m test areas) were missed due to 'operator error' (Figure 35). On average, System A lost 2.8 g (SE = 0.8 g) of fouling material per 1 m^2 area of vessel treated, while System B failed to retain 10.2 g (SE = 4.0), representing approximately 3 to 9% of the total material defouled. However, the difference in the performance of the two systems in retaining defouled biomass was not statistically significant ($F_{1,10} = 3.22$, $P = 0.103$, Table A12). Viable material 'lost' to the environment included crustaceans (copepods, amphipods, tanaid shrimps, cumaceans, caprellids), bryozoan fragments (e.g. *Bugula* sp., *Watersipora* sp.), ascidians fragments (*Diplosoma listerianum*), nematodes, polychaete worms and flatworms (platyhelminthes). Non-viable material comprised detritus, paint chips and various fragmented organisms (refer Appendix 5).

Figure 35: Representative photographs of 1m x 1m test areas on a fouled vessel. (A) before and (B) after defouling; (C) sections missed by divers.

4 Discussion

4.1 EFFICACY OF ROTATING BRUSH SYSTEMS

4.1.1 Removal of fouling material

The present study assessed the efficacy of two independently developed rotating brush systems in removing and retaining fouling material from experimental surfaces over a range of fouling levels and seasons. High performance (ca. 90% biomass removal) was achieved on experimental surfaces with low levels of fouling present. However, the efficacy of both systems decreased on settlement plates deployed for > 3 months; predominantly due to changes in total biomass and the morphology of dominant fouling taxa. In relation to the latter, our assessments revealed that both systems were highly effective at removing soft/erect organisms from experimental plates; but hard/encrusting organisms (particularly calcareous tubeworms and flat oysters) were more persistent and often remained on settlement plates following treatment. In fact, at least 50% of tubeworms on plates deployed for 12 months were resilient to rotating brush treatments. Seasonal changes in operational performance were also evident, with both systems removing a slightly higher proportion of fouling during the spring and summer trials. This was largely due to the morphology of organisms fouling plates at different times of the year. Plates sampled in winter and autumn were colonised by a higher proportion of hard/encrusting organisms than plates sampled during the summer and spring trials, of which 10-15% remained partially intact on the plates following treatment.

Despite the limitations of the brush systems against calcareous taxa, their efficacy on soft/erect fouling communities was highlighted during trials on the fouled vessel, in which both systems removed 100% of fouling biomass. This result clearly reflected the nature of the treated assemblages, which comprised erect bryozoans, hydroids and other soft-bodied taxa that were vulnerable to mechanical treatment effects. Such findings highlight the importance of taking into account the fouling community composition and morphology when rotating brush-type tools are being considered in incursion response. Even if target high-risk species are soft-bodied, however, fouling characterisation still remains an important consideration, as observations during our trials suggest that removal of soft organisms from areas dominated by hard/encrusting organisms can be compromised (Figure 36). For example, protection from the abrasive forces of the brushes appears to occur when their relatively stiff bristles pass up and over dense areas of calcareous serpulid tubes, preventing contact with the fouling cover in the gaps among tubes. Persistent fouling could possibly be removed by using different grades of brushes (Figure 37); however changes in the efficacy of removal and collection may occur as a result of coarser brushes and has not been addressed in this study. Furthermore, consideration would need to be given when treating antifouled or sensitive surfaces (e.g. fibreglass) that might be prone to damage from highly abrasive brush types.

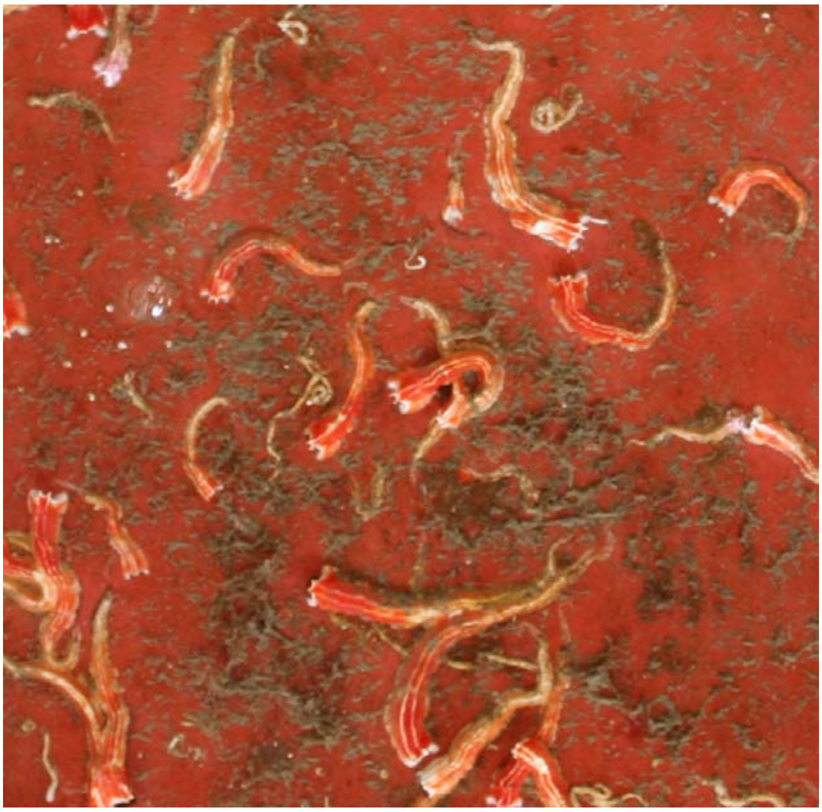

Figure 36: Soft/erect organisms remaining on settlement plates following a defouling treatment; presumably due to protection from bristles created by the dense cover of calcareous tubeworms (*Galeolaria hystrix*).

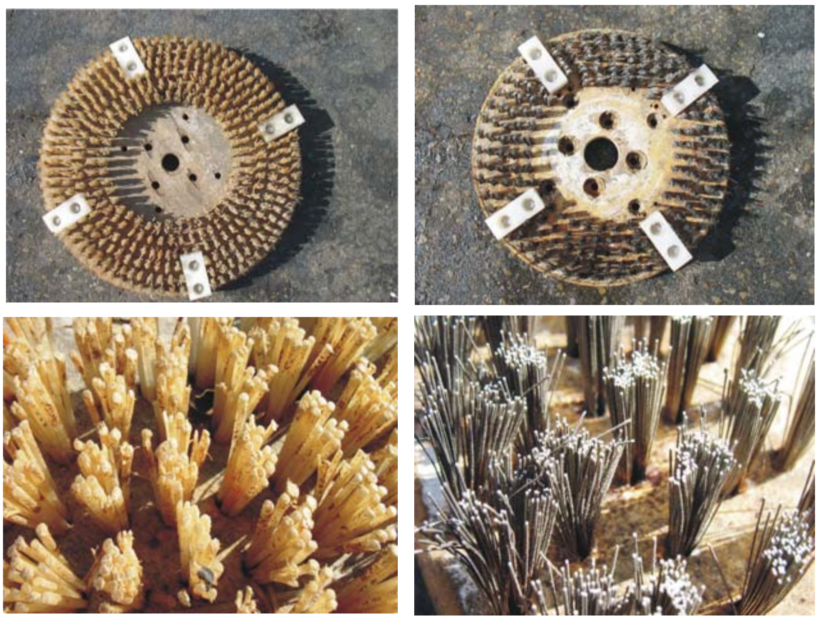

Figure 37: Examples of brush types (diameter of brush head = 360 mm) currently available to remove more persistent fouling organisms. Note: Polyethylene (left) and stainless steel (right) bristles. Higher grades (e.g. solid polyethylene blocks) are also available.

An additional consideration is whether microscopic or recently settled life stages of fouling organisms would survive rotating brush treatment, irrespective of their morphology as adults. Previous work has demonstrated that the microscopic gametophyte life stage of the invasive kelp *Undaria pinnatifida* is resistant to the effects of mechanical treatment in cases where micro-scale refugia are present (Forrest & Blakemore 2006). We suggest that micro-scale irregularities in surfaces treated by rotating brushes are also likely to provide refuge to treatment effects to early life stages on fouling organisms.

A final point relating to brush efficacy is the clear need for quality control regarding treatment effects. Even at the small scale of our trials on settlement plates and the fouled vessel, divers operating the brush units missed patches of fouling despite reasonable water clarity (~ 1-2 m) during field work. In the case of brush application to entire vessels or in turbid environments, the potential for missing patches of the hull would presumably be even greater. This situation indicates that strict quality control procedures would be advisable in marine biosecurity response, involving, for example, thorough diver inspections of the vessel following defouling and follow-up treatment of missed patches as necessary or where feasible.

4.1.2 Collection of defouled material

A diverse range of taxa was 'lost' to the environment in the form of fully intact organisms (e.g. juvenile mussels, barnacles, calcareous and non-calcareous polychaete worms) as well as potentially viable fragments (e.g. hydroids, colonial ascidians).
The amount of 'lost' material generally increased when treating curved plates with increasing biomass; whereas the material lost from flat plates was typically less and remained relatively constant throughout the trials. A potential explanation for this difference is that curved surfaces cause a partial loss of contact with brush devices, resulting in a decrease in suction created by the systems; it was noted in the field that the leading edge of the brush unit often lost contact with the experimental backing plate when treating curved plates. The greater loss of viable material from System B than System A is partially attributable to shroud design, as the leading edge of the large shroud on System B (refer Figure 1) was observed to 'scrape off' barnacles (without collection) during trials undertaken in winter and autumn. By contrast, the shroud on System A was much smaller and did not come into contact with the fouling communities.

Overall, the amount of defouled material 'lost' to the environment was very low in our trials; however we expect that this would be significantly greater in the case of defouling an entire vessel with relatively advanced fouling. Based on data from the ground-truthing trials we estimated the total mass of viable organisms that would be lost if the entire hull of the *Pacific Wind* (not including niche areas) had been cleaned. This estimate was based on total wetted surface area (TWSA), which was calculated according to Woods et al. (2007) as:

$$TWSA = (2 \times length \times draft) + (beam \times draft)$$

From this equation, we estimated that 1.2 kg (System A; SE = 0.4 kg) and 4.4 kg (System B; SE = 1.7 kg) of defouled material (wet weight) would be lost to the environment while cleaning the *Pacific Wind*, of which < 20% (based on efficacy trials) would be viable. Even though the predicted loss is reasonably small by mass, it can nonetheless represent the release of a wide range of viable taxa or fragments. Fouling organisms or viable fragments (*e.g.* of colonial organisms) dislodged during manual defouling may also survive and establish (ANZECC 1996; Forrest et al. 2007).

4.2 BROADER PERSPECTIVE ON TREATMENT EFFICACY AND ECOLOGICAL RISKS

Relative to no management, the total ecological risk of in-water defouling reflects the risk of the cleaning operation (e.g. release risk from loss of defouled material) combined with the residual risk posed by the reduced level of hull fouling (Figure 38; Hopkins & Forrest 2008). The release risk can theoretically be eliminated through the collection of all defouled material (Figure 38). The present study demonstrated that it was feasible to collect a high proportion (typically > 90%) of defouled material; however 100% retention was not achieved during our efficacy trials on moderate-to-heavily fouled surfaces, and may be difficult to achieve in practice for various reasons. For example, even if the material defouled by brush systems is all collected, our field observations revealed that fouling organisms were dislodged by diver surface-supply hoses. It is almost inevitable, therefore, that even the best cleaning systems will result in some release of viable organisms or fragmented material to the environment. Hence, the survival of such material is an important consideration in the evaluation of defouling risks.

4.2.1 Survivorship of defouled material

To our knowledge, the survival of organisms or fragments post-defouling has never been explicitly evaluated. However, the ability of many non-indigenous marine species to disperse or establish after fragmentation is recognised. For example, the ability of colonial ascidians to establish from fragments is often the means by which artificial cultures are created for experimental purposes (Johnston & Clark 2007; McCarthy et al. 2007; Osman & Whitlach 2007). Similarly, the dispersal or establishment of invasive macroalgae from fragments is documented for a number of species, including *Sargassum muticum* (Critchley et al. 1986), *Caulerpa taxifolia* (Smith & Walters 1999) and *Undaria pinnatifida* (Forrest et al. 2007; Sliwa et al. 2006). A key consideration in the establishment of defouled fragments or other viable material is the extent to which the recipient environment provides conditions that are suitable for attachment and survival. In situations where water currents disperse material into habitats typical of fouling communities (i.e. hard substrata) the likelihood of survival may be relatively high, unless factors such as sedimentation and benthic predation are limiting (G. Hopkins, unpublished data).

On the other hand, it is conceivable that a high proportion of fouling organisms associated with hard substrata will not survive in the primarily muddy and often contaminated sediments of high risk points of entry such as ports. While this contention may often be correct, our recent experience indicates that it is not always the case. In a recent New Zealand example, in-water high pressure (1200 psi) water blasters were used to remove organisms from an oil rig that was temporarily moored in Tasman Bay for defouling. Over a period of several weeks, divers systematically

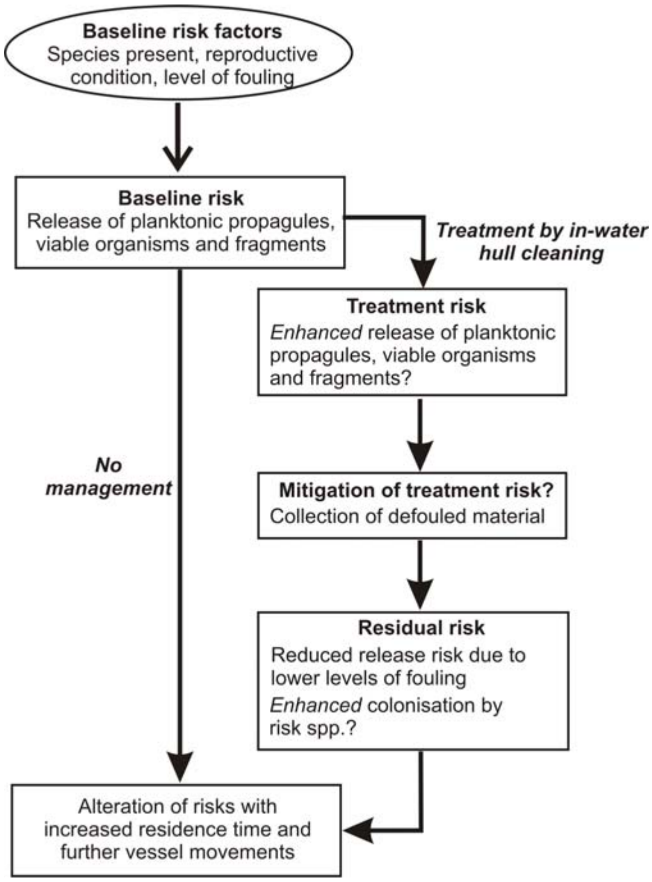

Figure 38: Conceptual diagram of risks posed by a fouled vessel if left unmanaged compared with risks from in-water hull cleaning. From Hopkins & Forrest 2008.

defouled the rig at two sites within the Bay, with no attempts made to collect the defouled material. Subsequent seabed surveys revealed a significant (> 20 tonnes) fouling biomass living on the seabed (firm muddy-sand) in the immediate vicinity of the defouling site (G. Hopkins unpublished data). Among the viable organisms were the non-indigenous brown mussel *Perna perna*, as well as numerous other non-indigenous taxa; including *Aulacomya atra* (mytilid bivalve), *Cnemidocarpa stolonifera* (solitary ascidian), *Mycale toxifera* (sponge) and the giant barnacle *Austromegabalanus cylindricus* (G. Hopkins, unpublished data). The presence of *Perna perna* led to a substantial eradication programme in which the site was repeatedly dredged to remove this potential pest species to densities that were not considered to be a significant biosecurity risk.

4.2.2 Post-defouling recruitment

In addition to the risk posed by the survival and establishment of defouled material, one of the residual risks post-defouling is that, unless antifouling paints are applied immediately following hull cleaning, mechanical treatment (including in-water and land-based) may increase the susceptibility of the surface to new fouling, thereby exacerbating future biosecurity risk (Figure 38). Floerl et al. (2005) found that defouled boat surfaces in a tropical region of Australia had up to six times more recruitment compared with surfaces that had been either chemically sterilised or contained intact fouling assemblages. Several theories were advanced to explain this finding, including the liberation of chemical or physical cues for settlement during defouling, reduced predation and reduced competition for space. It is conceivable that calcareous remnants as observed in our study could also enhance recruitment.

Fouling remaining on the vessel following treatment may also provide refugia for a range of fouling taxa. For example, Floerl et al. (2004) showed that *Watersipora subtorquata* acts as a foundation species for fouling assemblages colonising toxic paint coatings. In this study, the presence of *W. subtorquata* facilitated the recruitment of an additional 22 sessile species (including barnacles, bivalves, ascidians, bryozoans and calcareous tubeworms taxa) compared with adjacent toxic patches. Similarly, in studies with the colonial ascidian *Didemnum vexillum*, recruitment appeared to be facilitated by the presence of barnacles on settlement plates, as greater recruitment was evident on barnacles than bare space (L. Fletcher, pers. comm.).

Hence, in terms of vessel risk management, recruitment to defouled surfaces may require consideration when this activity is undertaken in regions where pest species are known to be present, particularly during their reproductive season. Defouling a vessel immediately prior to departure may also act to reduce the extent of recolonisation by resident taxa.

4.2.3 Release of gametes, larvae or propagules

Yet another consideration is that the act of defouling may itself exacerbate biosecurity risk by stimulating the release of planktonic gametes, larvae or propagules (Figure 38) due to physical disturbance (ANZECC 1996). The potential for inducing a spawning event is not unrealistic given that physical disturbance is used to induce the release of gametes from several marine invertebrate taxa during experimental studies. For example, gametes from *Hydroides elegans* (a common fouling organism) are reliably obtained by breaking open the tube (Wong et al. 2006; Xie 2005). This potentially represents a high-risk mode of gamete release, given that tubes of polychaete taxa (including *H. elegans*) were often damaged by the rotating brush systems during plate trials in the present study. Brooding taxa (including various ascidian, polychaete, crustacean, anemone and oyster species) may also present a high risk, particularly if fully developed larvae are stripped (and not collected) from the organism during the defouling process. However, despite recognition of these release risks, the degree to which in-water defouling may exacerbate biosecurity risks relative to natural spawning cues (e.g. water temperature, salinity, food availability, light, tide/currents) remains unknown and is part of our ongoing research.

4.3 UTILITY OF THE BRUSH SYSTEMS IN MARINE BIOSECURITY

Effective marine biosecurity response ideally requires application of rapid and cost-effective tools that completely eliminate risks from new incursions. Based on the preceding discussion it is evident that the rotating brush systems trialled in our study are not suited to this purpose. For example, complete removal of fouling is difficult (especially as fouling biomass increases), and some organisms or life-stages are inherently resistant to mechanical effects. Furthermore, we reiterate a point made at the outset of this report that the two systems tested were not designed to clean niche areas of a vessel, such as sea chests, anode straps and gratings (Figure 39). Recent research has demonstrated that such areas can have a higher biomass and taxa richness (per unit area) than regular sections of a hull (Coutts & Dodgshun 2007, Coutts & Taylor 2004; Floerl et al. 2008). Cawthron is presently developing trials to test the efficacy of steam as a treatment method for sea chest areas as part of our FRST-funded OBI research. However, there remains the need for the development of alternative cleaning strategies for other niche areas on a vessel.

Figure 39: An example of a niche area of a vessel that would be inaccessible to rotating brush systems.

As a minimum therefore, for an effective biosecurity response, the application of brush systems would need to be supported by complementary methods for niche areas, or for resistant fouling on the main part of the hull. For such purposes, Wotton et al. (2004), for example demonstrated the efficacy of heat treatment of a sunken vessel hull for eliminating the microscopic gametophyte life-stage of the kelp *Undaria*. Probably the main disadvantage of these types of methods are that they are highly labour intensive. An emerging and arguably preferable approach to vessel treatment is *in situ* encapsulation using plastic wrapping. This method has now been trialled on vessels in many size categories (e.g. yachts, barges, merchant-size vessels), and is also effective for treating infected artificial structures (e.g. wharf piles, moorings, fish farming cages) fouled with pest species (Coutts & Forrest 2005, 2007; Denny 2007; Pannell & Coutts 2007). The method relies on the development of anoxic conditions in the water encapsulated by the wraps. Previous studies reveal that anoxia can take several days to develop and further work is required to clarify the factors that influence this so that treatment guidelines can be developed. Where necessary, mortality can be accelerated through the addition of eco-friendly (i.e. non-persistent) chemical agents such as acetic acid and bleach to the encapsulated seawater (Coutts & Forrest 2005). A major advantage of encapsulation methods is that risk organisms are contained once the wrap is in place, although fouling material may be detached during the wrapping process (Denny 2007). An additional major advantage is that the treatment is effective against the wrapped vessel or structure in its entirety, hence has the potential to eliminate all life-stages of organisms from the encapsulated area, including niche areas such as sea chests.

In the absence of a biosecure in-water cleaning option, removal of a fouled vessel to land (i.e. dry-docking or haul-out) is arguably the most desirable alternative. Land-based treatment provides the ability to prevent defouled material from re-entering the marine environment through the installation of barriers such as filters and containment tanks (Woods et al. 2007). Also, residual risks can be eliminated through the treatment of niche areas, and the enhanced elimination of microscopic life-stages, for example through passive desiccation. Furthermore, the reapplication of antifouling paint reduces subsequent recolonisation (Floerl & Inglis 2005).

Despite the limitations of the rotating brush systems in incursion response where complete elimination of pest organisms for quarantine purposes is required, these tools have applications in other circumstances. For example, in cases where a target pest species is already present in the port or region, successful removal of the majority of organisms may reduce the likelihood that the pest will be spread to non-infected regions. This is especially important in situations where natural barriers to spread arise and infected vessel movements represent the primary means of inter-regional transport across internal borders (Forrest et al. in press). Furthermore, when coupled with collection capabilities, only a relatively small mass of material is likely to be lost to the environment during defouling, thus vessel treatment is unlikely to significantly add to an existing pest population.

It should also be kept in mind that in-water hull cleaning has benefits beyond marine biosecurity incursion response. Hull fouling can reduce a vessel's speed due to a reduction in hydrodynamics and manoeuvrability, causing increased fuel and maintenance costs (Townsin 2003). In order to maintain/optimise fuel efficiency, many vessels routinely undertake in-water maintenance, typically in the form of hull scrubbing using devices similar to those trialled in this study (without the suction/collection capability). Rising fuel costs and copper-resistant organisms (Russell & Morris 1970; Ng & Keough 2003; Dafforn et al. 2008) are expected to result in a worldwide increase in the amount of in-water hull cleaning undertaken. The use of non-stick fouling release paint coatings, an alternative to biocidal paints (see Brady 1999 for a review), may also require the need for increased in-water maintenance (Brady 2001). A further consideration is that rotating brush systems may be useful in a range of other applications, such as defouling of oil platforms and marina pontoons.

Given the potential increase in demand for routine in-water hull cleaning, devices that retain defouled material should be favoured over non-collecting devices due to reduced environmental risks generally. Given that vessel hulls are not typically inspected for pest species prior to routine vessel maintenance, there is a risk that unwanted organisms or pest species may be present without the owners' knowledge. In such cases, the collection of defouled material by cleaning systems will almost certainly reduce the potential risk of incursion compared with a 'no collection' approach. In addition to biosecurity risk mitigation, the collection of defouled material reduces the amount of organic material discharged to the environment, of which a significant component may be viable, or lead to seabed enrichment from death and decay.

Collection-based systems have the added benefit that they reduce the mass load of antifouling contaminants that are typically prevalent in seabed sediments beneath vessel cleaning areas (Roberts & Forrest 1999; Srinivasan & Swain 2007; Dafforn 2008 and references therein). Of particular concern is the release of tributyltin (TBT) and derivative compounds which have a high ecotoxicity, and are still present on some vessels despite being regulated internationally since 1990 (Stewart et al. 1992; Svavarsson 2000). During ground-truthing trials on the *Pacific Wind*, we observed a temporary discolouration of the water, due to fine antifouling paint particles removed by the brush systems (Figure 40). A large amount of this paint material was collected by the systems, resulting in experimental collection bags (60 µm mesh) at the surface eventually becoming clogged. Several large chips (~ 2 x 0.5 mm) of paint were also captured by divers collecting defouled material lost to the environment during the trials.

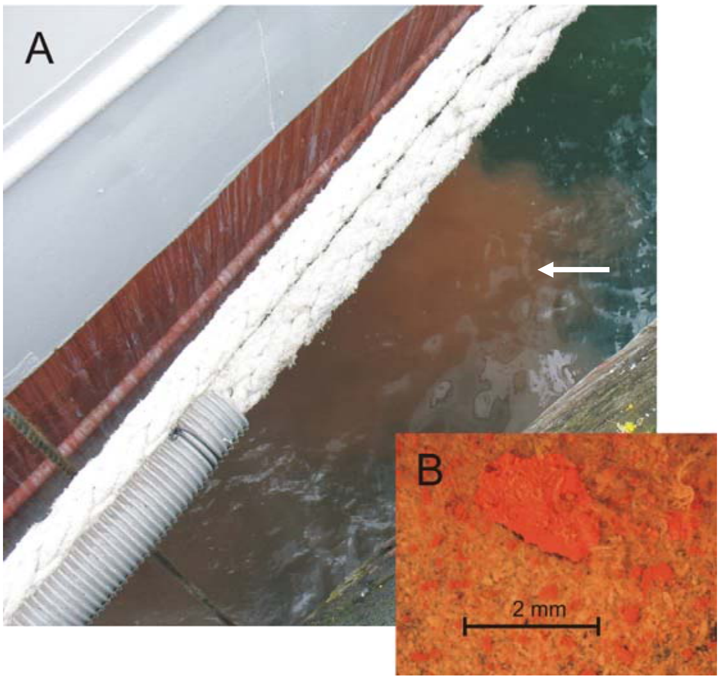

Figure 40: A (main photo): Discolouration of the water (arrow) by small paint particles during the defouling of the fishing vessel *Pacific Wind*. B (inset): photomicrograph of paint particles collected by divers during the defouling trials.

4.4 REGULATORY CONSIDERATIONS, PRACTICAL ISSUES AND COST OF TREATMENT

4.4.1 Regulatory considerations

Given that most incursion response operations are likely to occur with 12 nautical miles of the coastline[1], there is likely to be a requirement for operators to obtain a Resource Consent from the local Regional Council prior to treating a high risk vessel. At present, Diver Services Ltd and New Zealand Diving & Salvage Ltd have Resource Consents to operate their devices within the CMAs of the Greater Wellington and Auckland Regional Councils, respectively. A Ministerial decision can be made under Section 7A of the Biosecurity Act 1993 to treat a high-risk vessel immediately, prior to obtaining a Resource Consent from the relevant regional authority, but this approach would only be considered in extreme cases (P. Stratford, MAFBNZ, pers. comm.).

[1]12 nm is the extent of the New Zealand Coastal Marine Area (CMA), as defined under the Resource Management Act 1991.

4.4.2 Practical considerations and costs of treatment

Given the often short notice of a high risk vessel arriving in a port or region, it is important that effective incursion response tools can be mobilised quickly. Because of the infrequent use of the rotating brush systems trialled (in this study), both diving companies would ideally require at least one day to prepare and system-check the equipment prior to mobilisation (Matt Fabish & Tony Thew, pers. comm.). Both systems are relatively compact and can be transported around the country on a medium-sized truck (Figure 41). Infrastructure required to operate the brush systems is relatively modest. During the efficacy trials, equipment to operate the head unit (i.e. compressors, water pumps, hoses) was stationed at the surface on the wharf; however, both devices can also be setup and operated from onboard a medium sized vessel if required.

Costs associated with using rotating brush devices to treat a fouled vessel will vary with location, vessel size and degree of fouling, and operator availability. To give an indication of costs: personnel costs are likely to range between $600-$1000/day, hull defouling system hire $650-950/day, plus travelling and living costs (food and accommodation). Replacement filters/collection bags would be an additional charge, and would vary depending on the amount of defouling undertaken (refer to Appendix 1).

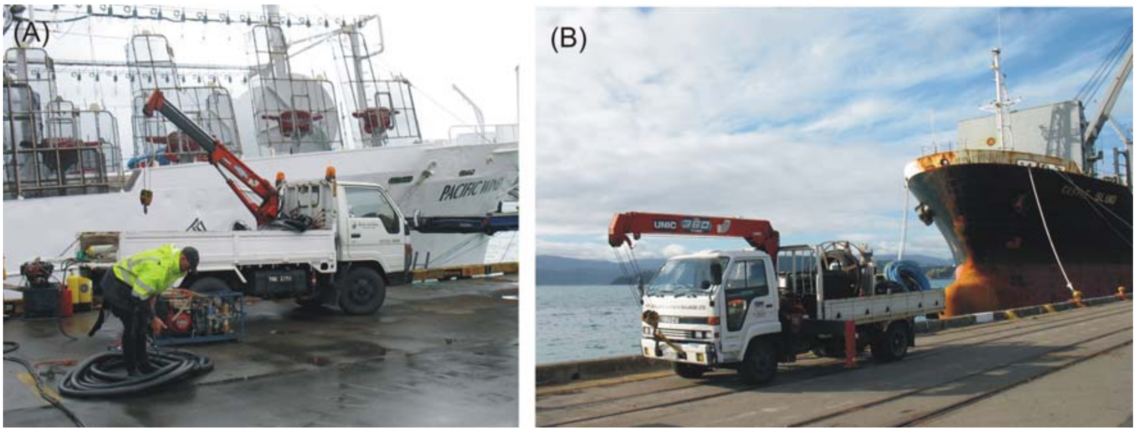

Figure 41: Rotating brush units used in the present study were transported to the dive site on small-medium sized trucks: (A) System A (developed by Diver Services Ltd), and (B) System B (developed by New Zealand Diving & Salvage Ltd).

5 Conclusions & future directions

The present study assessed the efficacy of two independently developed rotating brush systems in removing and retaining fouling material from experimental surfaces over a range of fouling levels and seasons. The main findings of this research were:

- Both systems were capable of removing up to 100% of cover/taxa richness from low-to-moderately fouled surfaces. However, as fouling became more advanced, mature hard/encrusting taxa were often resilient to the abrasive forces of the brushes.

- Both systems were capable of collecting a high proportion (> 95%) of defouled material. Of the material not collected, a wide range of taxa (including fragmented material) were lost to the environment, the fate of which is presently unknown.
- Efficacy trials on a fouled vessel highlighted the potential risk posed by unintentional detachment of fouling material by divers and the brush devices (and associated hoses).
- Ecological risks associated with treatment include (i) the release of defouled material (intact and fragmented organisms) to the environment, (ii) the potential release of gametes following physical disturbance of fouling communities, and (iii) residual risks. The latter includes remnant fouling on untreated surfaces (i.e. niche areas, missed patches), fouling resilient to brush treatment, and enhanced recolonisation of defouled surfaces.
- Release of antifouling paints, and to a limited extent fouling biomass (i.e. organic material), to the environment is also likely to occur during defouling. As such, the use of these devices will probably require a Resource Consent if undertaken within the CMA.

Incursion response tools need to be reliable, highly effective, mobile, available at relatively short notice, cost-effective and pose minimal risk to the wider environment. Both systems trialled in the present study meet most of these criteria. However, their inability to treat biosecurity risks posed by an entire vessel (e.g. niche areas, microscopic stages) limit their use in situations where complete elimination of risk (i.e. quarantine/elimination of a pest) is necessary. The brush systems could be used in combination with other response tools; however this may prove to be an inefficient approach. Other methods (e.g. encapsulation, dry docking or slipping, where feasible) are likely to achieve a more biosecure outcome.

Despite these limitations, the rotating brush systems represent one of the most biosecure in-water methods currently available to treat a low-to-moderately fouled vessel or structure that cannot be removed to land. For vessels that are expected to remain for only a short period of time (e.g. < 48 hours), it is likely that 'no treatment' is the lower risk management option than treating the vessel using the rotating brush systems that were tested. For vessels that intend to remain in a recipient port or region for an extended period of time (i.e. weeks to months), treatment risks associated with the rotating brush systems are potentially less than the biosecurity risks posed by leaving the vessel untreated, mainly due to reductions in fouling biomass (and hence inoculum pressure) following defouling. In such cases, alternative approaches (e.g. encapsulation) should also be considered.
Future research into the use of in-water methods should focus on gaining a better understanding of environmental factors affecting the survivorship of defouled material, the effects of cleaning disturbance on propagule release, and the colonisation of recently defouled surfaces by high risk species. The relative efficacy, costs and benefits of other in-water techniques (e.g. encapsulation) also need to be quantified. Gaining a better understanding of risks from in-water defouling methods, and the development of other in-water methods, will contribute to our existing biosecurity programmes. However, the effective management of hull fouling risks will ultimately require a broad suite of measures; including the development of specific management programmes for vessels visiting high value areas (e.g. Lewis et al. 2006), education and awareness among vessel operators; research to better understand the factors contributing to vessel risk; and targeted surveillance programmes for vessels or vessel types identified as high risk. Decisions on subsequent management options for high risk vessels will need to consider many factors, including the fouling

species present, level of fouling, residence time of a vessel in a recipient region, and risks from treatment (Hopkins & Forrest 2008).

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Appendix 1: Specifications of the rotating brush and pump system developed by New Zealand Diving & Salvage; including a summary of strengths, weaknesses and applications identified by NZDSL, as well as mobilisation and use charges.

Rotating brush unit	Divers Services Ltd	New Zealand Diving & Salvage Ltd
Make/model of unit	Phosmarine	Purpose built.
Brush type used during trials (bristle thickness and length)	406 mm wide brush head. Bristles are 50 mm long, 1mm in diameter and there are 36 bristle per sq. inch.	Commercial road sweeping brush head 35 mm long x 2 mm diameter.
Typical RPM during use	400 rpm.	700 rpm
Modifications to base unit purchased off the shelf	Collection shroud, suction lines, pump and collection bag	Whole unit purpose built.
Generator		
Make/model	Phosmarine (Hydraulic)	Purpose built
Minimum horsepower required	12 Hp	40 Hp
Pump unit		
Make/model	Stanley HP8	Casappa multi pump
Estimated flow rates (per minute)	1500 L	22.5 L
Filtration system		
Make/model of filters	The filtration system was designed by and manufactured for Diver Services Ltd.	FSI model BFNP12 316SS
Filter sizes available	30 to 1200 µm	1 µm upwards but normally 100 to 400 µm range.
Other considerations		
Cost of mobilisation (e.g. prep charges)	NZ$ 2000	Variable depending on location and timeframe
Day rates for cleaning a vessel (including personnel costs)	NZ$ 3000 to $6000, depending on size of crew.	Personnel rang from NZ$600 to $1000 per day per diver Hull cleaning gear costs range from $650 to 950 per day.
Do you think your system could be used on surfaces other than vessel hulls to remove fouling? If so, what types of surfaces?	This unit can be used for all kinds of recovery (e.g. dredged materials) as it has suction adaptors for use without the brush system.	On any steel structure with flat sides, as well as concrete surfaces such as slipways *etc*.
Does your system currently have any weaknesses that you are aware of, and can these be reduced/eliminated with further development?	The system is constantly being upgraded and many weaknesses have been eliminated as problems have arisen. The brush and pump unit are modified from standard equipment and purpose made would be more efficient.	The bulk of the suction hoses needs to be streamlined and the weight balanced.
Major strengths of your system	Easy to mobilise and transport to locations, large range of filter sizes available. The collection bags have been modified to complete a number of jobs and can be used for the collection of all kinds of materials.	Cleans very well.
Misc	We have notes that a similar system is now used for the collection of street rubbish	

Appendix 2: Outputs from statistical analyses.

A1: ANOVA examining differences in fouling biomass on flat and curved plates with increasing deployment time.

Effect	SS	df	MS	F	p
Intercept	2294618	1	2294618	757.0133	0.000000
Shape	94	1	94	0.0309	0.861052
Time	444214	3	148071	48.85	0.000000
Shape*Time	66485	3	22162	7.3113	0.000273
Error	193993	64	3031		

A2: Tukey's post-hoc test exploring statistical differences ($p<0.05$) in fouling biomass on flat and curved plates with increasing deployment time.

Cell no.	Shape	Time	{1} 46.122	{2} 203.22	{3} 309.89	{4} 150.29	{5} 100.37	{6} 123.22	{7} 278.11	{8} 216.94
1	Curved	3		0.000129	0.000127	0.003840	0.432365	0.075541	0.000127	0.000127
2	Curved	6	0.000129		0.002834	0.464452	0.004510	0.057088	0.092784	0.999496
3	Curved	9	0.000127	0.002834		0.000128	0.000127	0.000127	0.921656	0.014456
4	Curved	12	0.003840	0.464452	0.000128		0.540440	0.965832	0.000272	0.186952
5	Flat	3	0.432365	0.004510	0.000127	0.540440		0.986857	0.000127	0.000857
6	Flat	6	0.075541	0.057088	0.000127	0.965832	0.986857		0.000129	0.013235
7	Flat	9	0.000127	0.092784	0.921656	0.000272	0.000127	0.000129		0.280401
8	Flat	12	0.000127	0.999496	0.014456	0.186952	0.000857	0.013235	0.280401	

A3: Tukey's post-hoc test exploring statistical differences ($p<0.05$) in reductions of fouling biomass from flat and curved plates with increasing deployment time.

Cell no.	Time	Shape	{1} 92.800	{2} 89.600	{3} 81.667	{4} 89.800	{5} 73.500	{6} 71.300	{7} 83.567	{8} 36.067
1	3	1		1.000000	0.866195	1.000000	0.135042	0.059840	0.964764	0.000151
2	3	2	1.000000		0.990683	1.000000	0.361920	0.189153	0.999479	0.000151
3	6	1	0.866195	0.990683		0.988247	0.987797	0.916101	1.000000	0.000151
4	6	2	1.000000	1.000000	0.988247		0.343196	0.177092	0.999246	0.000151
5	9	1	0.135042	0.361920	0.987797	0.343196		1.000000	0.931816	0.000181
6	9	2	0.059840	0.189153	0.916101	0.177092	1.000000		0.768513	0.000249
7	12	1	0.964764	0.999479	1.000000	0.999246	0.931816	0.768513		0.000151
8	12	2	0.000151	0.000151	0.000151	0.000151	0.000181	0.000249	0.000151	
9	3	1	0.999995	1.000000	0.997458	1.000000	0.459529	0.255736	0.999935	0.000151
10	3	2	0.999910	1.000000	0.999570	1.000000	0.572725	0.343196	0.999997	0.000151
11	6	1	0.998989	1.000000	0.999972	1.000000	0.704207	0.463072	1.000000	0.000151
12	6	2	1.000000	1.000000	0.887383	1.000000	0.149749	0.067208	0.973151	0.000151
13	9	1	0.784438	0.973980	1.000000	0.968697	0.996422	0.959362	1.000000	0.000151
14	9	2	0.445458	0.787566	0.999995	0.768513	0.999997	0.999246	0.999479	0.000154
15	12	1	0.133484	0.358760	0.987333	0.340130	1.000000	1.000000	0.930173	0.000181
16	12	2	0.015584	0.058302	0.624600	0.053902	0.999836	1.000000	0.417844	0.000682

A3 (continued):

Cell no.	Time	Shape	{9} 88.633	{10} 87.600	{11} 86.400	{12} 92.500	{13} 80.700	{14} 77.533	{15} 73.467	{16} 68.033
1	3	1	0.999995	0.999910	0.998989	1.000000	0.784438	0.445458	0.133484	0.015584
2	3	2	1.000000	1.000000	1.000000	1.000000	0.973980	0.787566	0.358760	0.058302
3	6	1	0.997458	0.999570	0.999972	0.887383	1.000000	0.999995	0.987333	0.624600
4	6	2	1.000000	1.000000	1.000000	1.000000	0.968697	0.768513	0.340130	0.053902
5	9	1	0.459529	0.572725	0.704207	0.149749	0.996422	0.999997	1.000000	0.999836
6	9	2	0.255736	0.343196	0.463072	0.067208	0.959362	0.999246	1.000000	1.000000
7	12	1	0.999935	0.999997	1.000000	0.973151	1.000000	0.999479	0.930173	0.417844
8	12	2	0.000151	0.000151	0.000151	0.000151	0.000151	0.000154	0.000181	0.000682
9	3	1		1.000000	1.000000	0.999998	0.990683	0.868656	0.455996	0.084395
10	3	2	1.000000		1.000000	0.999957	0.997701	0.931816	0.569016	0.122984
11	6	1	1.000000	1.000000		0.999409	0.999730	0.974789	0.700684	0.185006
12	6	2	0.999998	0.999957	0.999409		0.811856	0.477508	0.148054	0.017722
13	9	1	0.990683	0.997701	0.999730	0.811856		1.000000	0.996250	0.728523
14	9	2	0.868656	0.931816	0.974789	0.477508	1.000000		0.999996	0.955839
15	12	1	0.455996	0.569016	0.700684	0.148054	0.996250	0.999996		0.999847
16	12	2	0.084395	0.122984	0.185006	0.017722	0.728523	0.955839	0.999847	

A4: ANOVA examining differences in the performance of the two rotating brush systems (System) in reducing fouling cover from flat and curved-shaped plates (Shape) with increasing deployment time (Time).

Effect	SS	df	MS	F	p
Intercept	303928.8	1	303928.8	5780.563	0.000000
System	250.7	1	250.7	4.768	0.036432
Time	4617.1	3	1539.0	29.272	0.000000
Shape	437.4	1	437.4	8.319	0.006962
System*Time	313.2	3	104.4	1.986	0.135911
System*Shape	318.8	1	318.8	6.063	0.019373
Time*Shape	1851.0	3	617.0	11.735	0.000024
System*Time*Shape	1015.7	3	338.6	6.440	0.001549
Error	1682.5	32	52.6		

A5: ANOVA examining differences in the performance of the two rotating brush systems (System) in reducing taxa richness from flat and curved-shaped plates (Shape) with increasing deployment time (Time).

Effect	SS	df	MS	F	p
Intercept	225433.5	1	225433.5	939.6580	0.000000
System	225.8	1	225.8	0.9410	0.339284
Time	11774.1	3	3924.7	16.3590	0.000001
Shape	1153.5	1	1153.5	4.8079	0.035716
System*Time	268.5	3	89.5	0.3731	0.772979
System*Shape	783.3	1	783.3	3.2649	0.080190
Time*Shape	6385.5	3	2128.5	8.8720	0.000200
System*Time*Shape	737.5	3	245.8	1.0247	0.394680
Error	7677.1	32	239.9		

A6: ANOVA examining differences in the performance of the two rotating brush systems (System) in reducing fouling cover from flat and curved-shaped plates (Shape) at different times of the year (Season).

Effect	SS	df	MS	F	p
Intercept	412403.8	1	412403.8	16295.98	0.000000
Season	1190.6	3	396.9	15.68	0.000002
System	4.4	1	4.4	0.18	0.678087
Shape	25.5	1	25.5	1.01	0.322809
Season*System	64.1	3	21.4	0.84	0.479663
Season*Shape	102.0	3	34.0	1.34	0.277840
System*Shape	15.0	1	15.0	0.59	0.447567
Season*System*Shape	34.0	3	11.3	0.45	0.720277
Error	809.8	32	25.3		

A7: ANOVA examining differences in the performance of the two rotating brush systems (Systems) in reducing taxa richness from flat and curved-shaped plates (Shape) at different times of the year (Season).

Effect	SS	df	MS	F	p
Intercept	400478.4	1	400478.4	4102.789	0.000000
Season	2207.4	3	735.8	7.538	0.000596
System	95.2	1	95.2	0.975	0.330763
Shape	462.5	1	462.5	4.738	0.036986
Season*System	575.9	3	192.0	1.967	0.138826
Season*Shape	486.0	3	162.0	1.660	0.195249
System*Shape	50.0	1	50.0	0.512	0.479272
Season*System*Shape	302.7	3	100.9	1.034	0.390806
Error	3123.6	32	97.6		

A8: ANOVA examining differences in the total mass of defouled material lost to the environment by the two rotating brush systems (System) treating flat and curved-shaped plates (Shape) with increasing deployment time (Time).

Effect	SS	df	MS	F	p
Intercept	48.20021	1	48.20021	92.28600	0.000000
Time	15.98229	3	5.32743	10.20011	0.000072
System	0.63021	1	0.63021	1.20662	0.280199
Shape	11.31021	1	11.31021	21.65497	0.000054
Time*System	6.37896	3	2.12632	4.07113	0.014757
Time*Shape	18.34229	3	6.11410	11.70629	0.000024
System*Shape	0.35021	1	0.35021	0.67052	0.418926
Time*System*Shape	4.00229	3	1.33410	2.55431	0.072736
Error	16.71333	32	0.52229		

A9: ANOVA examining differences in the viable mass of defouled material lost to the environment by the two rotating brush systems (System) treating flat and curved-shaped plates (Shape) with increasing deployment time (Time).

Effect	SS	df	MS	F	p
Intercept	0.350738	1	0.350738	59.02396	0.000000
Time	0.073549	3	0.024516	4.12574	0.013964
System	0.022912	1	0.022912	3.85573	0.058315
Shape	0.060840	1	0.060840	10.23853	0.003098
Time*System	0.019038	3	0.006346	1.06796	0.376437
Time*Shape	0.048297	3	0.016099	2.70924	0.061480
System*Shape	0.003480	1	0.003480	0.58562	0.449727
Time*System*Shape	0.018261	3	0.006087	1.02437	0.394830
Error	0.190154	32	0.005942		

A10: ANOVA examining differences in the total mass of defouled material lost to the environment by the two rotating brush systems (System) treating flat and curved-shaped plates (Shape) at different times of the year (Season).

Effect	SS	df	MS	F	p
Intercept	2.520833	1	2.520833	51.05485	0.000000
Season	1.075833	3	0.358611	7.26301	0.000754
System	0.030000	1	0.030000	0.60759	0.441422
Shape	0.040833	1	0.040833	0.82700	0.369939
Season*Operator	0.086667	3	0.028889	0.58509	0.629167
Season*Shape	0.242500	3	0.080833	1.63713	0.200245
Operator*Shape	0.163333	1	0.163333	3.30802	0.078313
Season*System*Shape	0.120000	3	0.040000	0.81013	0.497665
Error	1.580000	32	0.049375		

A11: ANOVA examining differences in the viable mass of defouled material lost to the environment by the two rotating brush systems (System) treating flat and curved-shaped plates (Shape) at different times of the year (Season).

Effect	SS	df	MS	F	p
Intercept	0.060947	1	0.060947	21.04227	0.000066
Season	0.012033	3	0.004011	1.38485	0.265184
System	0.016045	1	0.016045	5.53975	0.024896
Shape	0.010746	1	0.010746	3.71012	0.063006
Season*System	0.008812	3	0.002937	1.01414	0.399266
Season*Shape	0.004050	3	0.001350	0.46608	0.707979
System*Shape	0.006859	1	0.006859	2.36820	0.133661
Season*System*Shape	0.001758	3	0.000586	0.20237	0.893985
Error	0.092685	32	0.002896		

A12: ANOVA examining differences in the total mass lost to the environment by the two rotating brush systems (System) during efficacy trials on a fouled vessel.

Effect	SS	df	MS	F	p
Intercept	503.4443	1	503.4443	9.920873	0.010337
System	163.5851	1	163.5851	3.223609	0.102813
Error	507.4596	10	50.7460		

Appendix 3a: Organisms observed on flat settlement plates in relation to different seasons and levels of fouling (*=present, **=common, ***=abundant).

		Season				Time			
Taxa	**Common name**	**Winter**	**Spring**	**Summer**	**Autumn**	**3 mths**	**6 mths**	**9 mths**	**12 mths**
Unidentified sponge (Dendrilla-like)	Sponge			*			*	*	***
Unidentified hydroid (athecate)	Athecate hydroid		***				*		
Unidentified hydroid hydroid (thecate)	Thecate hydroid	***		*	*	***			
Unidentified hydroid (thecate)- Sertulariidae	Thecate hydroid								*
Unidentified bryozoan (encrusting)	Encrusting bryozoan	**	**	*	***	**	***	*	*
Watersiphora sp.	Encrusting bryozoan	*		*	**	*	*	*	
Unidentified bryozoan (erect)- leaf like	Erect bryozoan						**		
Unidentified bryozoan (erect)- branching	Erect bryozoan	**	**	***	**	**	***	***	**
Bugula sp.	Erect bryozoan	*	*	*	*	*	**		
Mytilus sp. (spat)	Blue mussel		*						
Ostrea chilensis (spat)	Flat oyster		*	*				*	*
Unidentified polychaete (Nereidae)	Polychaete		*						
Unidentified polychaete (Serpulidae)	Calcareous tubeworm								
Galeolaria hystrix	Calcareous tubeworm	*	**	*	*	*	***	***	***
Pomatoceros sp.	Calcareous tubeworm	*	*		***	*	*		*
Hydroides sp.	Calcareous tubeworm	*	*		**	*	*	*	*
Spirorbidae	Calcareous tubeworm	*	**		***	*	*	*	**
Unidentified solitary ascidian (juvenile)	Solitary ascidian	*	*		*	*			
Corella eumyota	Solitary ascidian	*	*			*			
Botrylloides sp	Colonial ascidian	*			**	*			*
Didemnum sp.	Colonial ascidian				**		*	**	**
Diplosoma listerianum	Colonial ascidian	**	**		***	**	*		*
Austrominius modestus	Barnacle	***	**	*	***	***	*	*	**
Unidentified macroalgae- 1 (juvenile)	Macroalgae		**		*		*	*	*
Unidentified macroalgae- 2 (juvenile)	Macroalgae		*						

Appendix 3b: Organisms observed on curved settlement plates in relation to different seasons and levels of fouling (*=present, **=common, ***=abundant).

		Season				Time			
Taxa	**Taxonomic Group**	**Winter**	**Spring**	**Summer**	**Autumn**	**3 mths**	**6 mths**	**9 mths**	**12 mths**
Unidentified sponge (Dendrilla-like)	Sponge				*		*	*	*
Unidentified hydroid (athecate)	Athecate hydroid		*						
Unidentified hydroid hydroid (thecate)	Thecate hydroid	**	*			**			
Unidentified hydroid (thecate)- Sertulariidae	Thecate hydroid								
Unidentified bryozoan (encrusting)	Encrusting bryozoan	**	**		***	**	***	***	*
Watersiphora sp.	Encrusting bryozoan	*		***	**	*	*		
Unidentified bryozoan (erect)- leaf like	Erect bryozoan			*	*		**		
Unidentified bryozoan (erect)- branching	Erect bryozoan	*	**		***	*	***	***	**
Bugula sp.	Erect bryozoan	*	*	***	**	*	***	*	
Mytilus sp. (spat)	Blue mussel		*	***			*		
Ostrea chilensis (spat)	Flat oyster	*				*	*	*	
Unidentified polychaete (Nereidae)	Polychaete			**					
Unidentified polychaete (Serpulidae)	Calcareous tubeworm								
Galeolaria hystrix	Calcareous tubeworm	**	*		*	**	**	***	**
Pomatoceros sp.	Calcareous tubeworm	*	*	*	*	*	*		
Hydroides sp.	Calcareous tubeworm	*	*		***	*	*	*	
Spirorbidae	Calcareous tubeworm	*	*		**	*		*	**
Unidentified solitary ascidian (juvenile)	Solitary ascidian	*	*	*		*			
Corella eumyota	Solitary ascidian	*	*			*			
Botrylloides sp	Colonial ascidian	*				*	*		*
Didemnum sp.	Colonial ascidian				**		*	**	**
Diplosoma listerianum	Colonial ascidian	*		**	*	*	**		
Austrominius modestus	Barnacle	***	*	**	***	***	***	***	*
Unidentified macroalgae- 1 (juvenile)	Macroalgae		*				*		*
Unidentified macroalgae- 2 (juvenile)	Macroalgae		*						

Appendix 4: Viable material lost during efficacy trials (1 of 4)

System	Shape	Deployment (months)	Rep	Athecate hydroid	Thecate hydroid	Barnacle	Algae (juv)	Gastropod	Blue mussel spat	Ribbed mussel spat	Other bivalve	Diplosoma sp.
B	Flat	3	1	0	0	1	0	0	0	0	0	0
B	Flat	3	2	0	0	1	0	0	0	0	0	0
B	Flat	3	3	1	0	1	0	0	0	0	0	0
B	Curved	3	1	1	1	1	0	0	0	0	0	0
B	Curved	3	2	1	0	1	0	0	0	0	0	1
B	Curved	3	3	0	0	1	0	0	0	0	0	0
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	1	0	0	0	0	1	0	1	1
A	Flat	3	3	1	0	0	0	0	0	0	0	1
A	Curved	3	1	0	0	1	0	0	0	0	0	1
A	Curved	3	2	0	0	0	1	0	0	0	0	0
A	Curved	3	3	0	0	0	0	0	0	0	0	1
B	Flat	3	1	0	0	0	0	0	0	0	0	0
B	Flat	3	2	0	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	0
B	Curved	3	1	0	0	0	0	0	0	0	0	0
B	Curved	3	2	0	0	0	0	0	0	0	0	0
B	Curved	3	3	1	0	0	0	0	0	0	0	0
B	Flat	6	1	0	0	0	0	0	1	0	0	0
B	Flat	6	2	1	0	0	0	0	1	0	0	0
B	Flat	6	3	1	0	0	1	0	1	0	0	0
B	Curved	6	1	1	0	0	0	0	0	0	0	1
B	Curved	6	2	0	0	1	0	0	1	0	0	1
B	Curved	6	3	0	0	0	0	0	1	0	1	0
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	1	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	0	0	0	0	0	0	0	0
A	Curved	3	2	1	0	0	0	0	0	0	0	0
A	Curved	3	3	1	0	1	0	0	0	0	0	0
A	Flat	6	1	1	0	0	0	0	0	0	0	0
A	Flat	6	2	1	0	0	0	0	0	0	0	0
A	Flat	6	3	1	0	0	1	0	1	0	0	0
A	Curved	6	1	0	1	0	0	0	1	0	0	1
A	Curved	6	2	1	0	0	0	0	1	0	0	1
A	Curved	6	3	1	0	1	1	0	1	0	0	1
B	Flat	3	1	1	0	0	1	0	0	0	0	0
B	Flat	3	2	1	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	0
B	Curved	3	1	0	0	0	0	0	0	0	0	1
B	Curved	3	2	0	0	1	0	0	0	0	0	0
B	Curved	3	3	0	0	1	1	0	0	0	0	0
B	Flat	9	1	1	0	0	1	0	0	0	0	1
B	Flat	9	2	0	0	0	0	0	1	1	0	0
B	Flat	9	3	1	0	0	1	1	1	1	0	0
B	Curved	9	1	1	0	0	0	0	1	1	0	0
B	Curved	9	2	0	0	0	0	0	0	0	0	0
B	Curved	9	3	0	0	1	0	0	1	1	0	0
A	Flat	3	1	1	1	0	0	0	1	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	0	0	0	0	0	0	0	0
A	Curved	3	2	0	0	0	0	0	0	0	0	0
A	Curved	3	3	0	0	0	1	0	0	0	0	1
A	Flat	9	1	0	0	0	0	0	0	0	0	0
A	Flat	9	2	0	0	0	1	0	0	0	0	0
A	Flat	9	3	1	0	0	0	0	0	0	0	0
A	Curved	9	1	1	0	0	0	0	1	1	0	0
A	Curved	9	2	1	1	0	0	0	1	1	1	0
A	Curved	9	3	1	0	1	0	0	0	0	0	0
B	Flat	3	1	0	0	0	0	0	0	0	0	0
B	Flat	3	2	0	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	0
B	Curved	3	1	0	0	1	1	0	0	0	0	0
B	Curved	3	2	0	0	0	0	0	0	0	0	0
B	Curved	3	3	0	0	1	0	0	0	0	0	1
B	Flat	12	1	1	0	0	0	0	0	0	0	0
B	Flat	12	2	0	0	0	0	0	0	0	0	0
B	Flat	12	3	1	0	0	1	0	0	0	0	0
B	Curved	12	1	1	0	0	1	1	0	0	0	0
B	Curved	12	2	1	0	0	1	0	0	0	0	1
B	Curved	12	3	1	0	0	0	0	0	0	0	0
A	Flat	3	1	1	0	0	1	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	1	0	0	0	0	0	0	0	0
A	Curved	3	2	0	0	0	0	0	0	0	0	0
A	Curved	3	3	0	0	0	0	0	0	0	0	0
A	Flat	12	1	1	0	0	0	0	0	0	0	0
A	Flat	12	2	0	0	0	1	0	0	0	0	0
A	Flat	12	3	0	0	0	1	0	0	0	0	0
A	Curved	12	1	0	0	0	0	0	0	0	0	0
A	Curved	12	2	0	0	0	0	0	0	0	0	0
A	Curved	12	3	0	0	0	0	0	0	0	0	0

Appendix 4 (continued): Viable material lost during efficacy trials (2 of 4)

System	Shape	Deployment (months	Rep	Didemnum sp.	Aplidium sp.	Crab larvae	Amphipod	Copepod	Isopod	Tanaid shrimp	Ostracod	Bryozoan (erect)
B	Flat	3	1	0	0	0	0	0	0	0	0	0
B	Flat	3	2	0	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	1
B	Curved	3	1	0	0	1	0	0	0	0	0	1
B	Curved	3	2	0	0	1	0	0	0	0	0	1
B	Curved	3	3	0	0	0	0	0	0	0	0	1
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	1
A	Flat	3	3	0	0	0	0	0	0	0	0	1
A	Curved	3	1	0	0	0	0	0	0	0	0	1
A	Curved	3	2	0	0	0	0	0	0	0	0	1
A	Curved	3	3	0	0	0	0	0	0	0	0	0
B	Flat	3	1	0	0	0	0	0	0	0	0	0
B	Flat	3	2	0	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	0
B	Curved	3	1	0	0	0	1	0	0	0	0	0
B	Curved	3	2	0	1	0	0	0	0	0	0	1
B	Curved	3	3	0	0	0	1	1	0	0	0	1
B	Flat	6	1	0	0	0	0	0	0	0	0	1
B	Flat	6	2	0	0	0	0	0	0	0	0	1
B	Flat	6	3	0	0	1	0	1	0	0	0	1
B	Curved	6	1	1	0	0	0	0	0	0	0	1
B	Curved	6	2	0	0	0	0	0	0	0	0	1
B	Curved	6	3	0	0	0	0	1	0	0	0	1
A	Flat	3	1	0	0	0	1	0	0	0	0	0
A	Flat	3	2	0	0	1	1	1	0	0	0	0
A	Flat	3	3	0	0	0	0	1	0	0	0	0
A	Curved	3	1	0	0	0	0	1	0	0	0	0
A	Curved	3	2	0	0	0	0	1	0	0	0	0
A	Curved	3	3	0	0	0	1	0	0	0	0	0
A	Flat	6	1	0	0	1	0	0	0	0	0	1
A	Flat	6	2	0	0	0	1	0	0	0	0	0
A	Flat	6	3	0	0	0	0	0	0	1	0	1
A	Curved	6	1	1	0	0	0	0	0	0	0	1
A	Curved	6	2	1	0	0	1	0	0	0	0	1
A	Curved	6	3	1	0	1	1	1	0	0	0	1
B	Flat	3	1	0	0	0	0	1	0	0	0	1
B	Flat	3	2	0	1	0	0	0	0	0	0	1
B	Flat	3	3	0	0	0	0	1	0	0	0	1
B	Curved	3	1	1	0	0	1	0	0	0	0	1
B	Curved	3	2	0	0	0	0	0	0	0	0	1
B	Curved	3	3	0	0	0	0	0	0	0	0	1
B	Flat	9	1	0	0	0	0	0	0	0	0	0
B	Flat	9	2	0	0	0	1	1	0	0	0	1
B	Flat	9	3	1	0	0	1	0	0	0	1	1
B	Curved	9	1	1	0	0	1	1	0	0	0	1
B	Curved	9	2	0	0	0	0	0	0	0	0	1
B	Curved	9	3	1	0	0	1	1	0	0	0	1
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	0	0	0	0	0	0	0	1
A	Curved	3	2	0	0	0	0	1	0	0	0	1
A	Curved	3	3	0	0	0	0	0	0	0	0	1
A	Flat	9	1	0	0	0	0	1	0	0	0	1
A	Flat	9	2	0	0	0	1	1	0	0	0	1
A	Flat	9	3	0	0	0	0	0	0	0	0	1
A	Curved	9	1	1	0	1	1	0	0	0	0	1
A	Curved	9	2	0	0	0	1	1	0	0	0	1
A	Curved	9	3	1	0	0	0	0	0	0	0	1
B	Flat	3	1	0	0	0	0	0	0	0	0	1
B	Flat	3	2	0	0	0	0	0	0	0	0	1
B	Flat	3	3	0	0	0	1	1	0	0	0	1
B	Curved	3	1	0	0	0	1	0	0	0	0	1
B	Curved	3	2	0	0	0	1	1	0	0	0	1
B	Curved	3	3	1	0	0	1	1	0	0	0	1
B	Flat	12	1	1	0	0	1	1	0	0	0	1
B	Flat	12	2	0	0	0	1	0	0	0	0	1
B	Flat	12	3	1	0	0	0	0	0	0	0	1
B	Curved	12	1	0	0	0	0	1	0	0	0	1
B	Curved	12	2	1	0	0	0	0	0	0	0	1
B	Curved	12	3	0	0	0	0	0	0	0	0	1
A	Flat	3	1	0	0	0	0	0	0	0	0	1
A	Flat	3	2	0	0	1	1	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	1
A	Curved	3	1	0	0	0	1	0	0	0	0	1
A	Curved	3	2	0	0	0	0	0	0	0	0	1
A	Curved	3	3	0	0	0	0	0	0	0	0	1
A	Flat	12	1	0	0	1	0	0	0	0	0	1
A	Flat	12	2	0	0	0	0	0	0	0	0	0
A	Flat	12	3	0	0	0	0	0	0	0	0	1
A	Curved	12	1	0	0	0	0	0	0	0	0	1
A	Curved	12	2	0	0	0	0	0	0	0	0	0
A	Curved	12	3	0	0	0	0	0	1	0	0	0

Appendix 4 (continued): Viable material lost during efficacy trials (3 of 4)

System	Shape	Deployment (months)	Rep	Bryozoan (flat)	Sponge	Fish egg	Gastropod egg	Nematode	*Galeolaria hystrix*	Pomatoceros sp.	Spirorbidae	Sabellidae
B	Flat	3	1	0	0	0	0	0	0	0	0	0
B	Flat	3	2	0	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	0
B	Curved	3	1	0	1	0	0	0	0	0	0	0
B	Curved	3	2	0	0	0	0	0	0	0	0	0
B	Curved	3	3	0	1	0	0	0	0	0	0	0
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	0	0	0	0	0	0	0	0
A	Curved	3	2	0	0	0	0	0	0	0	0	0
A	Curved	3	3	0	0	0	0	0	0	0	0	0
B	Flat	3	1	0	0	0	0	0	0	0	0	0
B	Flat	3	2	0	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	0
B	Curved	3	1	0	0	0	0	0	0	0	0	0
B	Curved	3	2	0	0	0	0	0	0	0	0	0
B	Curved	3	3	1	0	1	0	0	0	0	0	0
B	Flat	6	1	0	0	0	0	0	0	0	0	0
B	Flat	6	2	0	0	0	0	0	0	0	0	0
B	Flat	6	3	1	0	1	0	0	0	0	0	0
B	Curved	6	1	1	0	0	0	0	0	0	0	0
B	Curved	6	2	0	0	1	0	0	0	0	0	0
B	Curved	6	3	1	0	1	0	0	0	0	0	0
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	0	0	0	0	0	0	0	0
A	Curved	3	2	0	0	0	0	0	0	0	0	0
A	Curved	3	3	0	0	0	0	0	0	0	0	0
A	Flat	6	1	0	0	0	0	0	0	0	0	0
A	Flat	6	2	1	0	1	0	0	0	0	0	0
A	Flat	6	3	1	0	0	0	0	0	0	0	0
A	Curved	6	1	1	0	0	0	0	0	0	0	0
A	Curved	6	2	1	0	0	0	0	0	0	0	0
A	Curved	6	3	1	0	1	0	1	0	0	0	0
B	Flat	3	1	1	0	0	1	1	0	0	0	0
B	Flat	3	2	1	0	0	0	0	0	0	0	0
B	Flat	3	3	1	0	0	0	0	0	0	0	0
B	Curved	3	1	1	0	1	0	0	0	0	0	0
B	Curved	3	2	0	1	0	0	0	0	0	0	0
B	Curved	3	3	0	0	0	0	0	0	0	0	0
B	Flat	9	1	1	0	0	0	0	0	0	0	0
B	Flat	9	2	1	0	0	0	0	0	0	0	0
B	Flat	9	3	1	0	0	0	0	0	0	0	0
B	Curved	9	1	1	1	0	0	0	0	0	0	0
B	Curved	9	2	0	0	0	0	0	0	0	0	0
B	Curved	9	3	0	0	0	0	0	0	0	1	0
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	0	0	0	0	0	0	0	0
A	Curved	3	2	0	0	1	0	0	0	0	0	1
A	Curved	3	3	1	0	0	0	0	0	0	0	0
A	Flat	9	1	1	0	0	0	0	0	0	0	0
A	Flat	9	2	1	0	1	0	0	0	0	0	0
A	Flat	9	3	0	0	0	0	0	0	0	0	0
A	Curved	9	1	1	0	0	0	0	0	1	1	0
A	Curved	9	2	1	0	0	0	0	0	0	1	1
A	Curved	9	3	1	0	0	0	0	0	0	0	1
B	Flat	3	1	0	1	0	0	0	0	0	0	0
B	Flat	3	2	0	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	0
B	Curved	3	1	1	0	0	0	0	0	0	0	0
B	Curved	3	2	1	0	0	0	0	0	0	0	0
B	Curved	3	3	0	0	0	0	0	0	0	0	0
B	Flat	12	1	1	1	0	0	0	0	0	0	0
B	Flat	12	2	1	0	0	0	0	0	0	0	1
B	Flat	12	3	0	1	0	1	0	0	0	0	1
B	Curved	12	1	0	1	0	0	0	0	0	0	0
B	Curved	12	2	0	1	0	0	0	1	0	0	0
B	Curved	12	3	1	0	0	1	0	0	0	0	0
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	0	0	0	0	0	0	0	0
A	Curved	3	2	1	0	0	0	0	0	0	0	0
A	Curved	3	3	0	0	0	0	0	0	0	0	0
A	Flat	12	1	0	0	0	0	0	0	0	0	0
A	Flat	12	2	0	0	0	0	0	0	0	0	0
A	Flat	12	3	1	0	0	0	0	0	0	0	0
A	Curved	12	1	1	0	0	0	0	0	0	0	0
A	Curved	12	2	0	0	0	0	0	0	0	0	0
A	Curved	12	3	0	0	0	0	0	0	0	0	0

Appendix 4 (continued): Viable material lost during efficacy trials (4 of 4)

System	Shape	Deployment (months	Rep	Sphaerosyllus	Syllidae	Eunicidae	Nereidae	Spionidae	Phylodosidae	Armandia maculata	Hesionidae	Mite
B	Flat	3	1	0	0	0	0	0	0	0	0	0
B	Flat	3	2	0	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	0
B	Curved	3	1	0	0	0	0	0	0	0	0	0
B	Curved	3	2	0	0	0	0	0	0	0	0	0
B	Curved	3	3	0	0	0	0	0	0	0	0	0
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	0	0	0	0	0	0	0	0
A	Curved	3	2	0	0	0	0	0	0	0	0	0
A	Curved	3	3	0	0	0	0	0	0	0	0	0
B	Flat	3	1	0	0	0	0	0	0	0	0	0
B	Flat	3	2	0	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	0
B	Curved	3	1	0	0	0	0	0	0	0	0	0
B	Curved	3	2	0	0	0	0	0	0	0	0	0
B	Curved	3	3	0	0	0	0	0	0	0	0	0
B	Flat	6	1	0	0	0	0	0	0	0	0	0
B	Flat	6	2	0	0	0	0	0	0	0	0	0
B	Flat	6	3	0	1	0	0	0	0	0	0	0
B	Curved	6	1	0	0	0	0	0	0	0	0	0
B	Curved	6	2	0	0	0	0	0	0	0	0	0
B	Curved	6	3	1	1	0	0	0	0	0	0	0
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	0	0	0	0	0	0	0	0
A	Curved	3	2	0	0	0	0	0	0	0	0	0
A	Curved	3	3	0	0	0	0	0	0	0	0	0
A	Flat	6	1	0	0	0	0	0	0	0	0	0
A	Flat	6	2	0	0	0	0	0	0	0	0	0
A	Flat	6	3	0	0	0	0	0	0	0	0	0
A	Curved	6	1	0	0	0	0	0	0	0	0	0
A	Curved	6	2	0	1	0	0	0	0	0	0	0
A	Curved	6	3	0	1	0	0	0	0	0	0	0
B	Flat	3	1	0	0	0	0	0	0	0	0	0
B	Flat	3	2	0	0	0	0	0	0	0	0	0
B	Flat	3	3	0	0	0	0	0	0	0	0	0
B	Curved	3	1	1	0	0	0	0	0	0	0	0
B	Curved	3	2	0	0	0	0	0	0	0	0	0
B	Curved	3	3	0	0	0	0	0	0	0	0	0
B	Flat	9	1	0	0	0	0	0	0	0	0	0
B	Flat	9	2	0	0	0	0	0	0	0	0	0
B	Flat	9	3	0	0	0	0	0	0	0	0	0
B	Curved	9	1	1	0	1	0	0	0	0	0	0
B	Curved	9	2	0	0	0	0	0	0	0	0	0
B	Curved	9	3	0	0	0	0	0	0	0	0	0
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	0	0	0	0	0	0	0	0
A	Curved	3	2	1	0	0	0	0	0	0	0	0
A	Curved	3	3	0	0	1	0	0	0	0	0	0
A	Flat	9	1	0	1	0	0	0	0	0	0	0
A	Flat	9	2	0	0	0	0	0	0	0	0	0
A	Flat	9	3	1	1	0	0	0	0	0	0	1
A	Curved	9	1	1	1	0	0	0	0	0	0	0
A	Curved	9	2	0	1	0	0	0	0	0	0	0
A	Curved	9	3	0	1	0	1	0	1	0	0	0
B	Flat	3	1	0	0	0	0	0	0	0	0	0
B	Flat	3	2	1	0	0	0	0	0	0	0	0
B	Flat	3	3	1	0	0	0	0	0	0	0	0
B	Curved	3	1	1	0	0	0	0	0	0	0	1
B	Curved	3	2	1	0	0	0	0	0	0	0	0
B	Curved	3	3	0	0	0	0	0	0	0	0	0
B	Flat	12	1	1	0	0	0	0	0	0	0	0
B	Flat	12	2	0	0	0	0	0	0	1	1	0
B	Flat	12	3	1	1	0	0	0	0	0	0	0
B	Curved	12	1	1	1	0	0	0	1	1	0	0
B	Curved	12	2	1	0	0	0	0	0	0	0	0
B	Curved	12	3	1	0	0	0	0	0	0	0	0
A	Flat	3	1	0	0	0	0	0	0	0	0	0
A	Flat	3	2	0	0	0	0	0	0	0	0	0
A	Flat	3	3	0	0	0	0	0	0	0	0	0
A	Curved	3	1	0	1	0	0	1	0	0	0	0
A	Curved	3	2	0	0	0	0	0	0	0	0	0
A	Curved	3	3	0	0	0	0	0	0	0	0	0
A	Flat	12	1	1	0	0	0	0	0	0	0	0
A	Flat	12	2	0	0	0	0	0	0	0	0	0
A	Flat	12	3	0	0	0	0	0	0	0	0	0
A	Curved	12	1	0	0	0	0	0	0	0	0	0
A	Curved	12	2	0	0	0	0	0	0	0	0	0
A	Curved	12	3	0	0	0	0	0	0	0	0	0

Appendix 5: Viable material lost during ground-truthing exercise on the fishing vessel *Pacific Wind.*

	Sys A1	Sys A2	Sys A3	Sys A4	SysA5	Sys A6	Sys B1	Sys B2	Sys B3	Sys B4	Sys B5	Sys B6	Retained Sys A	Retained Sys B
Non-viable														
Detritus (muddy biogenic material)							A	A	A	A	C		VA	VA
Detritus	C	A	A	A	VA	A	A	A	A	A	A	C	A	A
Red paint fragments	C	A	A	A	A	A	A	A	A	A	C	C	A	A
Athecate hydroid (fragments)				L	L		C	L	L	L	VA		A	
Thecate hydroid (fragments)	C	A	A	A	VA	A	A			L			A	
Bugula sp. (fragments)	C	C	C	C	A			C	L				C	
Watersipora sp.								L	L	L	L			
Encrusting bryozoan												L		
Hesionidae (fragments)	L		L	L	L									
Syllidae (fragments)	L													
Red seaweed (fragments)				L										
Caprillidae (fragments)		L		L										
Diplosoma listerianum													L	
Nudibranch slug				L									L	
Potentially viable														
Copepod	A	A	A	A	VA	L	A	L	L	C		A	C	A
Amphipod		L	C	C	A	C	A	C	C	C	A	C	VA	C
Nematode	A	A	C	A	VA	A	L			L		A	VA	A
Tanaid shrimp							A	VA	VA	A		L	L	VA
Cumacean														L
Platyhelminthes													L	
Bugula sp. (fragments)						VA	VA			A	VA			
Caprillidae						L					L			
Syllidae							L						L	
Diplosoma listerianum				L										
Watersipora sp.							C							