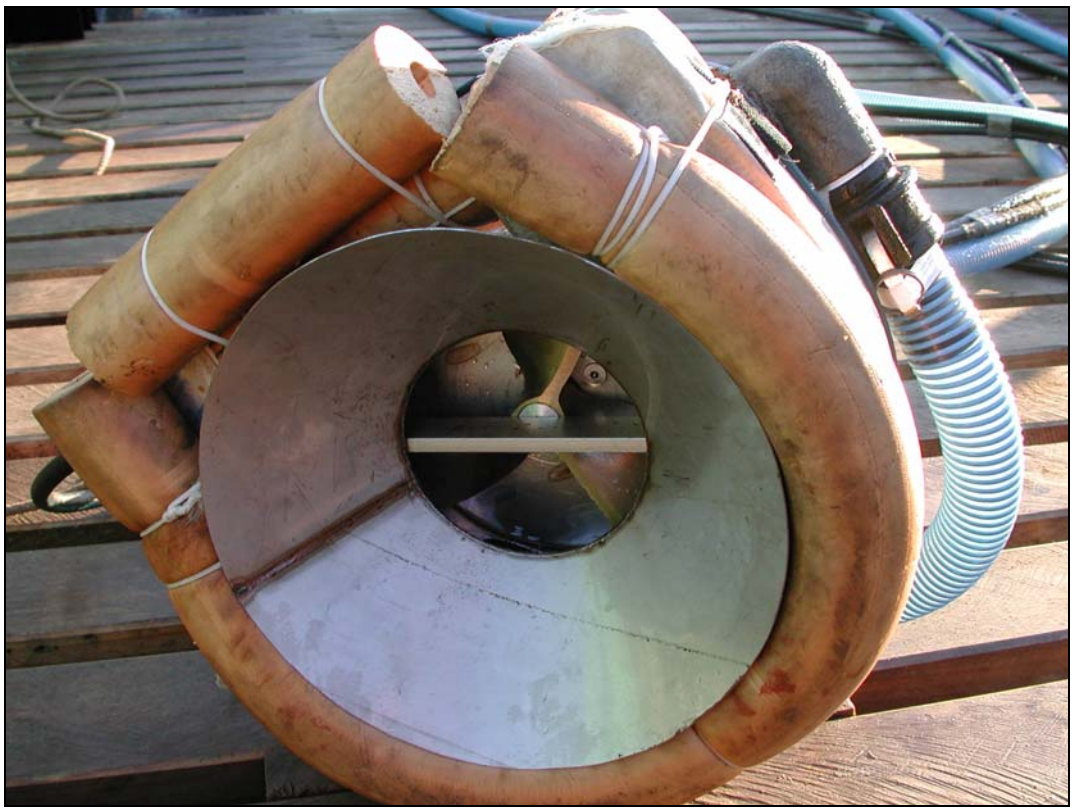
Cawthron Report No. 755

# The development of incursion response tools - underwater vacuum and filter system trials



Prepared for:

August 2002

# The development of incursion response tools – underwater vacuum and filter system trials

Prepared for

New Zealand Diving and Salvage Ltd

Prepared by

Ashley D. M. Coutts

Cawthron Institute
98 Halifax Street East
Private Bag 2
NELSON
NEW ZEALAND

Phone: +64-3-548 2319
Fax: +64-3-546 9464
Email: info@cawthron.org.nz



Cover Photo: The vacuuming cutting head designed to remove the bulk of the *Didemnum vexillum* from the hull of the 'Steel Mariner', Picton. (Photo courtesy of New Zealand Diving and Salvage Ltd).

## EXECUTIVE SUMMARY

In 1998 the Government released a strategy describing ways in which it would address marine biosecurity risks from ships and other vessels. The strategy stressed the need for an Import Health Standard for ship's ballast water and also directed the Department of Conservation and Ministry of Fisheries (MFish) to investigate means of controlling the threat from hull de-fouling.

The opportunity to design, test and document the efficacy of an underwater vacuum system for collecting and filtering de-fouled material from a vessel's hull presented itself in late December 2001. At this time, during a routine survey of Shakespeare Bay, Cawthron Institute divers noticed a heavily fouled steel barge, the 'Steel Mariner', moored west of Kaipupu Point, Picton. They observed a colonial ascidian or sea squirt, *Didemnum* sp., smothering the bottom of the barge and the seabed immediately below.

DNA sequence analysis undertaken by Dr Vicki Webb, National Institute of Water and Atmospheric Research (NIWA), confirmed that the *Didemnum* sp. on the barge and on the seabed surrounding it, was the same as the invasive ascidian found in Whangamata Harbour in October 2001. A world authority on ascidian taxonomy, Dr Patricia Mather (Queensland Museum, Australia), believed that the ascidian had never been described from anywhere in the world and she has subsequently named it *Didemnum vexillum* (Kott in press). Dr Mather believes that *D. vexillum* is indigenous to New Zealand and that it had had an extraordinary season due to favourable environmental conditions. However, the New Zealand Mussel Industry Council and the New Zealand Marine Farmers Association are particularly concerned about the possible spread of *D. vexillum* to marine farming areas in the Marlborough Sounds, where it has the potential to smother mussel lines.

On 16 July 2002, a group of stakeholders met and agreed to trial the use of an underwater vacuum device to remove the bulk of *D. vexillum* from the 'Steel Mariner's' hull and the seafloor below. In late July 2002, New Zealand Diving and Salvage Ltd (NZDS), Wellington, were commissioned by MFish to design, test and document the efficacy of an underwater vacuum system and filtering system for the removal of the bulk of the *D. vexillum* from the 'Steel Mariner' and the seabed below. Cawthron Institute was subsequently commissioned by NZDS to meet the following specific objectives:

- determine the reproductive state of *D. vexillum* colonies on the 'Steel Mariner' and the seabed below before any vacuuming commenced;
- determine whether *D. vexillum* releases larvae when mechanically disturbed by the vacuuming operation;
- determine the particle dynamics during vacuuming (*i.e.* material being expelled into the water around the vacuum heads);
- test the filtering effectiveness of various sized filter liners and bags (200, 100, 50, 25, 10, 5 and 1 μm);
- undertake a delimitation survey of *D. vexillum* on the seabed surrounding the 'Steel Mariner';
- and determine the wet biomass weight of *D. vexillum* removed from the 'Steel Mariner' during the vacuuming operation.

It was found that although the *D. vexillum* colonies possessed predominantly developing embryos

and undeveloped larvae, a variety of stages of larval development were observed in the basal or central test core. However, the presence of the occasional mature larvae suggests that the species is either approaching spawning time as the water temperatures increase or that the species develops some mature larvae all year around.

The original vacuum cutting head configuration was not effective at removing the *D. vexillum* colonies. The diver-operated nozzle proved to be both a very selective and efficient method for removing a wide size range of *D. vexillum* colonies from the hull. The divers completed vacuuming the bulk (approximately 473 kgs) of the *D. vexillum* as well as other fouling organisms from the hull of the 'Steel Mariner' in just two days (2-3 August 2002). A post-vacuuming quantitative survey revealed that the vacuuming operation removed an estimated 80% of the original *D. vexillum* wet biomass weight. Approximately 200 g of *D. vexillum* was hand-scraped from the hull of the support barge the 'Waimarie I'.

It took more than two days to achieve an approximate 75% clean-up of the *D. vexillum* from within a 7-10 m wide strip from the eastern to the western boundary (approximately 70 m long). It was decided, therefore, that the vacuuming operation would cease and a re-evaluation of the seabed was undertaken. Approximately 147 kgs of wet biomass weight of *D. vexillum* was removed from the seabed, which also included *D. vexillum* as well as other fouling organisms collected at Stage 1 of the filtering process. The distribution of the *D. vexillum* on the seabed below the 'Steel Mariner' was defined as: 71.1 m long on the western boundary; 76.9 m on the eastern boundary; 28.60 m on the shoreline; and 67.60 m at the seaward (*i.e.* deeper) boundary.

Nine filter sizes and configurations (mussel bag, 200, 100, 50, 25, 10, 5, BOS5 and 1µm) were tested over 5 days of the vacuuming operation. The filtering plant designed and used by NZDS clearly illustrated that de-fouled material can be successfully filtered to 50 µm. Given that the trunk of the *D. vexillum* larvae is approximately 300 µm across, there was little likelihood that the filtering process would have enabled the release of viable larvae back into the environment. This was further verified by the fact that no *D. vexillum* larvae were found amongst the Stage 3 effluent samples.

It is recommended that the system be tested during a merchant vessel's routine hull cleaning operation to determine a) what filter sizes (e.g. 200, 100, 50, 25, 10, 5 or 1 µm) are practical, b) whether or not the system is capable of accommodating the flow rates necessary to undertake a hull cleaning operation within an allocated timeframe, and c) determine whether or not it is a practical tool for removing, collecting and filtering de-fouled material within areas protected from strong laminar flows.

It is also recommended that a simple procedure be developed for on-site testing of the size of the particulate matter in the effluent. Hull cleaning operators could then ascertain whether or not their filtering system is performing correctly.

An estimated 120 kgs of the *D. vexillum* remains on the hull of the 'Steel Mariner' and the seabed below. The biomass of these remaining colonies is likely to increase, and the ascidian is likely to reach sexual maturity, as the water temperatures increase during the following months. It is strongly recommended, therefore, that a stakeholder meeting be arranged as soon as possible to discuss management options for the treatment of the 'Steel Mariner' and the seabed.

# TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF FIGURES

## LIST OF FIGURES CONTINUED.

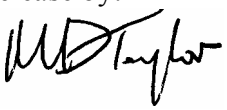


---

Report reviewed by:

Dr Michael Taylor
(Cawthron Institute, Biosecurity Research Manager).

Approved for release by:



Dr Michael Taylor
(Cawthron Institute, Biosecurity Research Manager).

# 1.0 INTRODUCTION

The introduction of non-indigenous marine species (NIMS) into new areas can have major impacts on: our natural marine ecosystems; commercial; recreational and customary fisheries; shipping; marine recreational and amenity values; and human health. Cranfield et al. (1998) stated that around 150 NIMS have been introduced to New Zealand and the total number is likely to be higher. However, only a relatively small number of these including the Japanese seaweed *Undaria pinnatifida*, the Asian mussel *Musculista senhousis*, the sea squirt *Ciona intestinalis* and the encrusting tubeworm *Ficopomatus enigmatica* are considered to have reached pest status at this point in time. There are also, however, a large number of cryptogenic (*i.e.* origin unknown) marine species and many other potential marine pests not yet thought to be established in New Zealand; *e.g.*, the Mediterranean fanworm *Sabella spallanzanii*, the European shore crab *Carcinus maenas*, the northern Pacific seastar *Asterias amurensis*, the Chinese mitten crab *Eriocheir sinensis*, the green seaweed *Caulerpa taxifolia* and the Asian clam *Potamocorbula amurensis*.

NIMS can arrive in New Zealand and be transported around the coast via several intentional and unintentional pathways including hull fouling, ballast water, mariculture and the aquarium trade. Interestingly, Cranfield et al. (1998) and Thresher et al. (1999) both stated that hull fouling on vessels has been responsible for introducing around 75% of the NIMS to both New Zealand and Port Phillip Bay, Australia, respectively.

Fouling organisms may be transferred from a vessel's hull to the marine environment by propagation, or fall off or be scraped off when a vessel bumps against a wharf. Vessel owners also often undertake hull cleaning activities, which also can result in the introduction of NIMS to new locations.

In 1998 the Government released a strategy describing ways in which it would address marine biosecurity risks from ships and other vessels. The strategy stressed the need for an Import Health Standard for ships' ballast water and also directed the Department of Conservation and Ministry of Fisheries (MFish) to investigate means of controlling the threat from hull de-fouling. MFish is New Zealand's leading marine biosecurity agency and proposes to minimise the biosecurity risks posed by hull cleaning activities in the coastal environment by putting in place regulations under the Biosecurity Act 1993. The proposed regulations would:

- require collection and containment of fouling material removed from hulls;
- control the disposal of material from hulls cleaned in or near the coastal marine area;
- and require treatment of any discharge resulting from hosing, water blasting or in-water cleaning of hulls to remove organisms, spores, or fragments of organisms before the water is discharged into the coastal marine area.

In-water cleaning of hulls (by divers or using remotely operated equipment) is considered a special case and may require additional controls. In March 2001, MFish commissioned Kingett Mitchell and Associates Ltd to undertake a desk-top study to establish a standard for hull cleaning. The standard aimed to minimise the chances of exotic organisms being returned to the coastal marine area in effluent from hull cleaning to the greatest extent practicable. The research recommended that the standard be based on a minimum particle size so as to prevent viable fragments of plants or

animals, spores or other reproductive material being discharged into the marine environment. It was recommended that all particles above 60 µm be collected or filtered from cleaning water and disposed of safely to land (McClary 2001).

The opportunity to design, test and document the efficacy of an underwater vacuum system for collecting and filtering de-fouled material from a vessel's hull presented itself in late December 2001. At this time, during a routine survey of Shakespeare Bay, Cawthron divers noticed a heavily fouled steel barge, the 'Steel Mariner', moored west of Kaipupu Point, Picton. They observed a colonial ascidian or sea squirt, *Didemnum* sp., smothering the bottom of the barge and the seabed immediately below. A biosecurity investigation of the barge, including its voyage history, is presented in Coutts (2002) and a brief overview of relevant findings is given below.

DNA sequence analysis undertaken by Dr Vicki Webb, NIWA, confirmed that the *Didemnum* sp. on the barge and on the seabed surrounding it, was the same as the invasive ascidian found in Whangamata Harbour in October 2001. A world authority on ascidian taxonomy, Dr Patricia Mather (Queensland Museum, Australia), believed the ascidian had never been described from anywhere in the world and she has subsequently named it *Didemnum vexillum* (Kott in press). Dr Mather believes that *D. vexillum* is indigenous to New Zealand and that it had had an extraordinary season due to favourable environmental conditions (Mather 2002).

Coutts (2002) estimated the total wet biomass of the *D. vexillum* on the barge to be 2,923±628 kgs with a further 460±180 kgs on the seabed within an estimated 40 x 80 m area surrounding the barge. Furthermore, a second barge moored next to the 'Steel Mariner', the 'Waimarie I', has also recently been colonised by what also appears to be *D. vexillum*. This barge is also a biosecurity risk as it has recently transported Greenshell™ mussels from East Bay in the outer Queen Charlotte Sound to Picton and has been towed to Napier, North Island. The New Zealand Mussel Industry Council and the New Zealand Marine Farmers Association are particularly concerned about the possible spread of *D. vexillum* to marine farming areas in the Marlborough Sounds, where it has the potential to smother mussel lines.

On 16 July 2002, a group of stakeholders met and agreed to trial the use of an underwater vacuum device to remove the bulk of *D. vexillum* from the 'Steel Mariner's' hull and the seabed below. In late July 2002, New Zealand Diving and Salvage Ltd (NZDS), Wellington, were commissioned by MFish to design, test and document the efficacy of an underwater vacuum and filter system for the removal of the bulk of the *D. vexillum* from the 'Steel Mariner', the 'Waimarie I' and the seabed below. Cawthron was subsequently commissioned by NZDS to meet the following specific objectives:

- determine the reproductive state of *D. vexillum* colonies on the 'Steel Mariner' and the seabed below before any vacuuming commenced;
- determine whether or not *D. vexillum* releases larvae when mechanically disturbed by the vacuuming operation;
- determine the particle dynamics during the vacuuming operation (*i.e.* material being expelled into the water around the vacuum heads);
- test the filtering effectiveness of various sized filter liners and bags (200, 100, 50, 25, 10, 5 and 1 µm);
- undertake a delimitation survey of *D. vexillum* on the seabed surrounding the 'Steel Mariner';

- and determine the wet biomass weight of *D. vexillum* removed from the 'Steel Mariner' during the vacuuming operation.

# 2.0 METHODS

## 2.1 *D. vexillum* reproductive status

On 30 July 2002, prior to the commencement of any vacuuming, approximately five *D. vexillum* colonies (approximately 500 g) were randomly collected from the hull of the 'Steel Mariner' and the seabed below. The colonies were placed in separate 10 L buckets containing approximately 8 L of seawater. Five 30 g pieces of *D. vexillum* were randomly selected from each bucket and placed in petri dishes containing distilled water for closer examination. The colonies were dissected cross-sectionally at various locations along the entire length of the colonies using a scalpel and forceps. The *D. vexillum* cross-sections were viewed on-site using a Nikon SMZ-1B microscope to observe and document their reproductive status. A micrometer graticule was used to calibrate the microscope eye piece graticule at various levels of magnification (x 40, 100 and 200), and the size (width and length) of the *D. vexillum* embryos and larvae was recorded. These measurements were then used to define the minimum filter size to be used in the filtering system during the vacuuming operation (see below). Tearing away the outer skin of the colonial test longitudinally using forceps proved to be the most efficient way of searching for the presence of embryos and larvae.

The remaining *D. vexillum* colonies not used for dissection were relaxed in a menthol/ethanol/seawater solution for one hour then preserved in 5% Formalin and 95% seawater. These specimens were later dissected at Cawthron and viewed under an Olympus SZH10 stereo microscope. Embryos and developing larvae were photographed using a microscope-mounted Nikon Coolpix 995 digital camera.

## 2.2 *D. vexillum* response to the vacuuming operation

Before any vacuuming commenced, a further five *D. vexillum* colonies (approximately 500 g) were randomly collected from both the 'Steel Mariner' and the seabed below. The samples were placed in separate 10 L buckets containing approximately 8 L of sterilized filtered seawater (38.4 ppt at 14.2$^{o}$C). The colonies in each bucket were mechanically disturbed using two hands to squeeze and tear them in an attempt to replicate the action of the proposed vacuuming operation. Approximately 15 minutes after being disturbed, 75 ml sterile plastic vials were used to collect water samples from the surrounding water inside each of the buckets. The ten seawater samples were viewed on site using the microscope described above to determine if any propagules were released. These observations were used to help establish the vacuuming technique required for removing the *D. vexillum* colonies from the 'Steel Mariner' and the seabed below. Water samples were preserved in 5% Formalin for archiving at Cawthron.

Also on 30 July 2002, before the vacuuming operation had started, a further experiment was undertaken to determine if any larvae that might have been released as a result of the operation were capable of settling. Twelve household bricks, which had been pre-conditioned in the marine environment for three weeks, were used as artificial settlement surfaces. The bricks were pre-

labelled using cable ties and tags, and placed separately inside 10 L plastic buckets with the lids placed on firmly. Three buckets were taken underneath the 'Steel Mariner' using SCUBA and the lids removed within 30 cm of the *D. vexillum* colonies, which allowed the surrounding water to fill the buckets. The lids were then replaced firmly and the buckets placed nearby on the seabed at 3 m depth. These replicates will be viewed over the next six months to establish whether or not *D. vexillum* larvae might have been present in the water column prior to the vacuuming operation.

Another three buckets, each with lids and a pre-conditioned brick inside, were taken underneath the barge using SCUBA, and approximately 500 g of *D. vexillum* was gently removed from the hull and placed inside each one. The lids were then replaced firmly, and the buckets transported to the seabed and placed next to the others. These replicates controlled for the release of *D. vexillum* larvae in the absence of any significant disturbance effects. A final three buckets were then taken underneath the barge and approximately 500 g of *D. vexillum* placed inside each one. The *D. vexillum* was mechanically disturbed as described above, the lids replaced firmly, and the buckets transported to the seabed and placed next to the others. These replicates will be viewed over the next six months to determine if the *D. vexillum* released any larvae as a result of the mechanical disturbance and, if so, whether or not they were capable of settling.

After 24 hours, the buckets were transported to an area on the seabed outside of the boundary of the *D. vexillum* population. Each of the bricks used for the control described above were then removed from the buckets and tied to a stainless steel frame using rope, approximately 500 cm from the seabed. A further three pre-conditioned bricks were suspended from the frame to control for any natural *D. vexillum* settlement that might occur over the following six months (*i.e.* during the monitoring phase).

Five 75 ml sterile plastic vials were also used to collect water samples from the immediate area surrounding a number of the *D. vexillum* colonies hanging from the hull of the 'Steel Mariner'. These samples were viewed on-site using the microscope described above to determine if any *D. vexillum* larvae might have been present in the water column prior to the commencement of the vacuuming operation. The samples were then preserved with 5% Formalin for archiving at Cawthron.

## 2.3 The vacuuming operation

NZDS designed and constructed an underwater vacuum system for the operation. The diver operated system was hydraulic powered, and had a vacuum cutting head to capture and shred the *D. vexillum* before pumping it into the filtration system, which was mounted on the 'Waimarie 1' (support barge) (Figure 1). Within an hour of being deployed, however, the diver was not able to manoeuvre the vacuum cutting head up the hanging *D. vexillum* colonies as expected. Therefore the divers proceeded to hand pick the colonies from the hull and place them into catch bags. The divers then carefully fed the colonies into the vacuum cutting head, which was suspended nearby using a rope. On 3 August 2002 the cutting head was replaced with a simple diver-operated nozzle (Figure 2). This nozzle was used for the remainder of the vacuuming operation on the 'Steel Mariner' and the seabed below.

On 6 August 2002, Cawthron staff used a paint scraper and catch bag to remove visible *D. vexillum* colonies from the hull of the 'Waimarie 1'. These specimens were relaxed in

menthol/ethanol/seawater for one hour then preserved in 5% Formalin and 95% seawater for archiving at Cawthron.

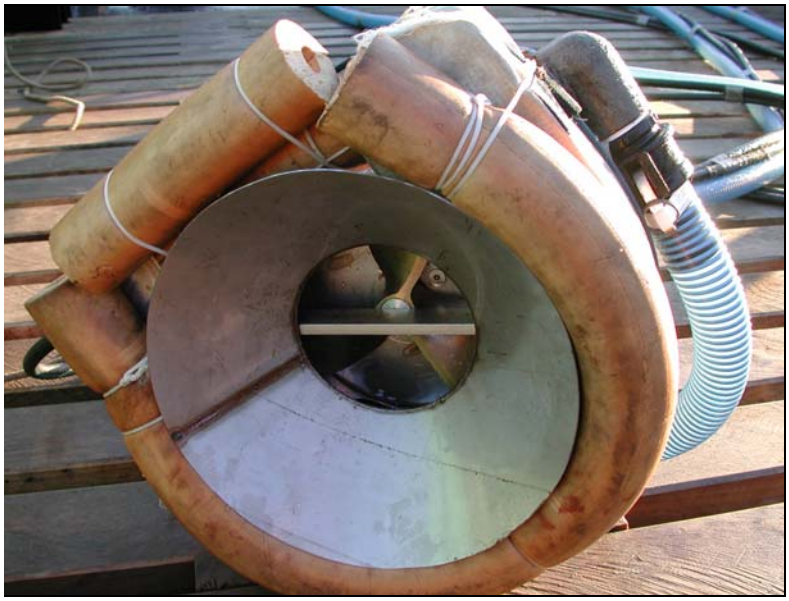


**Figure 1.** The original vacuum cutting head used to remove the *D. vexillum* from the 'Steel Mariner'. Photo courtesy of New Zealand Diving and Salvage Ltd.

**Figure 2.** The diver-operated nozzle used to vacuum the *D. vexillum* colonies from the 'Steel Mariner' and the seabed below. Photo courtesy of New Zealand Diving and Salvage Ltd.

## 2.4 *D. vexillum* delimitation survey

Two divers surveyed the seabed surrounding the 'Steel Mariner' to determine the outer boundary of the *D. vexillum* population. The divers surfaced at each of the boundary corners and personnel in a runabout deployed weights attached to buoys. Divers later repositioned the location of these weights according to the exact locations of the boundary corners. This exercise assisted the operations manager to determine the boundary of the *D. vexillum* in relation to the position of the 'Steel Mariner', and enabled a lead core polypropylene rope to be used to mark out the *D. vexillum* boundary on the seabed. Divers used metal pegs to staple the lead core rope to the seabed. During the vacuuming operation divers used a second rope, which was attached to the lead core rope, as a guide for systematically vacuuming the *D. vexillum* from the seabed. Divers used a measuring tape to measure the four dimensions of the boundary. The marked boundary was left in position to assist Cawthron divers with future monitoring of the *D. vexillum* distribution on the seabed.

## 2.5 The filtering process

*D. vexillum* and seawater that was also vacuumed during the operation, were treated using three filtering Stages; 1, 2 and 3 (Figures 3 and 4). All vacuumed material was passed through a Sandpiper flapper valve water pump into Stage 1, where a mussel bag and a 200 µm filter liner captured all gross-sized de-fouled material (Figure 5). The filtered effluent then flowed into a surrounding 1 mm thick impermeable polypropylene bag (Ultraflex) supported inside an aluminium frame. The effluent from Stage 1 then gravity fed into Stage 2, where 200 and 100 µm filter liners were tested (Figures 3, 4 and 6). The effluent then flowed into a surrounding 1 mm thick impermeable polypropylene bag (Ultraflex), supported by an aluminium frame. The effectiveness of Stages 1 and 2 at capturing the *D. vexillum* was tested using the procedures described in section 2.7.

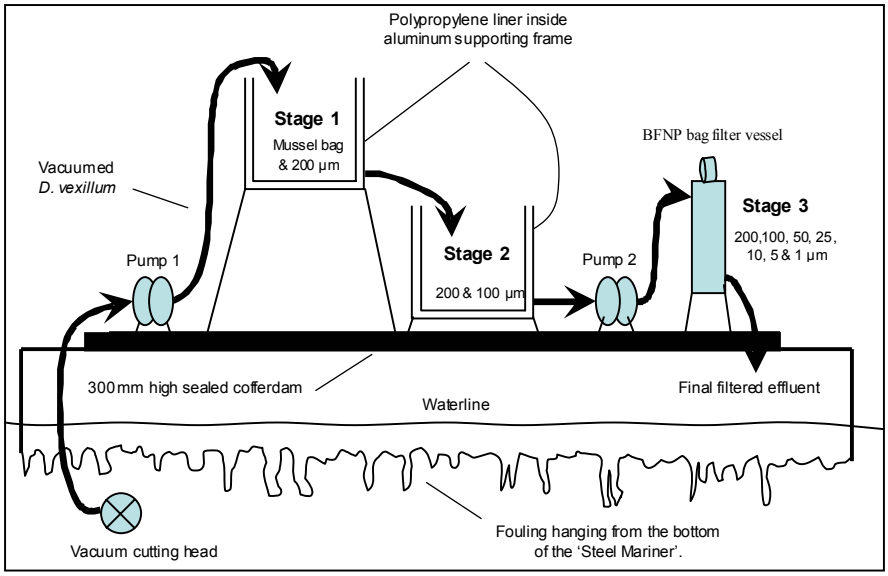



**Figure 3.** The vacuumed *D. vexillum* and seawater passed through three main stages of filtration where various sized filters were tested.

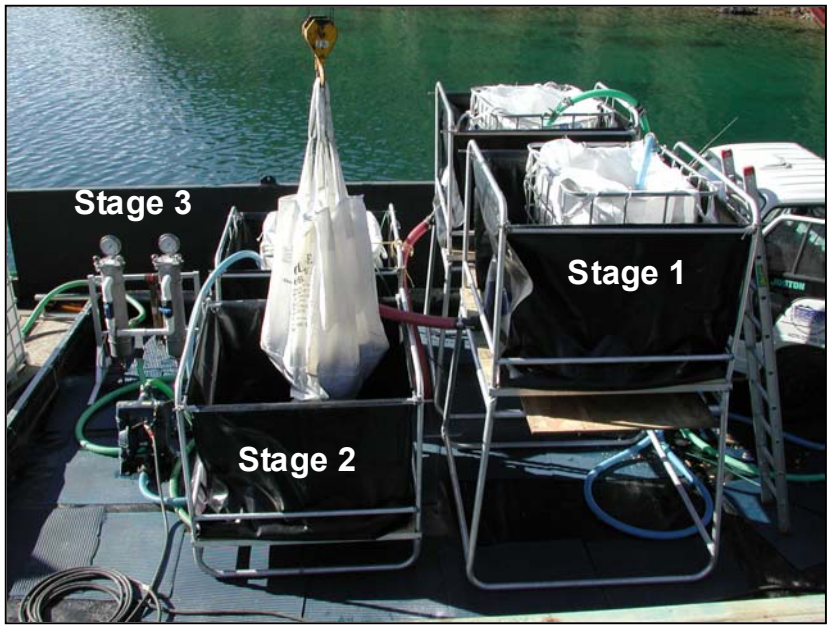



**Figure 4.** The layout of the three filtering stages used during the vacuuming operation. Photo courtesy of New Zealand Diving and Salvage Ltd.

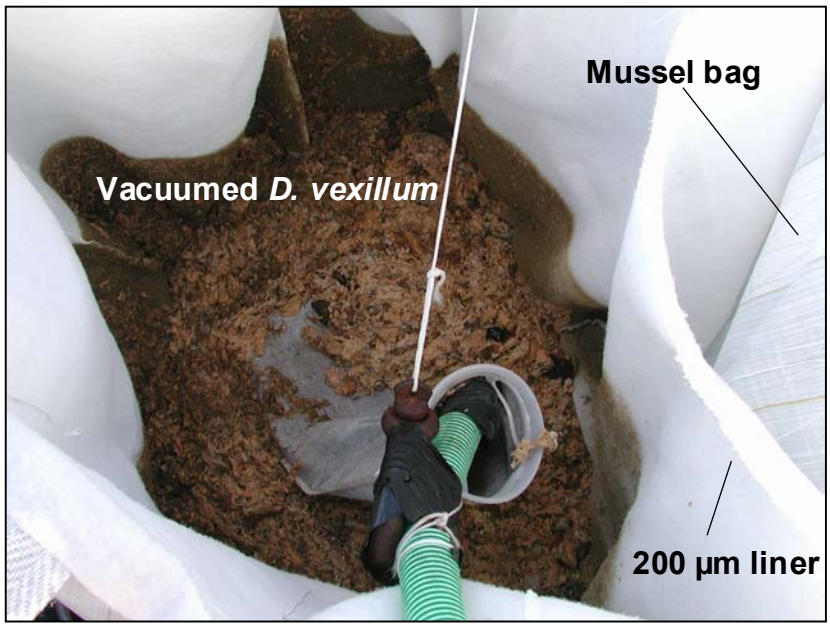



**Figure 5.** Stage 1 of the filtering process consisted of capturing gross sized de-fouled material inside a 200 µm filter liner, which was held within a mussel bag supported by an aluminium frame.

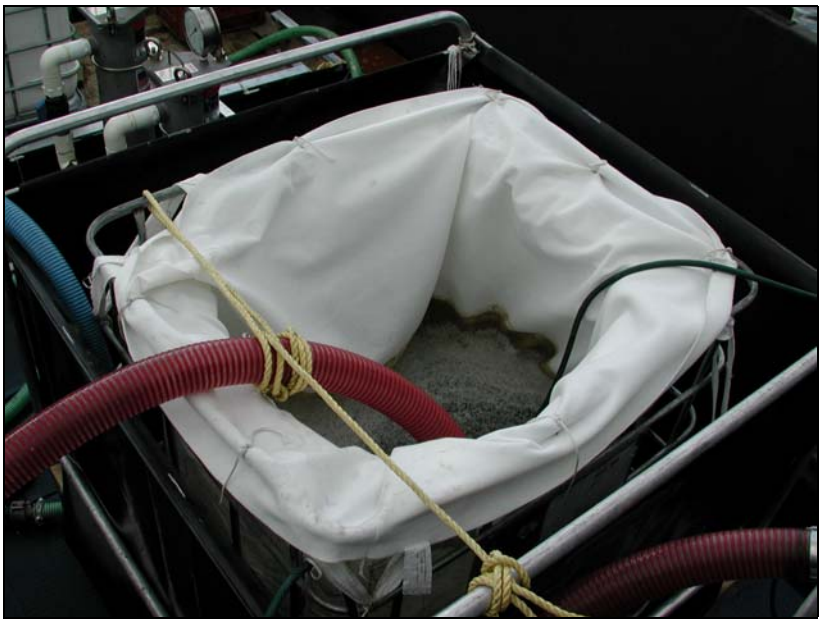


**Figure 6.** The effluent from Stage 1 gravity fed into Stage 2 where 200 and 100 µm filter liners were tested. Photo courtesy of New Zealand Diving and Salvage Ltd.

Stage 3 comprised of the effluent from Stage 2 being passed through another Sandpiper flapper valve water pump into a BFNP bag filter vessel. Filter bags were housed, under pressure, inside a perforated stainless steel basket within the BFNP bag filter vessel (Figures 7 and 8). The effluent from Stage 3 then flowed through a single hose back into the sea. All de-fouled *D. vexillum* collected by the filters during the vacuuming operation was taken to the Blenheim landfill for disposal. The effectiveness of 200, 100, 50, 25, 10, 5, BOS5 and 1 µm filter bags was tested using the procedures described in section 2.7. The BOS5 refers to a double lined 5 µm filter bag, while all other filter bags were single lined.

Effluent from the Stage 3 100 µm filter bag was tested at time 0 and after 15 and 45 minutes to determine whether or not the effectiveness of the filter improved over time. To check for the possibility of a faulty seal within the BFNP bag filter vessel, the 100 µm filtered effluent from Stage 2 was poured into 5 and 1 µm filter bags outside of the BFNP bag filter vessel, and the effluent collected and tested using the procedures described in section 2.7.

The entire filtering system was contained within a 300 mm high sealed cofferdam made of 1 mm thick impermeable polypropylene (Ultraflex) to contain any spillage or overflow from the operation (Figure 9). Any subsequent overflow was returned to the Stage 2 filtration bag via marine bilge pumps. Any blockages in the filter pipes between the vacuuming head and Stage 1 were cleared by flow reversal and blow out.

**Figure 7.** The effluent from Stage 2 was pumped into the BFNP bag filter vessel where 200, 100, 50, 25, 10, 5, BOS5 and 1 µm filter sizes were tested. Photo courtesy of New Zealand Diving and Salvage Ltd.

**Figure 8.** During Stage 3 of the filtering process 200, 100, 50, 25, 10, 5, BOS5 and 1 µm filter bags were tested inside the BFNP bag filter vessel. Photo courtesy of New Zealand Diving and Salvage Ltd.

**Figure 9.** The entire filtering system was contained inside a 300 mm high sealed cofferdam made of 1 mm thick impermeable polypropylene (Ultraflex) to contain any spillage or overflow during the vacuuming operation. Photo courtesy of New Zealand Diving and Salvage Ltd.

## 2.6 Total suspended solids

Prior to the commencement of the vacuuming operation, SCUBA was used to collect five 1 L seawater samples from underneath the 'Steel Mariner' for total suspended solids (TSS) analysis. Plastic bottles were used to collect the samples, and at each collection site the lid was removed from one of the bottles then replaced firmly once the bottle was full. The bottle was then returned to the surface. The samples were taken from within 30 cm of the *D. vexillum* colonies hanging from the barge.

The sampling procedure described above was repeated at various stages throughout the vacuuming operation: five 1 L seawater samples were collected from within 30 cm of the vacuuming cutting head during the initial vacuuming operation; a further five samples were collected immediately prior to modifying the vacuuming cutting head; and ten more were collected from within 30 cm of the diver-operated nozzle during the vacuuming of the *D. vexillum* colonies from the 'Steel Mariner' and the seabed below. All 25 seawater samples were placed in a cool shaded area on the working barge immediately after collection and then kept overnight in a fridge at 4°C. The samples were couriered to Cawthron the following day for TSS analysis using the APHA 20th Edition 2540D method (American Public Health Association 1998). The average TSS for each sampling time (n=5) was determined and comparisons made between the results.

## 2.7 Filter effluent tests

Three replicate 75 ml vials were used to collect samples of the effluent expelled from the various filter sizes at Stages 1, 2 and 3. All samples were labelled by date, filtering stage, and filter size/type tested. Samples were allowed to settle for a minimum of 15 minutes prior to being viewed on-site using the microscope described above. During viewing the contents of the samples were retained within the 75 ml vials with the lids removed. The microscope graticule was used to measure the approximate size of the particulate matter within the samples. A conservative approach was taken to test the samples; effluent samples that contained any particulate matter greater than the filter size specifications resulted in a failed test. Conversely, effluent samples that contained particulate matter equal to or less than the filter size specifications resulted in a passed test. The results were immediately conveyed to the on site operations manager, so that any necessary adjustments to the filtering system could be made. All samples were preserved using 5% Formalin for archiving at Cawthron.

## 2.8 Effectiveness of the vacuuming operation

On 12 August 2002, approximately 8 days after the completion of the vacuuming operation, the wet biomass weight of *D. vexillum* remaining on the 'Steel Mariner' and the seabed was assessed. All *D. vexillum* colonies within each of four 4 $m^2$ quadrats, which were randomly placed along each of four port to starboard transects that had been randomly placed along the length of the hull, were hand-picked by two divers and placed into collection bags (Figure 10). The *D. vexillum* collected from each quadrat was then transported to the surface. As much of the *D. vexillum* as possible was separated from the other fouling organisms and the *D. vexillum* collected from each quadart was weighed inside 10 L buckets using hand held scales.

The wet weight of *D. vexillum* within each quadrat was recorded in kilograms, then packaged and disposed of at the Blenheim landfill. An overall estimate of the quantity of *D. vexillum* remaining on the barge was determined by calculating the mean and standard error of *D. vexillum* wet biomass weight per $m^2$ amongst the 16 quadrats, then scaling these by the submerged area of the hull. The approximate wet weight of the *D. vexillum* vacuumed from the hull of the 'Steel Mariner' was then added to the wet biomass weight of *D. vexillum* remaining and an estimate made of the percentage removed from the hull by the vacuuming operation.

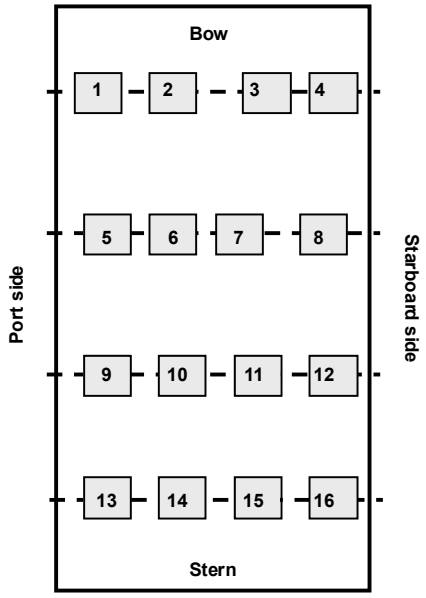



**Figure 10.** The hull of the 'Steel Mariner' was surveyed for the wet biomass weight of the *D. vexillum* using 16 randomly chosen 4 $m^2$ quadrats (numbered grey squares) on four random port-starboard transects (dashed lines).

# 3.0 RESULTS

## 3.1 *D. vexillum* reproductive status

There was no apparent difference in the stage of larval development between the *D. vexillum* specimens collected from the 'Steel Mariner' and those collected from the seabed. Although the *D. vexillum* colonies contained predominantly developing embryos and undeveloped larvae, a variety of stages of larval development were observed in the basal or central test core (Figures 11 and 12). Embryos had developed into tailed larvae with the tail wound from halfway to the whole way around the trunk. The trunk of the larvae was approximately 300 (width) x 400 (length) µm in size.

## 3.2 *D. vexillum* response to the vacuuming operation

No embryos or developed *D. vexillum* larvae were detected in any of the seawater samples collected from the buckets containing the mechanically disturbed *D. vexillum*. Similarly, no *D. vexillum* larvae were detected in any of the 75 ml seawater samples collected from around the *D. vexillum* colonies hanging from the hull of the 'Steel Mariner'.

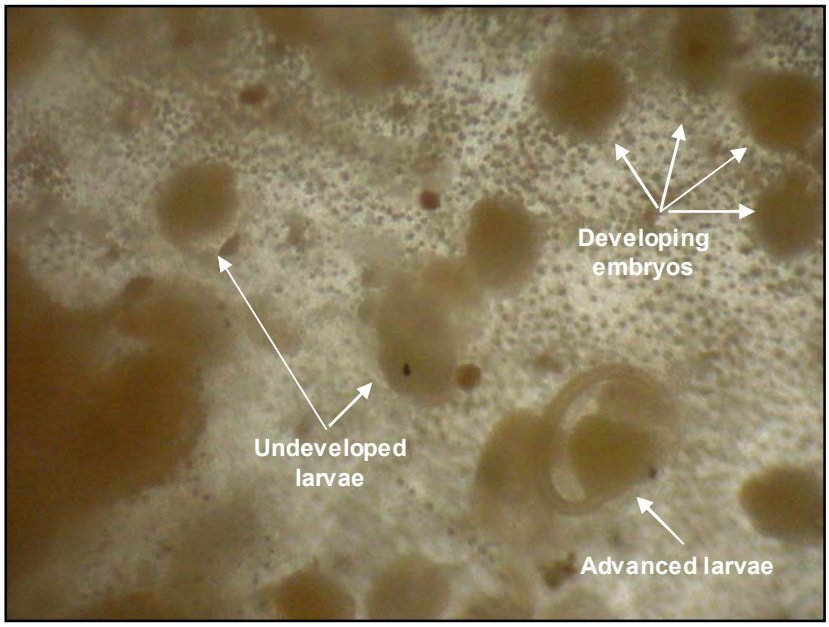



**Figure 11.** The colonies of *D. vexillum* collected from the 'Steel Mariner' and the seabed contained predominantly developing embryos and undeveloped larvae in the central test core.

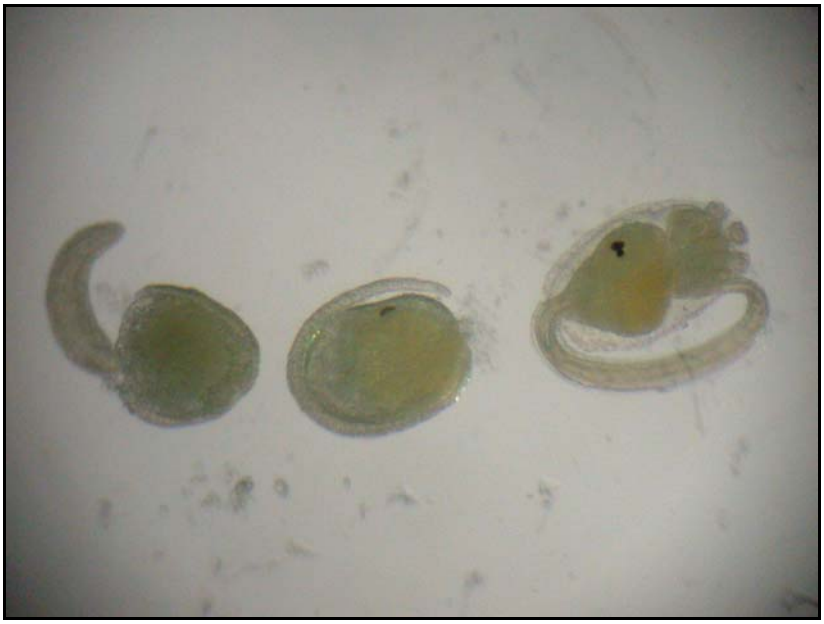


**Figure 12.** The *D. vexillum* colonies contained larvae that ranged in stages of development from undeveloped (left) to the very occasional well developed mature larvae (right).

## 3.3 The vacuuming operation

The original vacuum cutting head configuration was not practical for divers to use, nor was it effective at removing the *D. vexillum* colonies hanging from the hull of the 'Steel Mariner'. The housing surrounding the chopper prevented the removal of the remaining approximately 30 cm of each colony. While hand picking by divers did target the *D. vexillum*, it was found to be inefficient. The diver-operated nozzle targeted the ascidian and it also proved to be an efficient method for removing a wide range of sizes of *D. vexillum* colonies from the hull.

While the vacuuming operation targeted the *D. vexillum* colonies, other fouling organisms smothered by the *D. vexillum* were inevitably removed and collected during the vacuuming process, which often resulted in blockage of the pipes. Whilst some of these blockages occurred at the nozzle opening and were often cleared by the diver turning off the valve, the flow sometimes needed to be reversed in order to clear the system. This resulted in some of the mussels and oysters damaging the diaphragms within the Sandpiper flapper valve water pump.

The divers completed vacuuming the bulk of the *D. vexillum* from the hull of the 'Steel Mariner' in just two days (2-3 August 2002). Approximately 473 kgs of total wet biomass weight was removed from the hull, which included *D. vexillum* as well as other fouling organisms collected at Stage 1 of the filtering process. Approximately 200 g of *D. vexillum* was hand-scraped from the hull of the 'Waimarie I'.

Many other fouling organisms, sticks and small pieces of rock on the seabed that were smothered by the *D. vexillum*, caused frequent blockages of the vacuuming system and damage to the diaphragms within the Sandpiper flapper valve water pump. In some places the divers missed up to an estimated 60% of the *D. vexillum* on the seabed, as a result of poor visibility. This was caused by the physical contact of the diver and the dragging action of the vacuum pipe on the seabed. Hence, it took more than two days to achieve an approximate 75% clean-up of the *D. vexillum* from within a 7-10 m wide strip spanning the eastern to the western boundary (approximately 70 m long). It was decided, therefore, that the vacuuming operation would cease at this stage and a re-evaluation of the seabed undertaken. Approximately 147 kgs of wet biomass weight of *D. vexillum* was removed from the seabed, which also included *D. vexillum* as well as other fouling organisms collected at Stage 1 of the filtering process.

## 3.4 *D. vexillum* delimitation survey

The distribution of the *D. vexillum* on the seabed below the 'Steel Mariner' was defined as: 71.1 m long on the western boundary; 76.9 m on the eastern boundary; 28.60 m on the shoreline; and 67.60 m at the seaward (*i.e.* deeper) boundary (Figure 13). The highest quantity of *D. vexillum* colonies on the seabed appeared to be in the area closest to the barge. Furthermore, colonies closest to the 'Steel Mariner' had also successfully colonized other organisms such as red seaweeds, the Greenshell™ mussel *Perna canaliculus*, the blue mussels *Mytilus edulis galloprovincialis*, the horse mussels *Atrina pectinata zelandica*, the saddle squirts *Cnemidocarpa bicornuata*, rocks and pieces of wood (Figure 14). However, *D. vexillum* colonies towards the outer edges of the boundary seemed to be recently de-fouled colonies that had not yet colonized the seabed (Figure 15).

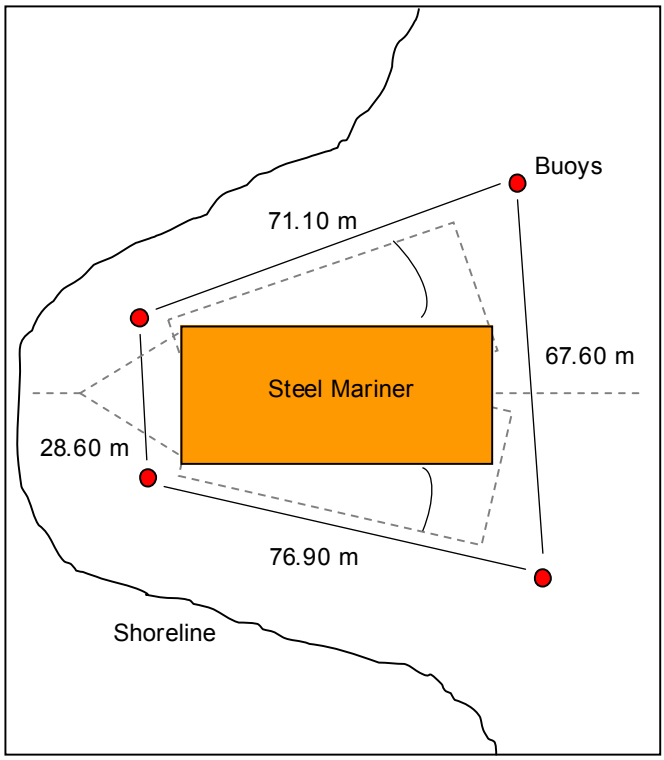



**Figure 13.** The distribution of the *D. vexillum* on the seabed was 71.1 m on the western boundary, 76.9 m on the southern boundary, 28.6 m on the shoreline and 67.6 m on the seaward boundary.

**Figure 14.** *D. vexillum* colonies closest to the 'Steel Mariner' had successfully colonized other fouling organisms such as red seaweeds, mussels, saddle squirts, rocks and pieces of wood.

**Figure 15.** *D. vexillum* colonies towards the outer edges of the boundary appeared to be recently de-fouled colonies from the 'Steel Mariner', which had not yet colonized the seabed. Photo courtesy of New Zealand Diving and Salvage Ltd.

### 3.5 Total suspended solids

The mean TSS in the water column surrounding the 'Steel Mariner' before the vacuuming operation was 28.40±0.98 (se) g/m$^3$ (Table 1). Interestingly, the TSS surrounding the cutter head during the vacuuming operation was found to be lower at 16.80±2.08(se) g/m$^3$ (however note the higher standard error). Similarly, the TSS underneath the 'Steel Mariner' before vacuuming commenced on the second day was also relatively low at 16.40±0.98 g/m$^3$ (Table 1).

**Table 1.** Total suspended solids (g/m$^3$) from five replicate 1 L water samples collected before and during the vacuuming operation.

| Replicate sample time and location of sample collection | Before vacuuming underneath the 'Steel Mariner'. (30/7/02) | During vacuuming around the vacuum cutting head. (2/8/02) | Before vacuuming underneath the 'Steel Mariner'. (4/8/02) | During vacuuming around the vacuum nozzle underneath the 'Steel Mariner. (4/8/02) | During vacuuming around the vacuum nozzle on the seabed. (5/8/02) |
|---|---|---|---|---|---|
| **1** | 28 | 25 | 15 | 16 | 32 |
| **2** | 31 | 16 | 15 | 16 | 30 |
| **3** | 29 | 14 | 17 | 16 | 18 |
| **4** | 25 | 15 | 15 | 14 | 53 |
| **5** | 29 | 14 | 20 | 17 | 35 |
| **Mean** | **28.40** | **16.80** | **16.40** | **15.80** | **33.60** |
| **se** | **0.98** | **2.08** | **0.98** | **0.49** | **5.64** |

### 3.6 Filter effluent tests

Nine filter sizes and configurations (mussel bag, 200, 100, 50, 25, 10, 5, BOS5 and 1µm) were tested over the 5 days (2-6 August 2002) of the vacuuming operation (Tables 2 and 3). No *D. vexillum* embryos or larvae were detected in any of the 25 effluent samples collected and viewed under the microscope. It was predicted that the mussel bag at Stage 1 would filter effluent to approximately 500 µm, however samples collected from the filtered effluent showed that only particles <150 µm were present (Table 3). The 200 µm filter liners and bags, which were tested at all three filtering stages, performed very well and no particulate matter >200 µm was detected amongst the effluent samples.

The 100 µm filter liners and bags were tested at Stages 2 and 3. One of the three 75 ml effluent samples collected from Stage 2 possessed two particles >200 µm, while no particulate matter >200 µm was detected in the other samples (Table 3). Stage 3 effluent samples from the first 200 µm filter bag trial contained some particulate matter >200 µm. This filter bag was later replaced and no particulate matter >200 µm was detected in the subsequent samples. The Stage 3 200 µm filter bag test showed that filtering effectiveness did not appear to improve with time (*i.e.* time = 0, 15 and 45 minutes) (Table 3).

The first two 50 µm filter bags tested at Stage 3 failed, since particulate matter up to 100 µm was detected in the effluent samples. The following two trials passed since no particulate matter >50 µm was detected. All the remaining filter bag (25, 10, 5, BOS5 and 1 µm) trials at Stage 3 failed since particulate matter in the effluent samples exceeded the specified size of the various filter bags (Table 3). For example, 100 µm particles were detected in the effluent from the 10 µm filter bags. Particulate matter as large as 200 µm was found in the 100 µm filtered effluent from Stage 2, which had been poured into the 5 and 1 µm filter bags for testing outside of the BFNP bag filter vessel.

**Table 2.** A total of 25 different filter tests of 9 various filter sizes and configurations were tested over the 5 days of the vacuuming operation.

| Filter size tested (µm) | Filter type/construction | Stage 1 | Stage 2 | Stage 3 |
|---|---|---|---|---|
| Mussel bag | Single bag | 1 | - | - |
| 200 | Bag liner, single lined | 2 | 1 | 1 |
| 100 | Bag liner, single lined | - | 2 | 4 |
| 50 | Single lined bag | - | - | 4 |
| 25 | ″ | - | - | 3 |
| 10 | ″ | - | - | 1 |
| 5 | ″ | - | - | 3 |
| BOS5 | Double lined bag | - | - | 1 |
| 1 | Single lined bag | - | - | 1 |
| **Total** | | **3** | **3** | **19** |

**Table 3.** Summary of results from the filtering trials conducted over the five days of the vacuuming operation. Filtering stage and filter size refer to the three main filtering stages (1, 2 and 3) and filtering sizes (mussel bag, 200, 100, 50, 25, 10, 5, BOS5 and 1 µm) tested respectively. Three replicate samples of the filtered effluent were viewed under a microscope to test the effectiveness of each filter size (see sections 2.3 & 2.4 for details). N/A = result not applicable; Pass = maximum particle size in effluent =/< filter size specifications; Fail = particles size in effluent > filter size specifications (see comments column for details).

| Filtering stage | Date tested | Filter size | Replicate | Location of *D. vexillum* | Result | Comments |
|---|---|---|---|---|---|---|
| **1** | 2/8/02 | Mussel bag | 1 | Steel Mariner | N/A | Particles =/<150 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | N/A | " |
| | | " | 3 | " | N/A | " |
| **1** | 3/8/02 | 200 µm | 1 | Steel Mariner | Pass | Particles =/<200 µm with cysts. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Pass | " |
| | | " | 3 | " | Pass | " |
| **1** | 5/8/02 | " | 1 | Seabed | Pass | Particles =/<200 µm with cysts. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Pass | " |
| | | " | 3 | " | Pass | " |
| **2** | 5/8/02 | 200 µm | 1 | Seabed | Pass | Particles =/<200 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Pass | " |
| | | " | 3 | " | Pass | " |
| **2** | 2/8/02 | 100 µm | 1 | Steel Mariner | Fail | 2 particles = 120 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Pass | Particles =/<100 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 3 | " | Pass | " |
| **2** | 3/8/02 | " | 1 | Steel Mariner | Pass | Particles =/<100 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Pass | " |
| | | " | 3 | " | Pass | " |

**Table 3 continued.**

| Filtering stage | Date trialed | Filter size | Replicate | Location of *D. vexillum* | Result | Comments |
|---|---|---|---|---|---|---|
| **3** | 6/8/02 | 200 µm | 1 | Seabed | Pass | Particles =/< 200 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Pass | " |
| | | " | 3 | " | Pass | " |
| **3** | 2/8/02 | 100 µm | 1 | Steel Mariner | Fail | Samples @ T 0. Particles >100 µm. No *D. vexillum* embryos or larvae. |
| | | " | 2 | " | Fail | " |
| | | " | 3 | " | Fail | " |
| **3** | 2/8/02 | 100 µm | 1 | Steel Mariner | Pass | Samples @ 15 mins. Particles <100 µm. No *D. vexillum* embryos or larvae. |
| | | " | 2 | " | Pass | " |
| | | " | 3 | " | Pass | " |
| **3** | 2/8/02 | " | 1 | Steel Mariner | Pass | Samples @ 45 mins. Particles <100 µm. No *D. vexillum* embryos or larvae. |
| | | " | 2 | " | Pass | " |
| | | " | 3 | " | Pass | " |
| **3** | 5/8/02 | " | 1 | Seabed | Pass | Particles =/< 100 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Pass | " |
| | | " | 3 | " | Pass | " |
| **3** | 2/8/02 | 50 µm | 1 | Steel Mariner | Fail | Particles up to 70 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Fail | Particles up to 100 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 3 | " | Fail | " |
| **3** | 2/8/02 | " | 1 | Steel Mariner | Fail | Particles >100 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Fail | " |
| | | " | 3 | " | Fail | " |
| **3** | 2/8/02 | " | 1 | Steel Mariner | Pass | Particles =/< 50 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Pass | " |
| | | " | 3 | " | Pass | " |
| **3** | 3/8/02 | " | 1 | Steel Mariner | Pass | Particles =/< 50 µm. No *D. vexillum* embryos or larvae found. |
| | | " | 2 | " | Pass | " |
| | | " | 3 | " | Pass | " |

**Table 3 continued.**

| Filtering stage | Date trialed | Filter size | Replicate | Location of *D. vexillum* | Result | Comments |
|---|---|---|---|---|---|---|
| **3** | 3/8/02 | 25 µm | 1 | Steel Mariner | Fail | Particles =/< 50 µm. No *D. vexillum* embryos or larvae found. |
| | | ″ | 2 | ″ | Fail | ″ |
| | | ″ | 3 | ″ | Fail | ″ |
| **3** | 3/8/02 | ″ | 1 | Steel Mariner | Fail | Particles up to 60 µm. No *D. vexillum* embryos or larvae found. |
| | | ″ | 2 | ″ | Fail | ″ |
| | | ″ | 3 | ″ | Fail | ″ |
| **3** | 4/8/02 | 25 µm | 1 | Seabed | Fail | Particles up to 60 µm. No *D. vexillum* embryos or larvae found. |
| | | ″ | 2 | ″ | Fail | ″ |
| | | ″ | 3 | ″ | Fail | ″ |
| **3** | 5/8/02 | 10 µm | 1 | Seabed | Fail | Particles up to 100 µm. No *D. vexillum* embryos or larvae found. |
| | | ″ | 2 | ″ | Fail | ″ |
| | | ″ | 3 | ″ | Fail | ″ |
| **3** | 3/8/02 | 5 µm | 1 | Steel Mariner | Fail | Particles up to 50 µm. No *D. vexillum* embryos or larvae found. |
| | | ″ | 2 | ″ | Fail | ″ |
| | | ″ | 3 | ″ | Fail | ″ |
| **3** | 4/8/02 | ″ | 1 | Seabed | Fail | Particles up to 50 µm. No *D. vexillum* embryos or larvae found. |
| | | ″ | 2 | ″ | Fail | ″ |
| | | ″ | 3 | ″ | Fail | ″ |
| **3** | 4/8/02 | ″ | 1 | Seabed | Fail | ″ |
| | | ″ | 2 | ″ | Fail | ″ |
| | | ″ | 3 | ″ | Fail | ″ |
| **3** | 6/8/02 | *5 µm | 1 | Seabed | Fail | Particles up to 200 µm. No *D. vexillum* embryos or larvae found. |
| | | ″ | 2 | ″ | Fail | ″ |
| | | ″ | 3 | ″ | Fail | ″ |

*5 µm refers to a test where 100 µm filtered effluent from Stage 2 was poured into 5 µm filter bags out of the BFNP bag filter vessel and the effluent collected and viewed under the on site microscope (Refer Section 2.4).

**Table 3 continued.**

| Filtering stage | Date trialed | Filter size | Replicate | Location of *D. vexillum* | Result | Comments |
|---|---|---|---|---|---|---|
| **3** | 3/8/02 | BOS 5 µm | 1 | Steel Mariner | Fail | Particles =/< 50 but mostly < 10 µm. No *D. vexillum* embryos or larvae. |
| | | ″ | 2 | ″ | Fail | ″ |
| | | ″ | 3 | ″ | Fail | ″ |
| **3** | 3/8/02 | 1 µm | 1 | Steel Mariner | Fail | Particles up to 50 µm. No *D. vexillum* embryos or larvae found. |
| | | ″ | 2 | ″ | Fail | ″ |
| | | ″ | 3 | ″ | Fail | ″ |
| **3** | 6/8/02 | *1 µm | 1 | Seabed | Fail | Particles up to 200 µm. No *D. vexillum* embryos or larvae found. |
| | | | 2 | ″ | Fail | ″ |
| | | | 3 | ″ | Fail | ″ |

*1 µm refers to a test where 100 µm filtered effluent from Stage 2 was poured into 1 µm filter bags out of the BFNP bag filter vessel and the effluent collected and viewed under the on site microscope on site (Refer Section 2.4).

### 3.7 Effectiveness of the vacuuming operation

The vacuuming operation removed the bulk of the *D. vexillum* colonies from the hull of the 'Steel Mariner'. Any remaining *D. vexillum* on the hull consisted of small fragments only, which were either the remainder of holdfasts or small colonies intertwined amongst other fouling organisms. An average of 0.09±0.02 kg/m$^2$ was found amongst the 16 quadrats used to survey the hull after the vacuuming operation. Interestingly, the total wet weight of the *D. vexillum* collected from within each quadrat was reasonably consistent and evenly distributed over the hull (Figure 16). Given that the submerged area of the hull was approximately 1,296 m$^2$ (60 x 21.6 m), an estimated total of 120.49 kgs of *D. vexillum* remains on the 'Steel Mariner'. Given that an estimated 473 kg wet weight of *D. vexillum* was removed from the hull, the vacuuming operation removed approximately 80% of the original *D. vexillum* biomass.

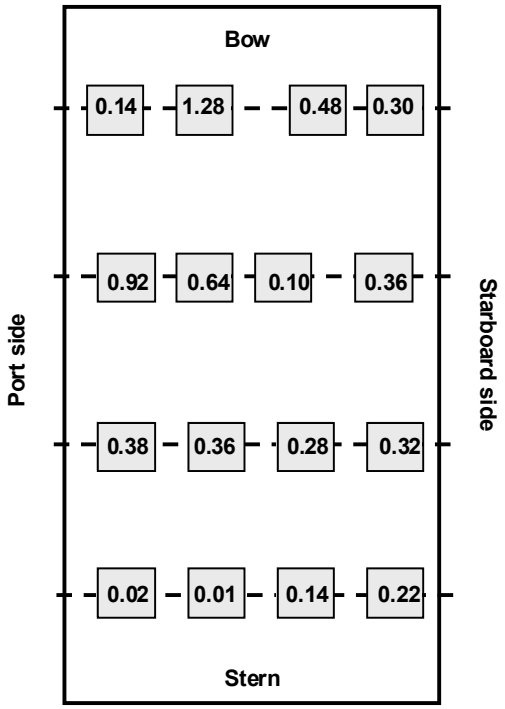



**Figure 16.** The wet weight (kg/m$^2$) of *D. vexillum* in quadrats used to survey the hull of the 'Steel Mariner' after the vacuuming operation. Transects are indicated by dashed lines.

## 4.0 DISCUSSION

### 4.1 *D. vexillum* reproductive status

The *D. vexillum* colonies on the hull of the 'Steel Mariner' appeared to be in a state of dormancy at the time of the vacuuming operation. The total biomass of the ascidian also seemed to have had declined significantly since it was first discovered on the barge in late December 2001. This is probably a consequence of the cold water temperatures (11.8$^o$C) reducing the rate of both sexual and asexual reproduction at this time of year. It was not surprising, therefore, to find predominantly

undeveloped embryos and larvae inside the central test core. The presence of the occasional mature larvae suggests, however, that the species is either approaching spawning time as the water temperatures increase, or that the species develops some mature larvae all year around. The former explanation seems most likely, given that the *D. vexillum* collected from the 'Steel Mariner' in January 2002 was full of mature larvae.

No larvae were detected in the seawater samples collected from the *D. vexillum* that was mechanically disturbed inside the buckets. This was unexpected given that undeveloped larvae as well as the occasional developed larvae were present in the *D. vexillum* colonies collected on 31 July 2002. Furthermore, no larvae were detected amongst the seawater samples collected from around the *D. vexillum* colonies on the hull of the 'Steel Mariner' both before and during the vacuuming operation. This result provided some evidence that the *D. vexillum* was not releasing larvae at that particular time. It will be interesting to see if any *D. vexillum* larvae settle on the suspended bricks from the disturbance experiment over the following six months, when the warmer water temperatures would promote their growth.

### 4.2 The vacuuming operation

The configuration of the original vacuum cutting head appeared to contain all de-fouled *D. vexillum* colonies, since no larvae or fragments were detected in the seawater samples collected from around the cutting head. However, the housing surrounding the cutting head prevented the removal of the remaining 30 cm of the *D. vexillum* colonies hanging from the hull.

Hand-picking *D. vexillum* colonies and then feeding them into the cutting head was not efficient, and such a handling process could easily increase the chances of larval release. This technique should be avoided, especially during the time of year when the *D. vexillum* is reproductively active (*i.e.* summer months).

The diver-operated vacuuming nozzle proved to be the most selective and efficient method for removing *D. vexillum* colonies from the hull of the 'Steel Mariner'. The vacuuming operation did not aim to eradicate the ascidian, and an estimated 80% removal of the *D. vexillum* biomass from the 'Steel Mariner' was an outstanding achievement given the quantity of biomass present. In fact, all the *D. vexillum* that remained on the barge consisted of small fragments intertwined amongst other fouling organisms. The remaining colonies are likely to increase in biomass again, however, as the seawater temperatures increase during the following months.

The diver-operated nozzle had some limitations. Fouling organisms (*e.g.* mussels and oysters) that had been smothered by the *D. vexillum* occasionally blocked the vacuuming equipment. While some of these blockages occurred at the nozzle and were often cleared by the diver turning the valve off, clearing of some of the blockages involved reversing the flow in order to clear the system. This inevitably resulted in the already disturbed *D. vexillum* being flushed back out into the surrounding seawater. These fragments were not captured and often settled onto the seabed below. This back washing procedure is undesirable, as viable larvae could have been released back into the water column if they were present. Hence, alternative clearing procedures or preventative measures may be required, especially during the time of year when the *D. vexillum* is reproductively active.

Initially, the diver-operated vacuuming nozzle proved to be an effective method for removing the

seabed material smothered by the *D. vexillum*. The effectiveness of the method decreased with time, however, as a result of poor visibility caused by the diver's contact with the seabed, the dragging of the vacuum pipe and the reverse flushing action used to clear blockages. As a consequence divers missed up to 60% of the *D. vexillum* in some areas. Hence, it took more than two days to achieve an estimated 75% clean-up of the *D. vexillum* from within a 7-10 m wide strip (approximately 70 m long). At that rate it was going to take approximately 14 days to remove up to 75% of the *D. vexillum* from the total area. The correct decision was made, therefore, to cease the vacuuming operation and review alternative methods for removing the *D. vexillum* from the seabed. These might include the use of freshwater or chlorine contained within tarpaulins or polypropylene covers, a similar technique utilised in California, U.S.A., to control the invasive seaweed *Caulerpa taxifolia*. Another suggestion is to smother the *D. vexillum* colonies with a fine layer of cement dust or dirt from the surrounding seabed.

### 4.3 *D. vexillum* delimitation survey

Coutts (2002) reported in late February 2002 that the *D. vexillum* on the seabed was distributed over an estimated 3,200 $m^2$ (40 x 80 m) surrounding the 'Steel Mariner'. This estimate appears to be reasonably accurate in light of the results from the present survey, which estimated the *D. vexillum* to be distributed over a 3,559 $m^2$ area. The distribution of the ascidian on the seabed appears to have increased, however, especially on the western boundary. Some of this spread would have been a result of the barge's back and forth motion during the prevailing north-easterly winds, however much of the *D. vexillum* on the seabed appears to be recently de-fouled colonies that have yet to take a foothold.

In comparison to the previous surveys (see Coutts 2002), the most striking observation made by the author during the present survey was the increase in the abundance of small *D. vexillum* colonies on other fouling organisms, rocks, and pieces of wood on the seabed. This is likely to be a consequence of de-fouled colonies falling from the 'Steel Mariner', which have subsequently reproduced asexually. Furthermore, it is highly likely that the *D. vexillum* has undergone at least one sexual reproductive cycle since April 2001, at which time the barge first arrived at its present location (see Coutts 2002 for information on the vessel's history). Hence, much of the increase in abundance of small established colonies could also be a result of larvae being attracted to the shaded area beneath the barge by a phototrophic response (*i.e.* attracted to the shade) (Mather 2002).

### 4.4 Total suspended solids

Interestingly, the mean TSS in the water surrounding the 'Steel Mariner' was higher before the vacuuming operation commenced than during the operation. It is unlikely that the vacuum and filter system reduced the TSS in the surrounding water. It may have been a result, however, of the suspended solids settling out after a period of rain that had occurred prior to the vacuuming operation. As expected, the TSS were highest during the vacuuming operation on the seabed, at which time the divers and vacuuming equipment were in contact with the seabed.

### 4.4 The filtering process

The filtering plant designed and used by NZDS clearly illustrated that de-fouled material can be successfully filtered to 50 µm, although some particulate matter >200 µm, but <250 µm, was detected in effluent samples from the Stage 3 filters. However, given that the trunk of *D. vexillum* larvae is approximately 300 µm across, there was little likelihood that the filtering process would have enabled the release of viable larvae back into the environment. This was further verified by the fact that no *D. vexillum* larvae were found amongst the Stage 3 effluent samples. The 300 µm high sealed cofferdam was also very effective at containing any spillage or overflow during the operation.

Successful filtering to 50 µm is a significant result given that MFish are currently proposing to implement hull de-fouling regulations and guidelines under the Biosecurity Act 1993. These include a requirement for the containment of de-fouled particles larger than 60 µm in diameter (see section 1.0). Some biosecurity scientists have suggested that this filtering standard should be as low as 10 µm, however, if the intent of the standard is to prevent the release of algal gametes and spores such as those of the Japanese seaweed *Undaria pinnatifida*. Although *Undaria* was not targeted during the present operation, it was found on the hull of the 'Steel Mariner'.

It is not known why effluent samples from the 25, 10, 5 and 1 µm filter bags tested at Stage 3, contained particulate matter that was significantly larger than the specified size of the filters. Stage 1 effluent was gravity fed through 5 and 1 µm filters in an attempt to eliminate the possibility of effluent by-passing the filters through the filter seating inside the BFNP bag filter vessel. However, the effluent from these tests contained particulate matter up to 200 µm. One explanation for this is that the effluent samples were contaminated by particulate matter on the outside of the filter bags.

## 5.0 RECOMMENDATIONS

The development of an underwater hull de-fouling mechanism capable of capturing and filtering all de-fouled material to a desired level is required. Current technology is moving closer towards capturing de-fouled material from the more uniform areas of the hull (flat sides), but the challenge now lies with cleaning the areas protected from strong laminar flows (APSLF) such as the gratings, pipes, sea chests, rope guards, rudders, bow thrusters and bilge keels on the hulls of vessels. It is recommended, therefore, that the diver-operated vacuuming nozzle be tested during a merchant ship's hull de-fouling operation to determine whether or not it is a practical tool for removing, collecting and filtering de-fouled material from these APSLF.

The filtering plant and diver-operated vacuuming nozzle designed by NZDS was capable of collecting, containing and filtering de-fouled *D. vexillum* and associated material to 50 µm. However, it is not yet known if the filtering plant can be utilised for de-fouling the hulls of other vessels including merchant ships. It is therefore recommended that the system be tested during a merchant ship's routine hull cleaning operation, to determine what filter sizes (e.g. 200, 100, 50, 25, 10, 5 or 1 µm) can be practically achieved, and whether or not the system is capable of accommodating the flow rates necessary to undertake the hull cleaning operation within the allocated timeframe. Furthermore, more rigorous tests of the 25, 10, 5 and 1 µm filter bags are recommended for future work.

Surprisingly, filtering effectiveness did not appear to improve with time (*i.e.* time 0, 15 and 45 minutes) when the effluent from a 200 µm filter bag was tested at Stage 3. Future tests might include sampling of the effluent over longer time periods.

It is also recommended that a simple procedure be developed for on-site testing of the size of particulate matter in the effluent. Hull cleaning operators could then ascertain whether or not their filtering system is performing correctly.

An estimated 120 kgs of the *D. vexillum* remains on the hull of the 'Steel Mariner' and the seabed below. The biomass of these remaining colonies is likely to increase and the ascidian reach sexual maturity as the water temperatures increase during the following months. It is strongly recommended, therefore, that a stakeholder meeting be arranged as soon as possible to discuss management options for the treatment of the 'Steel Mariner' and the seabed below.

## 6.0 ACKNOWLEDGEMENTS

I wish to thank the New Zealand Diving and Salvage Ltd team consisting of Richard Moore, Bill Humphries, James Brodie, Maurice Kapua, Brendon Hall and Rob Gasson for their assistance with collecting samples during the entire operation. A special thanks should also go to James and Val Brodie for their hospitality at their Divers Homestay at Picton. Finally, thank you to Dr. Michael Taylor (Cawthron Institute, Biosecurity Research Manager) for reviewing this report before release.

## 7.0 REFERENCES

Coutts, A. D. M. 2002. A biosecurity investigation of a barge in the Marlborough Sounds. Cawthron Report No. 744. July 2002: 59.

Cranfield, H. J.; Gordon, D. J.; Willan, R. C.; Marshall, B. C.; Battershill, C. N.; Francis, M. P.; Nelson, W. A.; Glasby, C. J.; Read, G. B. 1998. Adventive marine species in New Zealand. NIWA Technical Report, No. 34: 48.

Mather, P. 2002. Identification of a didemnid? Ascidian from Whangamata Harbour. Ministry of Fisheries Report. ZBS2001-08.

McClary. D. 2001. Alternative biosecurity management tools for vector threats – technical guidelines for acceptable hull cleaning facilities. Ministry of Fisheries Research Project ZBS 2000/03.

American Public Health Association. (1998). Standard Methods for Examination of Water and Wastewater. 20th Edition. Washington D.C. American Public Health Association, American Water Works Association and Water Environment Federation.

Thresher, R. E.; Hewitt, C. L. and Campbell, M. L. 1999. Synthesis: Introduced and cryptogenic species in Port Phillip Bay. In: Hewitt, C.L.; Campbell, M.L.; Thesher, R.E; Martin, R.B. (eds). Marine biological invasions of Port Phillip Bay, Victoria. Centre for Research on Introduced Marine Pests. Technical Report No. 20: 283-295.