# Empirical validation: Small vessel translocation of key threatening species

## Stage I – *Asterias amurensis*

---

Keith Hayes, Caroline Sutton, Rasanthi Gunasekera, Cath Sliwa, Sasha Migus, Felicity McEnnulty, Piers Dunstan, Mark Green and Jawahar Patil

October 2004



ISBN



**Authorship:**

This report is an independent review prepared by CSIRO Marine Research. The views and opinions expressed in this publication are those of the authors and do not necessarily reflect those of the Australian Government or the Minister for the Environment and Heritage.

The Australian Government does not accept responsibility for the accuracy or completeness of the contents of this publication, and shall not be liable for any loss or damage that may be occasioned directly or indirectly through the use of, or reliance on, the contents of this publication.

Natural Heritage Trust

*Helping Communities Helping Australia*

An Australian Government Initiative

# EXECUTIVE SUMMARY

The empirical validation project is a two year project designed to collect quantitative information on small vessel biofouling that supports the development and implementation of the new national system for preventing and managing marine pests in Australian waters. This document reports on the results of the first year of the project.

This project had four overall objectives:

1. to collect bio-fouling samples from small commercial and recreational vessels in order to test the predictions of the Infections Modes and Effects Analysis completed for small craft operating in southeast Australia;

2. to determine the presence or absence of *Asterias amurensis,* with a low probability of Type II error, by applying an existing gene probe to the samples collected from these vessels;

3. to develop a Bayesian journey survival model to estimate probability of survival as a function of vector dynamics and journey duration based on (1) and (2); and,

4. to develop quantitative risk factors for fouling biomass based on vessel activity, paint type and management practices that assist in the development and/or implementation of new national protocols designed to minimise the bio-invasion risks associated with small craft.

30 vessels were sampled, at two locations (Royal Hobart yacht club and the Domain slip yard) between the 12$^{th}$ August 2003 and the 11$^{th}$ June 2004, comprising 20 yachts/motor cruisers, 9 fishing vessels and 1 tug. The survey team sampled or inspected 68 different locations in and around the hull, propeller, rudder and anchor, internal spaces, fishing gear and deck of these vessels. A total of 750 samples were taken. A further 204 inspections were made on board the vessels that resulted in no sample being taken. An additional 120 plankton samples – 4 for each vessel sampled – were also taken at the sites. No samples were collected from the deck or fishing gear of any of the vessels taken – in all cases these locations were found to be clean and dry upon inspection.

Most of the inspected vessels were well maintained and relatively free of biofouling. There were, however, rare occurrences of very heavily fouled vessels. This pattern gives rise to zero inflated, positively skewed data that may be well described by a delta distribution. One of the key aims of the second stage of the project is to model the effect of potential explanatory co-variates (such as anti-fouling paint age and type, and vessel activity patterns) on the parameters of this distribution. A preliminary analysis of the results, however, suggests that significant hull fouling (greater than 50 grams per 0.5m$^2$) is likely to develop on anti-fouled vessels after approximately 400 days.

The Infection Modes and Effects analysis, completed two years ago, scores different parts of the vessels according to: a) their environmental suitability; b) the occurrence of marine organisms; and, c) the likelihood of detection. The wet weight of samples gathered in this project was compared to the multiple of the environmental suitability score and the occurrence score. In most cases the wet weight of biofouling supported the Infection Modes and Effects Analysis score. Notable exceptions include the garboard plank (which had much higher levels of fouling than suggested by the analysis), the block spaces and other small niche areas on the hull (which had much lower levels of fouling than suggested by the analysis) and the rudder surface (which had higher levels of fouling than predicted). These results will be re-assessed at the end of the second stage of the project. The level of fouling associated with fishing gear was also significantly different from that predicted by the Infection Modes and Effects Analysis. The

results reported here are not an accurate reflection of the translocation risks associated with fishing gear because this gear was washed prior to the vessels being slipped and inspected.

All of the 750 samples and 120 plankton samples were successfully processed and DNA extracted and probed. This project demonstrates that it is possible to process hull fouling samples for genetic probe application, but in some cases (e.g. large biomass samples) this requires extensive primary, and secondary, processing that is costly both in terms of materials and labour. Indeed the time associated with the pre-processing, coupled with the large number of samples led to much greater staff and operational costs than was originally anticipated.

The proportion of positive plankton samples broadly reflects the breeding cycle of *Asterias amurensis* in the Derwent estuary. There is a small window between the 3rd of February and the 18th of March during which time none of the plankton samples returned positive results. This is consistent with previous data on the life-cycle of *A. amurensis* in Derwent. The samples taken on the 23rd of April (boat 25) were also negative but thereafter, from the 28th of April onwards, one or more of the plankton samples tested positive. This suggests that the *A. amurensis* starts to spawn in the Derwent in mid-to late April, approximately two months earlier than previously reported and one month earlier than recorded in the ballast water Decision Support System databases.

The proportion of positive samples decreases quite dramatically between August and December, which may reflect the declining presence of *Asterias* larvae and gametes in the sea-water associated with the samples. Five vessels – 6, 18, 19, 20 and 25 - returned *Asterias* positive samples with negative plankton samples. The two vessels in the limit (6 and 25) do not provide strong evidence for settlement because the sample dates on both occasions (1st of October and 23rd of April) are within, or at least close to, the spawning season of *Asterias amurensis*, and hence the positive sample may simply reflect the presence of larvae or gametes in the seawater associated with the sample. The three remaining vessels sampled contiguously between the 3rd of February and the 25th, however, provide the first strong evidence of *A. amurensis* settlement on small recreational and commercial vessels. The plankton samples during this period are consistently negative, suggesting that the positive result is not due to contamination of the sample by larvae or gametes in the associated seawater.

Positive water samples were collected from the internal spaces of eight vessels. These positive results, however, indicated a potential translocation risk on only one occasion - water from an anchor well that was not self draining. Virtually all of the other internal spaces that are routinely filled with water, on the boats inspected to date, were self draining – particularly live catch circulation tanks and anchor wells. For these reasons, and a lack of prolonged access to a vessel, the project was unable to develop a journey survival model for *Asterias*.

# CONTENTS

# 1 INTRODUCTION

Introductions of non-indigenous species (NIS) are a serious threat to global biodiversity (Baltz, 1991) and in the marine environment have been occurring since humans started exploring the world's oceans. The transport and introduction of organisms throughout the world's oceans is happening at an unprecedented rate (Walford and Wicklund, 1973; Carlton, 1985; 1995). A number of anthropogenic vectors are, or have been, responsible for spreading marine organisms beyond natural bio-geographic boundaries (Carlton, 2001). The vectors responsible for the introduction of marine organisms vary with time and with geographical region. In Australia, hull fouling, accidental release associated with mariculture (predominately oysters) and the ballast water discharges of large commercial vessels are the most prominent sources of marine NIS (Thresher *et al*., 1999).

In addition to large commercial vessels, it is becoming increasingly clear that small craft, including yachts and fishing vessels, are capable of introducing NIS to new sites and contributing to their subsequent spread. A well known recent example is the introduction of the black-striped mussel *Mytilopsis* spp. to Darwin in 1999 (Willan *et al.,* 2000). It is almost certain that this species arrived on the hull of an ocean-going yacht, and spread to nearby marinas by other local yachts. Adult *Mytilopsis* mussels have subsequently been found on the hull and in the seawater piping of Indonesian fishing vessels and other ocean-going yachts (Willan *et al.,* 2000; *pers. comm.* A. Marshall, Northern Territory Aquatic Pests Program).

In southern Australia, NIS such as *Asterias amurensis* and *Undaria pinnatifida* threaten a variety of fishery resources. *A. amurensis* is an important predator of scallops, mussels and other commercially important molluscs (Ross, 2001). Fishing vessels, recreational vessels and aquaculture equipment may be inadvertently assisting the spread of this species. Spat collectors, for example, have recently been implicated in the introduction of *A. amurensis* into Spring Bay on the east coast of Tasmania (*pers comm.* C. Sutton, CSIRO).

*Asterias amurensis* arrived in Australia in the 1980s (Turner, 1992; Ward and Andrew, 1995). The first Australian record, from southern Tasmania, dates to 1986 (Turner, 1992). The seastar has since spread along Tasmania's southeast coast and has also become established in Port Phillip Bay, Victoria (Garnham, 1998). In Port Phillip Bay the species achieved phenomenal rates of population growth - the population increased from a small number of adults, first detected in 1995, to over 100 million five years later (Talman *et al.*, 1999). *A. amurensis* has broad temperature and salinity tolerances and therefore has the potential to spread throughout Australia's temperate marine ecosystems (Hewitt *et al.,* 2002). One of the aims of this project is to investigate the potential translocation of *Asterias amurensis* by fishing and recreational vessels.

This project was designed to complement the results of two other studies undertaken by the Bureau of Rural Sciences (BRS) and CSIRO Marine Research (CMR). BRS were undertaking an analysis of vector movements in the southeast of Australia, quantifying vector strength, mapping pathways and identifying nodes for a range of commercial and non-commercial vectors such as cargo ships, fishing vessels, yachts, aquaculture gear, oil rigs etc. This was to provide information on the relative risks posed by different vectors in terms of their dispersal capability and area of operations. The project was withdrawn before its completion, and therefore its data and results cannot be utilised by this project.

Two years ago, CMR in collaboration with the Department of Natural Resources and Environment in Victoria (DNRE), completed an Infection Modes and Effects Analysis (IMEA) for small craft (Hayes, 2002). This analysis identified (and ranked) locations on fishing vessels and recreational vessels that might be capable of transporting marine pests. The results were used to identify potential "hot-spots" on fishing vessels and recreational vessels operating in Tasmania and Victoria. One of the aims of this project was to collect water, sediment and biological samples from these "hot-spots" in order to test the predictions of the IMEA analysis.

## 2 Objectives

This project had four overall objectives:

1. to collect bio-fouling samples from small commercial and recreational vessels in order to test the predictions of the Infection Modes and Effects Analysis (Hayes, 2002) recently completed for small craft operating in southeast Australia;

2. to determine the presence or absence of *Asterias amurensis,* with a low probability of Type II error, by applying an existing gene probe (Deagle *et al.*, 2001), to the samples collected from these vessels;

3. to develop a Bayesian journey survival model to estimate probability of survival as a function of vector dynamics and journey duration based on (1) and (2); and,

4. to develop quantitative risk factors for fouling biomass based on vessel activity, paint type and management practices that assist in the development and/or implementation of new national protocols designed to minimise the bio-invasion risks associated with small craft.

This project is a two-year project, with each year funded separately. The first year (Stage I) collected samples that will be analysed for the presence of *Asterias amurensis.* Samples collected in the second year (Stage II) will be analysed for *Undaria pinnatifida* following the development of an *U. pinnatifida* specific gene probe, similar to that used for *A. amurensis* (Deagle *et al*., 2001). Samples collected in both years will be preserved and may be re-analysed for either *A. amurensis* or *U. pinnatifida* (depending on the year of collection) if sufficient resources are available in the future. This document reports on the objectives, deliverables and results of Stage I of the project.

The stage I deliverables listed in the project proposal are as follows:

1. a quantified estimate of the translocation potential of various internal and external spaces and surfaces on fishing vessels, recreational vessels and aquaculture equipment. These results will provide:

    a. an empirical verification of the Infection Modes and Effects Analysis;

    b. vessel infection estimates that support bio-invasion risk assessment and management strategy evaluation for preventing and minimising the spread of *A. amurensis* in southeast Australia;

    c. data to support education campaigns for recreational boaters, fisherman and aquaculture operators; and,

2. a Bayesian journey survival model for *A. amurensis* that supports bioinvasion risk assessment and management strategy evaluation

It is important to note that the delivery of the journey survival model is subject to the success of the vessel sampling protocols. The model cannot be developed without a sufficient number of positive samples, collected over a period of at least two or three days.

The objectives of the project have been modified slightly since the submission of the proposal to reflect and support the development of the new National System for the Prevention and Management of Marine Pests in Australia. In particular a fourth objective has been added to assist in the development and implementation of the national protocols for the management of bio-invasion risks associated with recreational vessels and small fishing vessels.

# 3 METHODS

## 3.1 Field methods

*Fishing vessels and recreational yachts*

Fishing vessels and recreational yachts were sampled at two locations: the Royal Hobart Yacht Club and the Domain Slip Yard. Vessels were selected based on the slipping schedules of the club and the slip yard. These two slip yards provided access to a range of vessels including fishing vessels, yachts, motor boats and tugs. Vessel selection, access and permission from boat owners was organised by liaising with the Bosun at each of the slip yards.

Five field staff were necessary for quick and efficient sample collection and to prevent delays on the slip. On average, sample collection took between 45 to 60 minutes depending on the size of the vessel. Upon introduction the vessel owners were given a brief explanation of the project and methodology, provided with a project summary sheet (Appendix A) and asked to close all sea-cocks prior to slipping so that internal water samples could be collected. Sampling commenced as soon as the boat was safely secured on the slipway.

Samples were collected from external surfaces and accessible internal surfaces using plastic putty knifes, metal putty knives (for excessive growth of barnacles etc.) and metal vegetable peelers (for water inlets and outlets). Samples were rinsed from the utensils with 0.2µm filter water into sample jars. Hull surface samples were taken by scraping 6 (three port and three starboard) 0.5$m^2$ quadrats placed haphazardly on the hull and keel. Rudder surface samples were taken from 2 (one port, on starboard) 0.5$m^2$ quadrats.

Garboard planks, keel cooling pipes and stabilisers were sampled by scraping the entire width of the structure along a 1m section, usually from the top and bottom surfaces wherever appropriate. All other external features, such as paddle wheels, water inlet/outlet cover plates, echo sounders, etc were sampled as completely as possible. The interior surfaces of water inlets and outlets were sampled to maximum extent allowable by the diameter of the inlet and the width of the vegetable peelers.

All vessels were boarded to inspect the deck, fishing gear (if applicable) and internal spaces, such as anchor wells, cockpit bins, rudder control rooms, bilges, etc. Samples of water (and sediment) were collected wherever possible. Water samples were collected using a 60 ml syringe, electric mono pump (depending on volume) or by simply opening the sea-cocks and collecting the water that drained from the vessel into a sample jar or bucket. All field equipment that was used more than once was thoroughly washed with 0.2µm filter water and dried between samples to prevent cross-contamination.

All vessel owners were interviewed to acquire supplementary information such as the dimensions of the vessel, hull material, sailing and cleaning activities, name of antifouling paint applied to the vessel, and the date last applied. The degree of fouling on the boot tops, vertical bottom and flat bottom surfaces of the hull (port and starboard) was scored using industry standard hull fouling indices. All information was recorded on survey sheet specifically designed for this project (Appendix B).

Plankton samples were also collected at the sampling site in order to confirm the presence or absence of *Asterias amurensis* in the surrounding waters. Four (one for visual inspection), five minute samples were collected with an electric mono pump (CP 25) and sieved through a 100µm mesh plankton net. The mono pump was calibrated at 1-2m head, delivering 31 litres per minute (range of 30 – 31.6 litres per minute). The total volume of water sampled for each plankton sample was approximately 155 litres.

During the initial stages of the project, the project team liaised with CMR engineers to design and build a vacuum-based suction sampler. The suction sampler features a two-stage, in-line filtration system, with suction provided via a pump driven venturi. The system can be used with an interchangeable head to suit vessel fouling conditions (Appendix C). The vacuum sampler was not subsequently used, however, because it quickly became apparent that small vessels must be hauled onto a slip in order to allow access to, and collection of samples from, the many small niche areas on the hull, propeller and rudder. The vacuum-based sampler is better suited to sampling fouling from large commercial vessels, and may be used for these purposes in the future.

### *Aquaculture equipment*

The original proposal specifications stated that samples would be collected from fishing vessels, recreational vessels and aquaculture equipment (section 2). Prior to the collection of samples from aquaculture equipment, the survey team started to survey aquaculture operations in southeast Tasmania in order to gather information on the protocols, procedures and frequency of equipment movements around the state. The aim of this survey was to quantify the types and amount of equipment moved, and thereby target our sampling methodology. We were particularly interested in cage movements between fish farms in the Derwent and D'entrecasteaux channel, and the movement of seed and spat (locally and interstate) from hatcheries on the south and east coast of Tasmania. Upon contacting the Tasmanian Department of Primary Industries Water and Environment to obtain marine farm records we were requested to delay our survey, until further notice, because the Department staff were planning a similar survey in the near future and preferred that farmers be contacted only once to prevent “survey exhaustion”. Prior to this the project had already obtained preliminary cage movement data from two marine fish farms and made one site visit to an oyster farm. No other contact was made with representatives of the aquaculture industry and we did not subsequently sample any aquaculture equipment.

## 3.2 Laboratory methods

Upon returning to the laboratory all field equipment was thoroughly washed in hot water and detergent and air dried. All samples were washed with 0.2µm pressure-filtered seawater and sieved through a 106µm sieve within a few hours of being collected. Sieved samples were then weighed and if the wet weight exceeded 5g were split by weight into approximately two halves. Samples were weighed on Sartorius BL3100 scales (readability = 0.1g, linearity = 0.2g) that were tarred prior to each sample weight measurement. Samples that weighed less than 1.5 grams were allocated a nominal weight of 0.5 grams biomass because of the unreliability of very low wet-weight measurements – i.e. the mass of the water associated with the sample contributes a significant (but un-quantified) proportion of the overall mass of the sample.

After weighing, all samples were preserved in 25X SET buffered ethanol fixative and placed into a fridge prior to DNA extraction or pre-extraction processing where necessary (see below). The SET buffer fixative comprises 25ml of 90% reagent grade ethanol, 2ml Milli-Q-water and 3ml of 25X SET (Table 1).

**Table 1 Preparation of 25X SET buffer**

| Reagent | Stock in 400ml | To make 400ml of 25X SET | Final molarity |
|---|---|---|---|
| 3.75M NaCl | | 87.66g | 3.75M |
| 0.5M Na2 EDTA (ph8) | 74.4g | 20ml | 25mM |
| 0.8M Tris HCl (ph 7.8) | 50.42g | 200ml | 0.4M |

### *Pre-extraction sample processing*

All samples with large biomasses or with bulky organic matter (e.g. barnacles) underwent a second processing stage prior to DNA extraction and amplification. The average wet weight of samples from all locations was approximately 80g, whereas the maximum biomass the genetic probe can process without sub-sampling is approximately 1g. It proved necessary therefore to remove as much of the biomass as possible to eliminate the need to sub-sample during DNA extraction and thereby preserve the sensitively of the probe. This process was undertaken as a second step, after initial preservation due to concerns that live organisms may not be readily washed off their substrates.

During the second stage processing, bulky samples were rinsed with un-buffered reagent grade ethanol; this ethanol was used for one rinse only to avoid contamination between samples. Samples were rinsed through a 2mm mesh sieve in plastic dish full of ethanol and agitated for approximately one minute to encourage the finer particles to move through the sieve. The contents of the dish were then sieved through a 106µm sieve. The contents of this sieve were then washed with 25X SET buffered ethanol into a sample jar and placed in the fridge ready for DNA-extraction.

### *DNA extraction and amplification*

All samples were concentrated by vacuum filtration through a 5 µm pore-sized hydrophilic Durapore Filter (Millipore). The residue was briefly air-dried, weight measured, transferred to a 2ml tube and DNA extracted using the DNeasy Plant Kit (QIAGEN) following suppliers instructions. DNA was retrieved in 200µl elution buffer and stored at 4°C.

The amount of DNA in each sample was quantified (Gene Quant) and samples were diluted to a concentration of 5 -10 ng/µl. Samples were then subjected to a two step nested Polymerase Chain Reaction (PCR) to enhance sensitivity of the gene probe. Standard PCR was conducted using the mitochondrial COI primer pairs ECOLa and HCO. Standard PCR reactions were done in a 25µl volume containing 0.2 µM of each primer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1X AmpliTaq Gold® buffer and 0.625 units AmpliTaq Gold® (Applied Biosystems). Thermal cycling conditions were: 94°C for 9 minutes then 35 cycles (94°C, 30s / 54°C, 30s / 72°C, 1 minute) followed by 72°C for 5 minutes.

A secondary *Asterias* specific PCR was carried out using the CASF1 and CASR1 primer pairs (mitochondrial COI) in a 25µl volume containing 0.4 µM of each primer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1X AmpliTaq Gold® buffer and 0.625 units AmpliTaq Gold® (Applied Biosystems). Thermal cycling conditions during the PCR were: 94°C for 9 minutes then 40 cycles (94°C, 30s / 61°C, 30s / 72°C, 15s) followed by 72°C for 5 minutes.

A separate PCR reaction was carried out on all samples using universal ribosomal DNA primers (NSF 1179 and NSR 1642) to confirm suitability of each sample for PCR (Standard PCR conditions were used). Aerosol-resistant pipette tips were used with all PCR solutions and negative and positive control reactions were performed with each PCR cocktail. The PCR products were run on a 1.8 % Agarose gel. All gels were stained with ethidium bromide and visualized under UV light and documented using a Nikon Coolpix digital camera.

Randomly selected Asterias positive samples were sequenced for further conformation. The amplified PCR products were purified using the QIAquick PCR purification kit (QIAGEN). Sequencing reactions were carried out on both strands, using the original amplification primers, with the ABI Big Dye dideoxy terminator cycle sequencing kit (Applied Biosystems). Electrophoresis was carried out on an ABI-377 automated DNA sequencer and sequence data were edited with Sequence Navigator software (Applied Biosystems).

## 3.3 Data Analysis

The data analysis was performed in 4 discrete steps:

1. An initial exploration of the data;

2. A comparison of the predictions of the Infection Modes and Effects Analysis with the actual biomass (measured as wet weight) collected at the various sample sites;

3. An analysis of the probe results, focussing in particular on the incidence of *Asterias* positive samples in the plankton and the vessel samples; and,

4. An initial exploration of the relationship between biomass and important co-variates such as vessel activity, slipping schedules and paint type.

Item 4 is an important pre-cursor to the development of predictive statistical models that we hope will identify quantitative risk factors for fouling of small recreational and commercial craft. These models will be developed during the second year of the project, building on the results gathered to date and the additional samples that will be collected during the second year of the project.

This document does not develop a journey survival model for *Asterias* because to date we have been unable to collect the data necessary to develop a journey survival model– i.e. we have been unable to gain access, follow and sample individual (positive) vessels. Furthermore we cannot guarantee access to individual vessels. The delivery of this milestone in the second year of the project is contingent on suitable vessel access.

All data summary and analysis was performed using the programming language R (R Development Core Team, 2004). R is an open source code (http://www.r-project.org/) similar to S. The analysis code is reproduced in Appendix D. This document also reports on our initial investigation into the movement of aquaculture movement in the southeast of Tasmania and the results of the limited discussion held with representatives of the aquaculture industry.

# 4 RESULTS

## 4.1 Initial data exploration

The project team sampled 30 vessels between the 12th August 2003 and the 11th June 2004[1], comprising 20 yachts/motor cruisers, 9 fishing vessels and 1 tug. The team sampled or inspected 68 different locations in and around the hull, propeller, rudder and anchor, internal spaces, fishing gear and deck of these vessels. A total of 750 samples were taken. A further 204 inspections were made on board the vessels that resulted in no sample being taken. An additional 120 plankton samples – 4 for each vessel sampled – were also taken at the two survey sites. No samples were collected from the deck or fishing gear of any of the vessels taken – in all cases these locations were clean and dry when inspected by the project team[2].

Figure 1 plots histograms of the wet weight (grams) of the samples taken from the hull, internal spaces, and propeller, rudder and anchor. The data are clearly zero-inflated and censored. The lower limit on the reliability of the wet weight of the samples introduces (left) censorship because samples weighing ≤ 1.5 grams were assumed to contain 0.5 grams of biomass. The large number of samples in this category gives rise to zero-inflation. The term "zero inflated" is used to describe datasets that contain an excessive number of zeros, or in this case near zeros. The word inflation is used to emphasise that the probability mass at the point zero exceeds that allowed under any standard parametric family of distributions (Wanzhu, 2002). For example, 38 of the 144 hull quadrats weighed less than 1.5 grams. These types of datasets are very common in environmental science and, if not carefully modeled, will invalidate the distributional assumptions of many of the usual statistical procedures used to analyse environmental data (see below).

Figure 2 plots the histogram of the log-transformed data $x = f(x)$ where $x$ is the wet-weight of the sample in grams, and $f(x)$ is given by:

$$f(x) = log_{10}(x + a) \quad . \qquad [1]$$

The parameter $a$ in equation [1] is a "shift-parameter" which is commonly used in if the data cannot be smaller than a certain bound different from zero (Limpert *et al*., 2001). In this instance the lower limit on the reliability of wet weights suggested an upper bound for small samples of 1.5 grams. All such samples were assumed to contain 0.5 grams biomass, hence in this analysis $a$ was arbitrarily set to 1 to reflect this bound.

The transformed data $x = f(x)$ are still clearly zero-inflated, and also show a positive skew caused by rare recordings of relatively high biomass. Data of this type may be adequately described by a delta distribution (Wanzhu, 2002) – see section 4.4 Again it is important to note that the usual statistical tests based on comparison of means - such as Analysis of Variance, F-tests and General Linear Models – may not provide correct inferences unless this data is carefully modelled.

Figure 3 summarises the transformed data in the form of box plots by sampling location. The sampling location codes are taken from the survey sheets (Appendix B). The box in each of the box plots shows the upper and lower quartiles of the data, with the median shown by a line inside the box. The largest and smallest values (outliers excepted) are shown by dotted lines

[1] Sampling has continued since this date in order to complete the year – the results from these vessels will be reported in the second stage of the project.

[2] Following discussions with skippers it became apparent that all fishing vessels surveyed during the project had washed their gear (mainly pots) as soon as they returned to port – i.e. prior to slipping and our inspections.

ending in a bar. Outliers are denoted by circles. Table 2 summarises the IMEA score (section 4.2), mean, variance and sample size for each of the sample locations.

**Figure 1 Size frequency histograms of the wet weight (grams) of the samples**

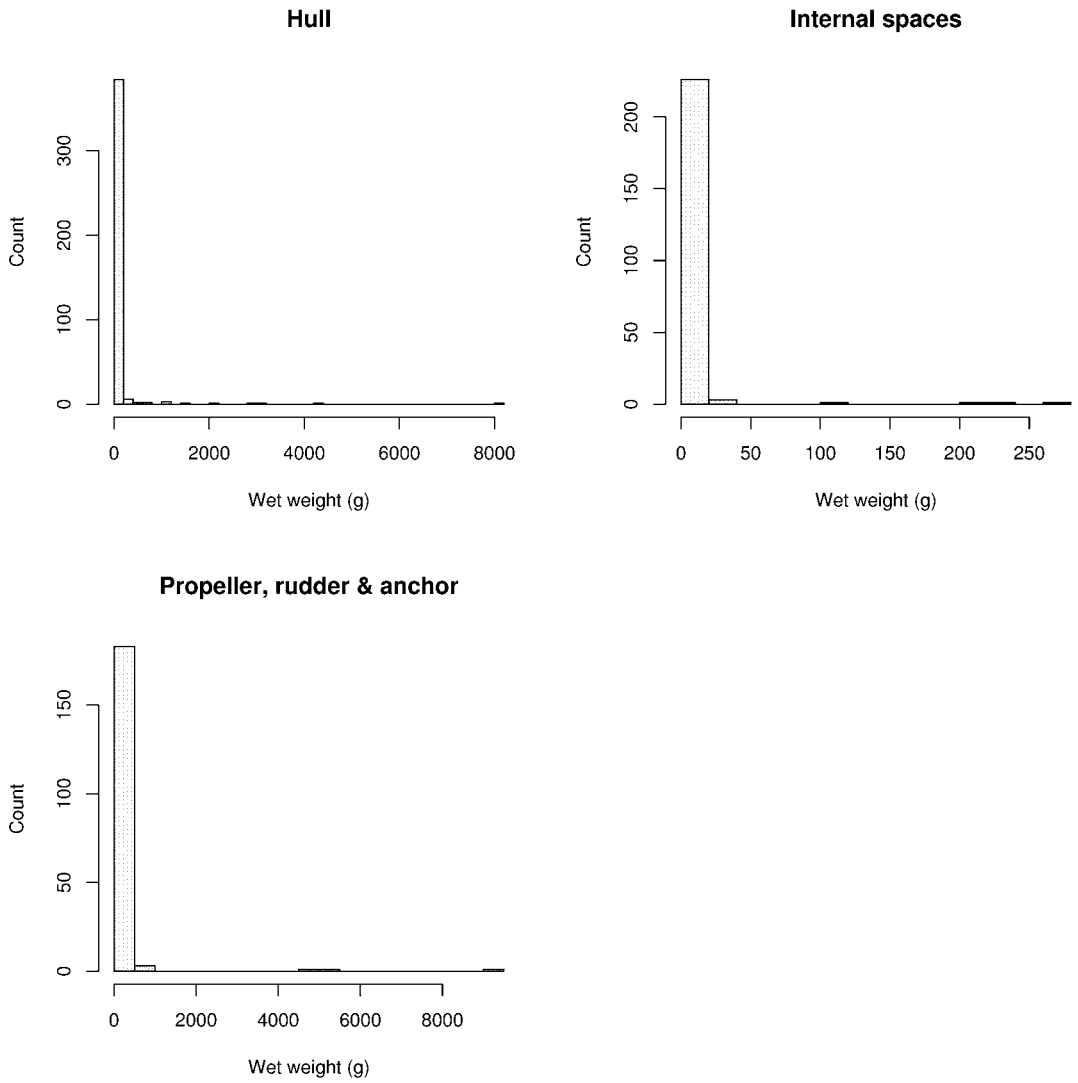


**Figure 2 Size frequency histograms of the transformed wet weight of samples**

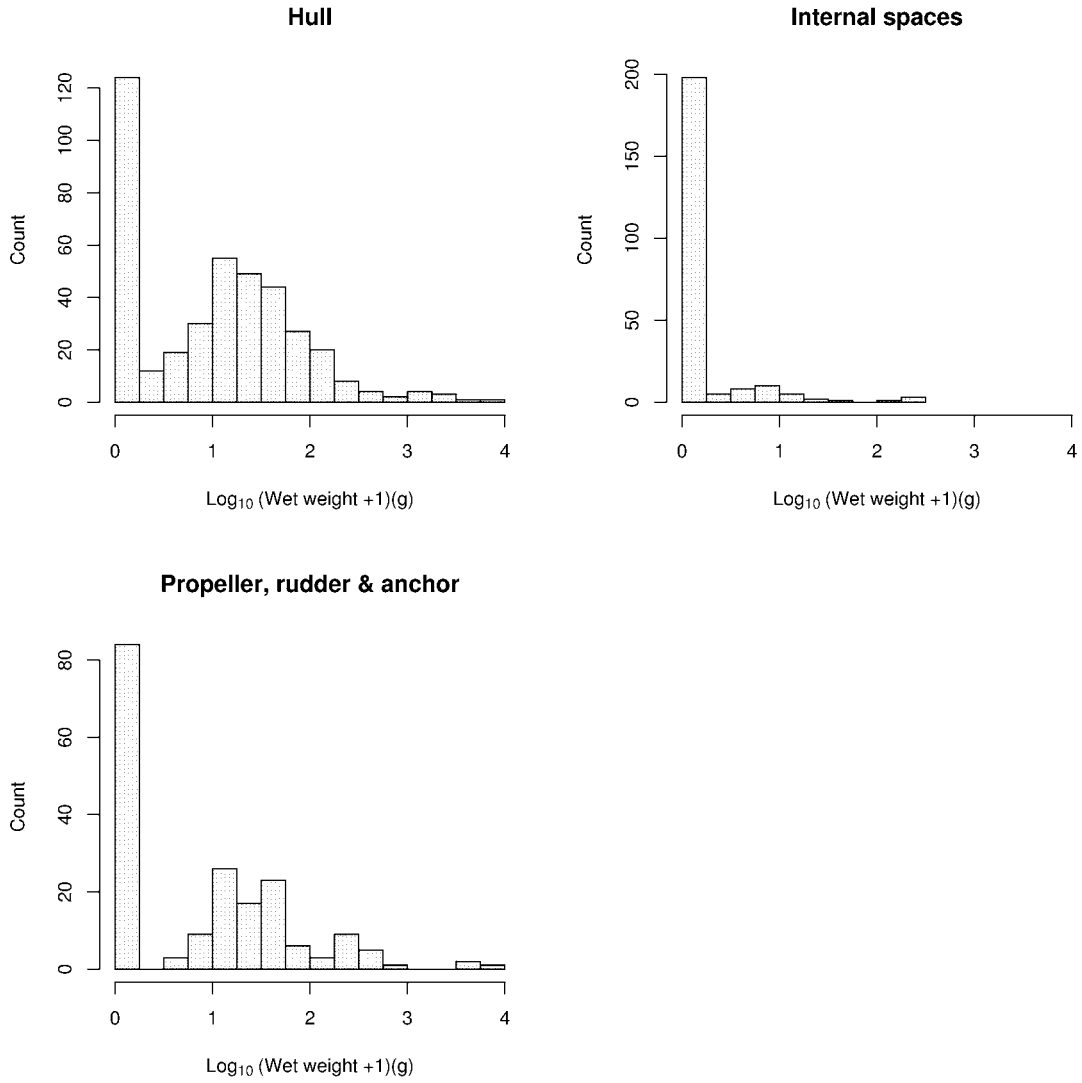


**Figure 3 Box plots of the transformed wet weight of samples summarised by sample location (see Table 2 and Appendix B for description of Location ID codes)**

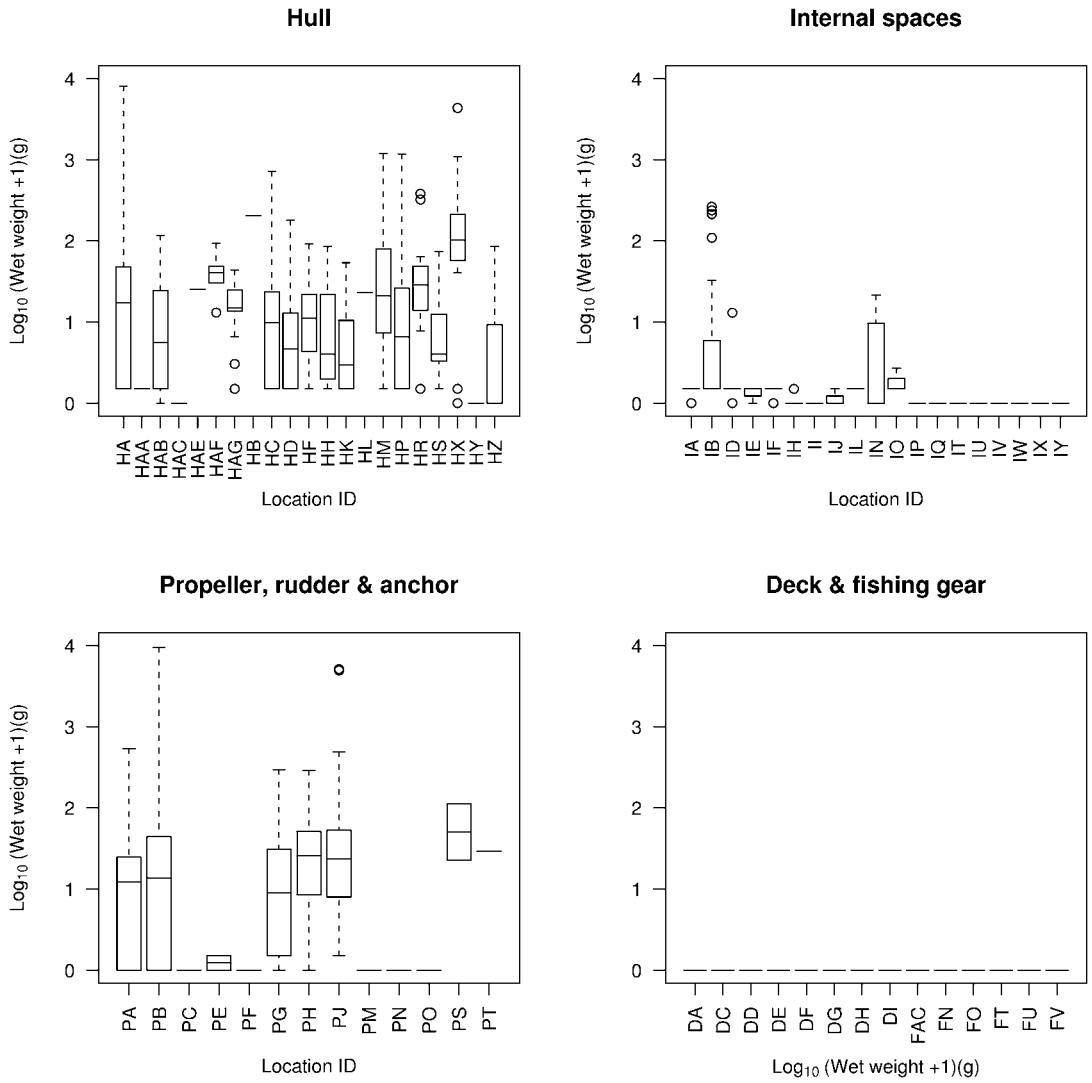


**Table 2 Mean, variance, sample size and IMEA score of the transformed wet weights of the samples**

| LocID | Location name | IMEA score | Mean | Variance | Sample size |
|---|---|---|---|---|---|
| HX | Garboard plank: external | 58.08 | 485.72 | 1229966 | 15 |
| PB | Propeller surface: external | 16.61 | 410.34 | 3076052 | 29 |
| PJ | Rudder surface: external | 7.44 | 228.58 | 875624.3 | 56 |
| HB | Sonar tubes: internal | 72 | 203 | NA | 1 |
| HA | Hull surface: external | 64.58 | 159.76 | 616909.2 | 143 |
| HM | Transducer: external | 90.25 | 147.5 | 101223.4 | 15 |
| HC | Skin fittings: external | 70.88 | 80.01 | 49865.81 | 10 |
| HR | Stabilisers: top | 48.12 | 68.74 | 13578.82 | 15 |
| PS | Propeller cowling: external | NA | 66.85 | 4077.04 | 2 |
| PA | Propeller shaft: external | 16.61 | 57.53 | 16708.29 | 24 |
| PH | Keel extension: external | 72.25 | 57.11 | 7534.83 | 13 |
| HP | Keel – fixed: external | 66.94 | 52.26 | 35109.68 | 40 |
| HAF | Radio earth plate: external | NA | 44.16 | 897.39 | 5 |
| PG | Stern tube: external | 63.07 | 31.61 | 4941.82 | 18 |
| PT | Rudder pintel: external | NA | 28.2 | NA | 1 |
| HZ | Marlin board: external | 41.11 | 28.19 | 2384.03 | 3 |
| HAE | Autopilot sensor: external | NA | 24.12 | NA | 1 |
| HL | Block space: external | 69.06 | 21.98 | NA | 1 |
| HH | Echo sounder booth: external | 65.95 | 19.74 | 923.39 | 11 |
| HD | Water inlet/outlet cover plates | NA | 18.82 | 1525.19 | 32 |
| HF | Keel cooling pipes: external | 70.72 | 18.7 | 647.02 | 11 |
| HS | Stabilisers: bottom | 48.12 | 17.98 | 950.29 | 5 |
| HAB | Zinc blocks: in front | 8.75 | 17.64 | 796.45 | 40 |
| HAG | Live catch tank inlet/outlet | NA | 16.72 | 103.62 | 21 |
| IB | Seawater/grey-water inlet/outlet: internal | 71.89 | 14.25 | 2351.61 | 73 |
| HK | Paddle wheel and booth: external | 72.22 | 8.9 | 214.47 | 20 |
| IN | Live catch circulation tank: internal | 48.75 | 5.82 | 81.21 | 5 |
| ID | Bilge – closed: water | 20.94 | 1.06 | 8 | 17 |
| IO | Live catch circulation tank: water | 29.25 | 0.9 | 0.48 | 3 |
| HAA | Zinc blocks: behind | 27.17 | 0.5 | 0 | 3 |
| IL | Live catch wet well: water | 29.25 | 0.5 | 0 | 2 |
| IA | Seawater/grey-water inlet/outlet: water | 75.08 | 0.48 | 0.01 | 33 |
| IF | Engine cooling water filter: water | 30.94 | 0.46 | 0.02 | 13 |
| IE | Bilge – open: water | 1 | 0.33 | 0.08 | 3 |
| PE | Outboard sail drive legs: external | 53.3 | 0.25 | 0.12 | 2 |
| IJ | Ballast tank: water | 41.25 | 0.17 | 0.08 | 3 |
| IH | Anchor well: water | 13.42 | 0.08 | 0.03 | 26 |
| DA | Cracks in deck: water | 6.19 | 0 | 0 | 8 |
| DC | Gunwale: sediment | 3.67 | 0 | 0 | 2 |
| DD | Hatches: water | 3.33 | 0 | 0 | 3 |
| DE | Cockpit bins/open storage: water | 3.75 | 0 | 0 | 3 |
| DF | Winch box: water | 3.75 | 0 | NA | 1 |
| DG | Deck surface: water | 2.75 | 0 | 0 | 2 |
| DH | Canvas screens: water | 1.5 | 0 | NA | 1 |
| DI | Bullwarks: sediment | 1.88 | 0 | NA | 1 |
| FAC | Marker bouys: water | 6.88 | 0 | NA | 1 |
| FN | Floats-pots: water | 22 | 0 | NA | 1 |
| FO | Floats-pots: external | 22 | 0 | NA | 1 |
| FT | Traps: water | 27.5 | 0 | NA | 1 |
| FU | Traps: external | 27.5 | 0 | 0 | 2 |

**Table 2 cont…**

| LocID | Location name | IMEA score | Mean | Variance | Sample size |
|---|---|---|---|---|---|
| HAC | Exhaust outlet: external | 12.25 | 0 | 0 | 8 |
| HY | Bob-stay fitting: external | 67.5 | 0 | 0 | 2 |
| II | Anchor well: sediment | 13.42 | 0 | 0 | 17 |
| IP | Rudder control room: water | 5.19 | 0 | 0 | 3 |
| IQ | Rudder control room: internal | 5.19 | 0 | 0 | 4 |
| IT | Storage rooms: water | 2.44 | 0 | 0 | 3 |
| IU | Storage boxes: water | 2.44 | 0 | 0 | 3 |
| IV | Wheelhouse: water | 3.75 | 0 | 0 | 11 |
| IW | Wheelhouse: sediment | 3.75 | 0 | 0 | 10 |
| IX | Dead catch store – spray room: water | 2.25 | 0 | 0 | 2 |
| IY | Dead catch store – insulated: water | 2.25 | 0 | 0 | 2 |
| PC | Propeller nozzle: external | 6.5 | 0 | NA | 1 |
| PF | Stern tube: internal | 12.86 | 0 | NA | 1 |
| PM | Anchor surface: external | 4.08 | 0 | 0 | 21 |
| PN | Anchor chain: external | 5.83 | 0 | 0 | 20 |
| PO | Sea anchors: external | 1.5 | 0 | NA | 1 |
| FV | Traps: internal | NA | 0 | NA | 1 |
| HAD | Exhaust outlet: internal | 1 | NA | NA | NA |
| HE | Echo sounder booth: internal | 43.97 | NA | NA | NA |
| HG | Water inlet/outlet cover plates: internal | 70.71 | NA | NA | NA |
| HJ | Paddle wheel and booth: internal | 72.22 | NA | NA | NA |
| HN | Keel – retractable: external | 76.5 | NA | NA | NA |
| HO | Keel – retractable: internal | 76.5 | NA | NA | NA |
| HQ | False keel: external | 52 | NA | NA | NA |
| HT | Rolling chock: top | 57.44 | NA | NA | NA |
| HU | Rolling chock: bottom | 57.44 | NA | NA | NA |
| HV | Head fitting: external | 76.5 | NA | NA | NA |
| HW | Head fitting: internal | 76.5 | NA | NA | NA |
| IC | Sewage holding tank: water | 27.5 | NA | NA | NA |
| IG | Engine cooling water filter: internal | 30.94 | NA | NA | NA |
| IK | Ballast tank: sediment | 41.25 | NA | NA | NA |
| IM | Live catch wet well: internal | 48.75 | NA | NA | NA |
| IR | Ice makers (sea water): water | 1 | NA | NA | NA |
| IS | Shower holding tank: water | 1.5 | NA | NA | NA |
| PD | Outboard sail drive legs: internal | 41 | NA | NA | NA |
| PI | Keel extension: internal | 72.25 | NA | NA | NA |
| PK | Tiller flat: external | 15 | NA | NA | NA |
| PL | Tiller flat: internal | 15 | NA | NA | NA |
| PP | Sea anchors: water | 1.5 | NA | NA | NA |
| PQ | Anchor buoys: external | 1.5 | NA | NA | NA |
| PR | Anchor buoys: water | 1.5 | NA | NA | NA |

The ten most significantly fouled locations on the vessels sampled during the first year of the project were (in descending order of the mean): the garboard plank; the surface of the propeller and rudder; the inside surfaces of the sonar tube; the surface of the hull; the external surfaces of transducer blocks bolted to the hull; hull surface skin fittings (usually water inlet/outlet blanks); the top surface of stabilizers; propeller cowlings; and, propeller shafts. It is important to note, however, the small sample sizes associated with the sonar tubes and propeller cowlings – the significance of the results for these locations must be treated with caution.

Sample locations whose mean and variance are zero represent areas that were inspected by the survey team but found to be free of water, sediment or fouling. Sample locations whose mean and variance is marked "NA" are areas that the team either did not encounter or locations that were inaccessible to us during sampling. For example none of the vessels sampled had retractable keels (location code HN). Similarly the survey team were unable unscrew the cover plates of seawater/grey-water inlets/outlets and were therefore unable to samples the interior surfaces (location code HG) of these areas.

Figure 4 plots the mean of the transformed data *f(x)*, averaged across all locations, against each of the three main location categories hull; propeller, rudder and anchor; and, internal spaces, distinguished by vessel type (yacht, fishing vessel and tug). There is clearly a reasonably close correlation between the hull samples and the samples taken from the propeller, rudder and anchor (Spearman's rho statistic = 0.74), whereas there is very little relationship between the samples taken from the internal spaces of the vessel and the hull (Spearman's rho statistic = 0.29), or the propeller, rudder and anchor (Spearman's rho statistic = 0.16).

Figure 5 plots the relationship between the hull fouling indices for the boot top, vertical bottom and flat bottom (averaged across the port and starboard) and mean wet weight of the transformed data *f(x)* for the hull category. Interestingly a very similar pattern is apparent in all three plots: from 0 to 30 the hull fouling indices appear unable to resolve mean bio-fouling less than approximately 50 grams – i.e. there is very little or no relationship between the hull fouling indices less than 30 and mean fouling biomass less than 50g. Thereafter, however, there appears to be a reasonably close correlation between the mean level of biofouling and the hull fouling indices – indicated by the points rising to the top right hand corner of each graph. It is also interesting to note that the hull fouling indice used in the project moves from weed (20 – 30) to calcareous fouling and fouling fauna (40 +) across this transition.

These results may have important implications for the new national protocols for the management of biofouling risks associated with small craft. For example the draft management process for international yachts and apprehended vessels relies heavily on the degree of biofouling around the water line (i.e. the boot top) to determine whether or not the vessel is issued a clearance certificate. These results suggest low levels of biofouling on the hull may not be distinguished by a qualitative assessment of the fouling along the boot-top. More over whilst the level of fouling on the hull is indicative for the rudder, propeller and anchor, it is unlikely to reflect to the level of fouling in the internal spaces.

The pattern of these results may also reflect the delta-like distribution of samples noted above. Determining predictive covariates (such as activity patterns, paint type and age, etc.) for the parameters of this distribution would represent an important step forward in the assessment of the bio-fouling risks associated with small craft (see section 4.4).

**Figure 4** **The relationship of the mean transformed data, averaged across all locations, between the three main location categories.**

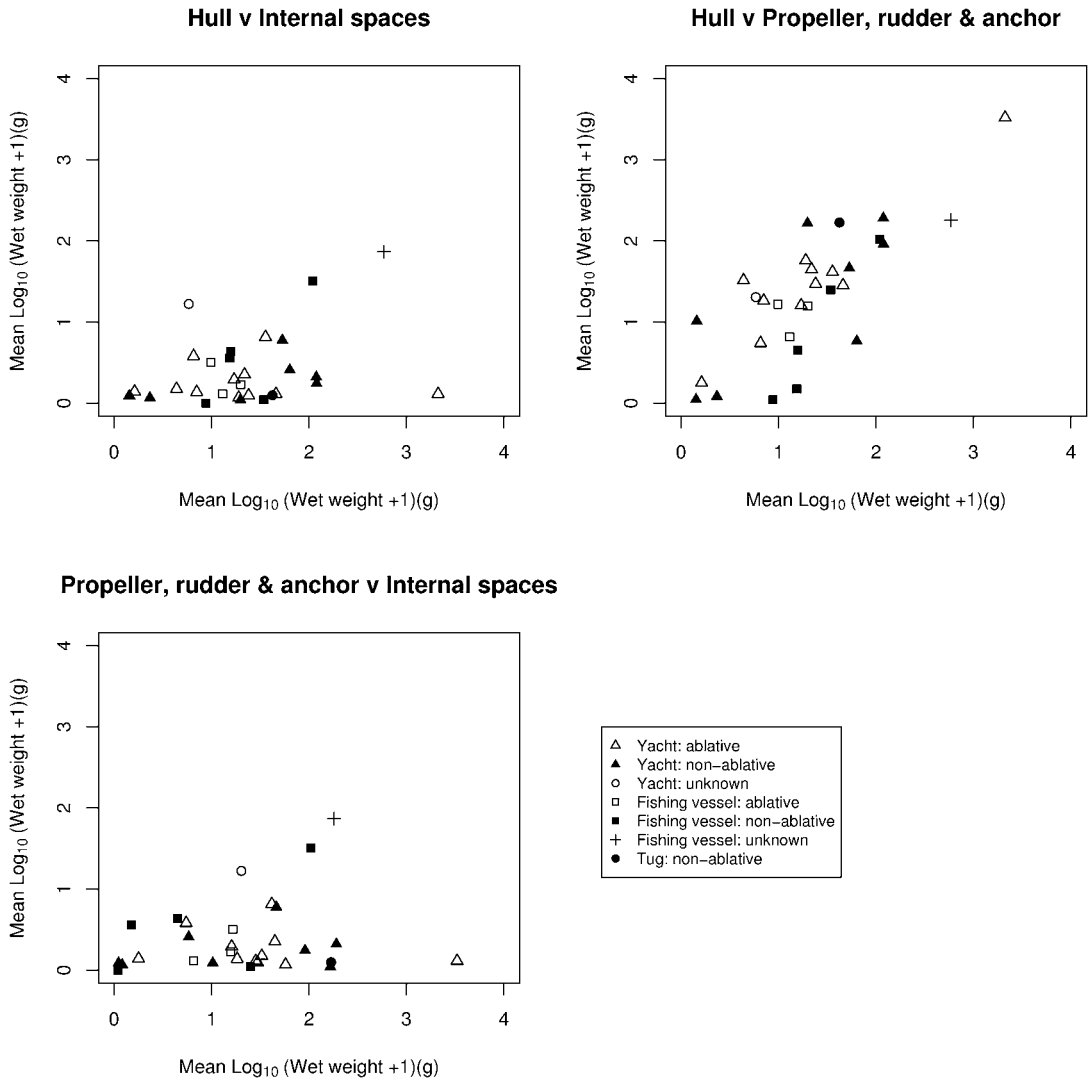
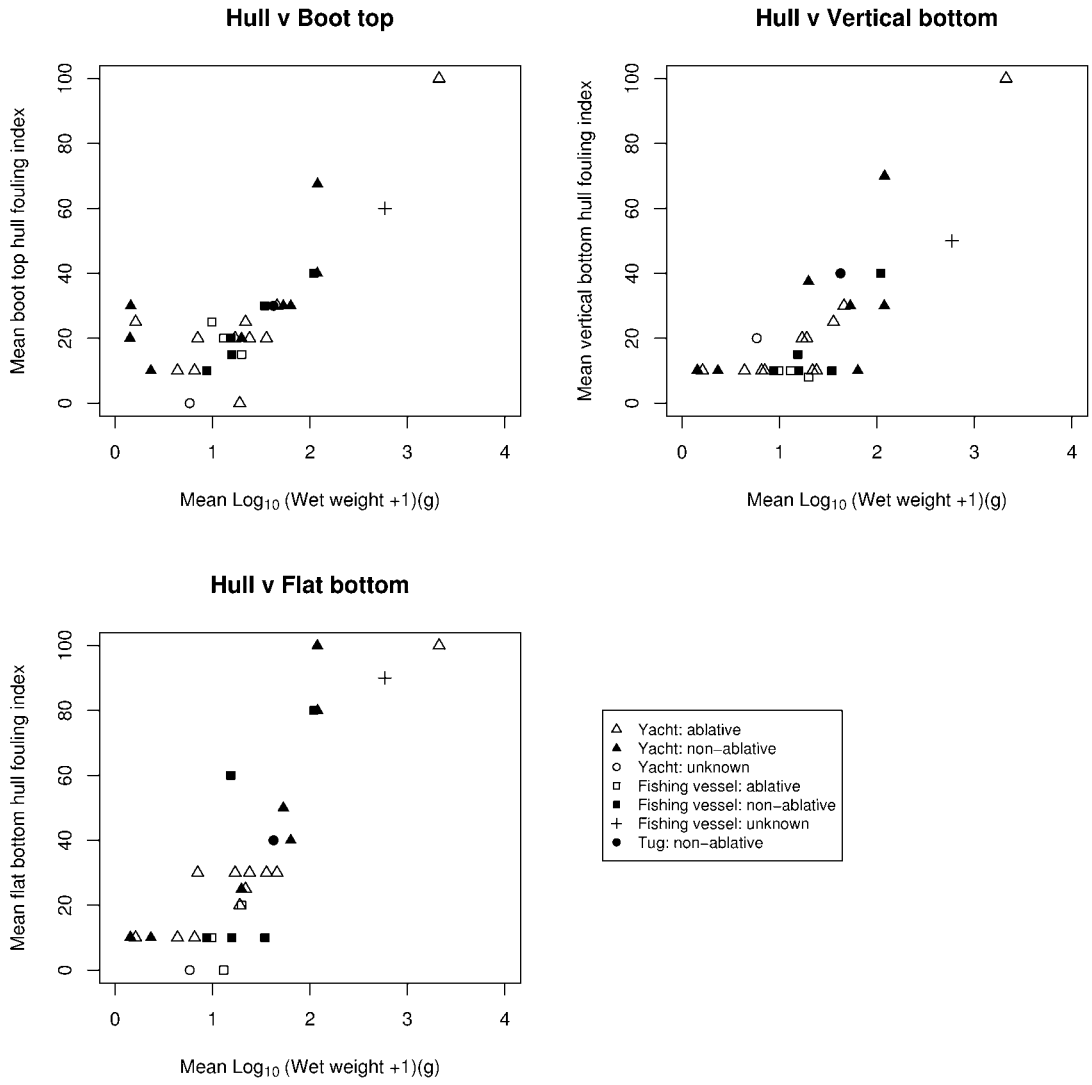


**Figure 5 Relationship between the three hull fouling indices and the mean transformed data of the hull category**



## 4.2 Infection Modes and Effects Analysis

The Infection Modes and Effects Analysis (IMEA) ranks vessel sub-components according to: a) their environmental suitability; b) the occurrence of marine organisms; and, c) the likelihood of detection (Hayes, 2002). The results gathered in this project verify the occurrence of marine organisms through their abundance measured via wet biomass, and to a lesser extent environmental suitability in so far as it is related to the presence/absence of marine organisms in certain locations of a boat. In this context we assume that the mean wet weight of biofouling taken from each location is a reasonable proxy for the bio-invasion hazard associated with that location

Figure 6 plots the mean wet weight of the log-transformed data *f(x)*, for each sample location, against the severity score x occurrence score allocated to that location in the IMEA analysis. Points that lie close to, or parallel to the line $y = x$ reflect accurate predictions, points which deviate from this line represent predictions that were either too high or too low. In this context it is important to note that the IMEA analysis does provide an absolute measure of biofouling wet-weight, but rather a relative measure of biofouling hazard –hence points that lie along any single line, parallel to the line $y = x$ represent good agreement between the two data sets.

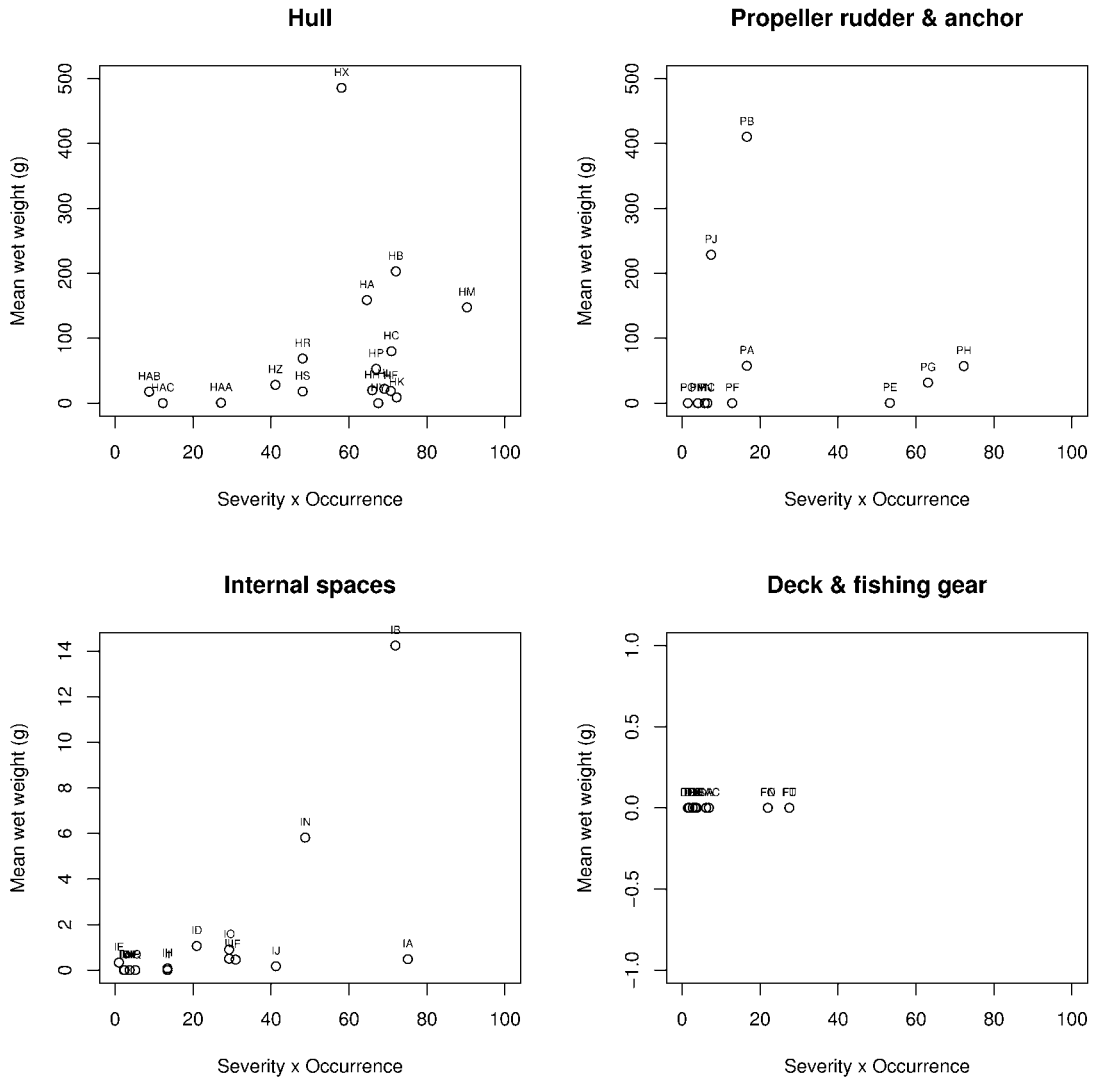
For the hull locations it is apparent that there is a reasonably good agreement between the IMEA predictions and wet weight of biomass found in the following locations (in ascending order of biofouling hazard): external surfaces of exhaust outlets, the inside surface of cathodic zinc blocks, the external surfaces of the marlin board, the top and bottom surfaces of fixed stabilizers, the hull surface and the internal surfaces of sonar tubes. Again some these results need to be treated cautiously due to the small sample size and high variance (Table 2).

There is a group of 8 hull locations that had less fouling that was suggested by the IMEA analysis, namely: the paddle wheel, the external surfaces of keel cooling pipes; the surface of the fixed keel, external surfaces of skin fittings, the bob-stay fitting, the external surfaces of echo-sounder booths, the block spaces, and the external surfaces of transducers bolted to the hull. The interpretation of some of these results, however, is complicated by differences between the surface areas of the locations concerned. For example skin fittings, transducers, block spaces and paddle wheels are very much smaller in area than the $0.5m^2$ quadrats taken on the hull, keel and rudder. It is not surprising therefore that these areas have less biofouling than areas scored equivalently in the IMEA analysis. The surface area of the keel quadrats, however, are exactly equivalent to the hull quadrats, thereby identifying this a truly anomalous result.

The amount of fouling on the garboard plank is also anomalous when compared to the predictions of the IMEA analysis. This location (1m scrape along the entire width of the plank) had significantly higher biofouling than other areas scored equivalently in the IMEA analysis – in effect the IMEA under emphasized the bio-fouling hazard posed by this location.

The results for the propeller, rudder and anchor fall into three distinct groups. The first group consists of two locations that were under-rated by the IMEA: the surface of the propeller and the rudder. Both of these areas scored low in the IMEA, but both proved to hold relatively large amounts of fouling (but again note the high variance). During the IMEA analysis, these areas scored lower than the hull surface because some of the participants believed that the high flow velocity associated with the propeller and rudder made these areas less suitable for fouling organisms. The variance of the IMEA score for the propeller surface, however, was relatively high (see Tables 3 and 4, Hayes, 2002) because of disagreement among the workshop participants about how stationary the propeller (which is not anti-fouled) may be, and therefore how much fouling might colonise it.

**Figure 6 IMEA predictions compared to the mean wet weight *f(x)* of the samples, aggregated by location**



With the exception of the water and internal surfaces of seawater/grey-water inlets/outlets, and ballast water, the level of biofouling found in the internal spaces is reasonably well reflected by the IMEA predictions. In this context it should be noted that the wet-weight of biofouling is not a reasonable reflection of the bio-invasion hazard associated with any of the water samples taken from vessels because this metric does not capture the bio-invasion hazards associated with microscopic organisms such as larvae, diatoms and dinoflagellates. The severity x occurrence score of all the other internal spaces cluster around 0 to 30 (relatively low) and this is reflected in the low mean wet weight of samples collected from these locations. The only exception to this pattern is the internal surfaces of live catch tanks, which scored quite highly and also had a moderate level of fouling as compared to other internal spaces. Again, however, the surfaces area of this sample (in this case $0.5m^2$) was much higher than other internal spaces.

No biomass samples were collected from either the deck or the fishing gear of the vessels sampled during the first year of the project. For the deck these results accord well with the predictions of the IMEA which allocated low scores to all of the locations within this category. For fishing gear, however, there is some departure from the predictions of the IMEA, particularly for the external surfaces and water collected in traps and floats and pots. The survey team found all traps, floats and pots to be clean and dry upon inspection. As noted above, however, virtually all of the fishing gear inspected by the project team had been cleaned by the crew prior to the vessel being slipped.

## 4.3 Gene probe results

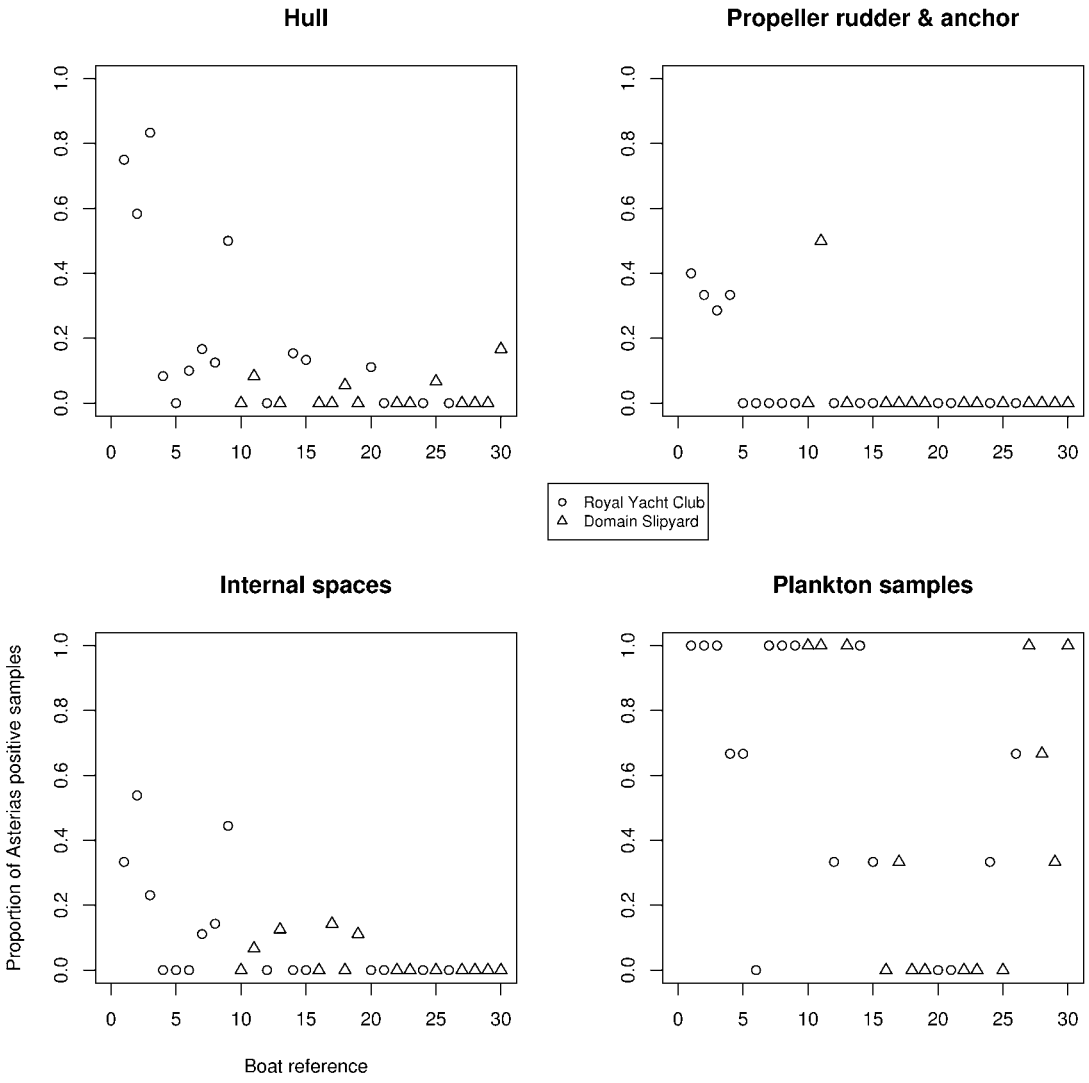
The large number of samples collected during the first stage of the project, coupled with the additional time need to pre-process biofouling samples prior to probing, conflicting project demands and insufficient genetics staff, caused a back log in the probing of the processed DNA. All of the vessel and plankton samples were processed and DNA extracted in June 2004, however, the quantification and DNA probing of all samples was not completed until September 2004.

Figure 7 plots the proportion of the probed samples that tested positive for the presence of *Asterias amurensis*. Interestingly there is a clear decline in the proportion of the positive samples from the hull and internal spaces, between the first vessel (sampled on the 12th August 2003) and the twentieth vessel (sampled on the 25th February 2004). This decline is less apparent in the samples obtained from the propeller, rudder and anchor because 3 of the 6 samples from this location taken from boat 11 (sampled on the 4th November 2003) tested positive. Whilst the total number of samples from each location category are similar between boats, they are not equal between location categories and cannot therefore be compared across categories.

The proportion of positive plankton samples broadly reflects the breeding cycle of *Asterias amurensis* in the Derwent estuary. There is a small window between the 3rd of February and the 18th of March during which time none of the plankton samples returned positive results. This is consistent with previous data on the life-cycle of *A. amurensis* in Derwent. The samples taken on the 23rd of April (boat 25) were also negative but thereafter, from the 28th of April onwards, one or more of the plankton samples tested positive. This suggests that the *A. amurensis* starts to spawn in the Derwent in mid-to late April, approximately two months earlier than previously reported (Bruce *et al*., unpub. data) and one month earlier than recorded in the ballast water Decision Support System databases.

Five vessels – 6, 18, 19, 20 and 25 - returned *Asterias* positive samples with negative plankton samples. The two vessels in the limit (6 and 25) do not provide strong evidence for settlement because the sample dates on both occasions are within the spawning season of *Asterias amurensis*, and hence the positive sample may simply reflect the presence of larvae or gametes in the seawater associated with the sample (despite the negative plankton samples) and we cannot discriminate between settled juveniles in the bio-fouling samples, and larvae/gametes in the seawater associated with the samples.

**Figure 7 Proportion of Asterias positive samples from the three main location categories and the plankton**



The positive samples associated with boats 18, 19, and 20, however, provide much stronger evidence for settlement because they are all contiguous throughout the period of negative plankton samples. The positive sample on boat 18 (sampled 3rd of February) came from a scrape of the external surface of a keel cooling pipe. The positive sample from boat 19 came from the internal surface of a seawater inlet (a live catch circulation tank). In both cases the wet weight of sample biomass was approximately 20 grams. The positive sample from boat 20 came from a keel quadrat but in this case the wet weight of the sample biomass was less than 1.5 grams

Table 3 summarises the proportion of *Asterias* positive samples by vessel location. Eight out of twenty hull locations sampled returned positive samples: the hull surface and keel, zinc anodes, skin fittings, water inlet/outlet cover plates, depth sounders, paddle wheels and the garboard plank. Only four locations on the propeller, rudder or anchor returned positive samples: the propeller shaft, propeller surface, stern tube and rudder surface. Most of these locations figure prominently in Table 2 – i.e. are among the most heavily fouled locations of the vessels sampled here. None of these locations, however, returned *Asterias* positive samples outside of the plankton period.

Five internal spaces returned positive samples: water and scrapes from seawater/grey-water inlets/outlets (including live catch circulation tanks), water from the anchor well and water from live catch circulation tanks. These areas are capable of moving (albeit very small) quantities of water from one location to another and may therefore represent a translocation risk even without settlement. Note that in this context water inlet/outlets are very unlikely to represent a translocation risk if the vessel's seacocks are open during the voyages. Evidence of settlement on the intake of a live catch circulation tank outside the plankton period of *Asterias amurensis* (see above) points to the further possibility of a translocation hazard.

All but two of the live catch storage tanks on the fishing vessels surveyed were circulation tanks, as opposed to wet wells (Table 2). Both of the wet well water samples collected by the project team were probed and found to be negative. The potential for circulations tanks to translocate water seems limited but cannot be confirmed without further analysis. The survey team collected water from 3 circulation tanks, one of which tested positive for *Asterias amurensis*. This vessel, however, was sampled during the spawning period of *A. amurensis* and hence this positive result may simply reflect water collected in the tank as the vessel was brought up on the slip.

Similarly most of the anchor wells inspected by the survey team were self draining and would not therefore represent a translocation risk. Indeed the project team inspected 26 anchor wells (Table 2) but only collected water samples from three. The *Asterias* positive anchor well of vessel 13 (a fishing vessel) was not self-draining and contained a relatively substantial amount of water. This situation might represent a translocation risk if this water were drained into a location free of *Asterias amurensis*.

## 4.4 Statistical modelling

The level of biofouling associated with small craft may be well explained by a delta distribution. The delta distribution with parameters $p$, $\mu$ and $\sigma$ is denoted as $X \sim \Delta(p, \mu, \sigma^2)$ and defined as

$$X \approx \begin{cases} 0 & \text{with probability } p \\ log\ normal\left(\mu, \sigma^2\right) & \text{with probability } (1-p) \end{cases} . \qquad [2]$$

The delta distribution is able to describe data that is zero-inflated and account for positive skew in the non-zero observations (see section 1). The (albeit limited) sampling conducted during the first stage of this project suggests that this may prove to be a useful predictive model for biofouling of small craft.

**Table 3 Proportion of Asterias positive samples by location**

| boatID | LocID | Sample size | Ast +ve | Prop. Ast +ve |
|---|---|---|---|---|
| 1 | HA | 6 | 5 | 0.83 |
| 2 | HA | 4 | 2 | 0.50 |
| 3 | HA | 4 | 4 | 1.00 |
| 6 | HA | 6 | 1 | 0.17 |
| 7 | HA | 4 | 1 | 0.25 |
| 9 | HA | 4 | 3 | 0.75 |
| 14 | HA | 5 | 1 | 0.20 |
| 30 | HA | 5 | 1 | 0.20 |
| 1 | HAB | 1 | 1 | 1.00 |
| 3 | HAB | 1 | 1 | 1.00 |
| 4 | HAB | 1 | 1 | 1.00 |
| 15 | HAB | 2 | 1 | 0.50 |
| 14 | HC | 1 | 1 | 1.00 |
| 2 | HD | 2 | 1 | 0.50 |
| 3 | HD | 1 | 1 | 1.00 |
| 8 | HD | 1 | 1 | 1.00 |
| 9 | HD | 1 | 1 | 1.00 |
| 11 | HD | 3 | 1 | 0.33 |
| 18 | HF | 2 | 1 | 0.50 |
| 2 | HH | 1 | 1 | 1.00 |
| 2 | HK | 1 | 1 | 1.00 |
| 3 | HK | 1 | 1 | 1.00 |
| 7 | HK | 2 | 1 | 0.50 |
| 9 | HK | 1 | 1 | 1.00 |
| 15 | HK | 2 | 1 | 0.50 |
| 2 | HP | 2 | 2 | 1.00 |
| 3 | HP | 2 | 2 | 1.00 |
| 20 | HP | 2 | 1 | 0.50 |
| 25 | HP | 2 | 1 | 0.50 |
| 30 | HP | 1 | 1 | 1.00 |
| 3 | HX | 1 | 1 | 1.00 |
| 1 | IA | 1 | 1 | 1.00 |
| 2 | IA | 4 | 4 | 1.00 |
| 3 | IA | 2 | 1 | 0.50 |
| 8 | IA | 2 | 1 | 0.50 |
| 9 | IA | 3 | 2 | 0.67 |
| 17 | IA | 2 | 1 | 0.50 |
| 2 | IB | 4 | 3 | 0.75 |
| 3 | IB | 4 | 2 | 0.50 |
| 7 | IB | 4 | 1 | 0.25 |
| 9 | IB | 6 | 2 | 0.33 |
| 13 | IH | 2 | 1 | 0.50 |
| 19 | IN | 2 | 1 | 0.50 |
| 11 | IO | 1 | 1 | 1.00 |
| 2 | PA | 1 | 1 | 1.00 |
| 4 | PA | 2 | 1 | 0.50 |
| 11 | PA | 2 | 1 | 0.50 |
| 1 | PB | 1 | 1 | 1.00 |
| 4 | PB | 2 | 1 | 0.50 |
| 3 | PG | 1 | 1 | 1.00 |
| 11 | PG | 2 | 2 | 1.00 |
| 1 | PJ | 2 | 1 | 0.50 |
| 2 | PJ | 2 | 1 | 0.50 |
| 3 | PJ | 2 | 1 | 0.50 |
| 4 | PJ | 2 | 1 | 0.50 |

The aim of a predictive bio-fouling model is to identify quantitative risk factors for bio-fouling of small craft. Quantitative risk factors are explanatory covariates those values potentially affect the parameters of the delta distribution. One of the aims of the second stage of the project is to develop a generalized linear model for these parameters. There are number of challenges to this approach, notably: the different sample sizes between locations, the high variance, the fact that only the hull, propeller and keel quadrats were collected in a uniform and comparable manner, and the large number of potential explanatory covariates – the most obvious being paint type and age, and vessel activity patterns.

An initial examination of the quadrat data suggests that the only significant covariate on the level of biofouling in the hull quadrats is the number of days since the vessel was last slipped (a proxy for age of the anti-fouling paint). Figure 8 plots the mean wet weight of the transformed data *f(x)* for the quadrat collected samples, against the number days between the sample date and the date the vessel was last slipped. The vessel type and paint type (ablative versus non-ablative) are distinguished here. There are clearly no outstanding vessel-type and paint-type effects but these need to be confirmed following a more extensive analysis of the data.

The tug sampled during the project is a clear outlier – particularly with respect to the keel quadrats. Subsequent discussions with the vessel operators revealed that this vessel is moored in a near-freshwater environment in the upper reaches of the Derwent estuary, but operates almost every day in the lower more saline reaches of the estuary. This dramatic change in salinity may explain the very low levels of fouling found on the vessel despite the fact that it had not been slipped for over 800 days.

A simple regression model for the mean wet weight of the hull quadrats in grams ($x$), excluding the tug data, is given by:

$$log_{10}(x+1) = 0.0024(days) + 0.658 \quad . \qquad [3]$$

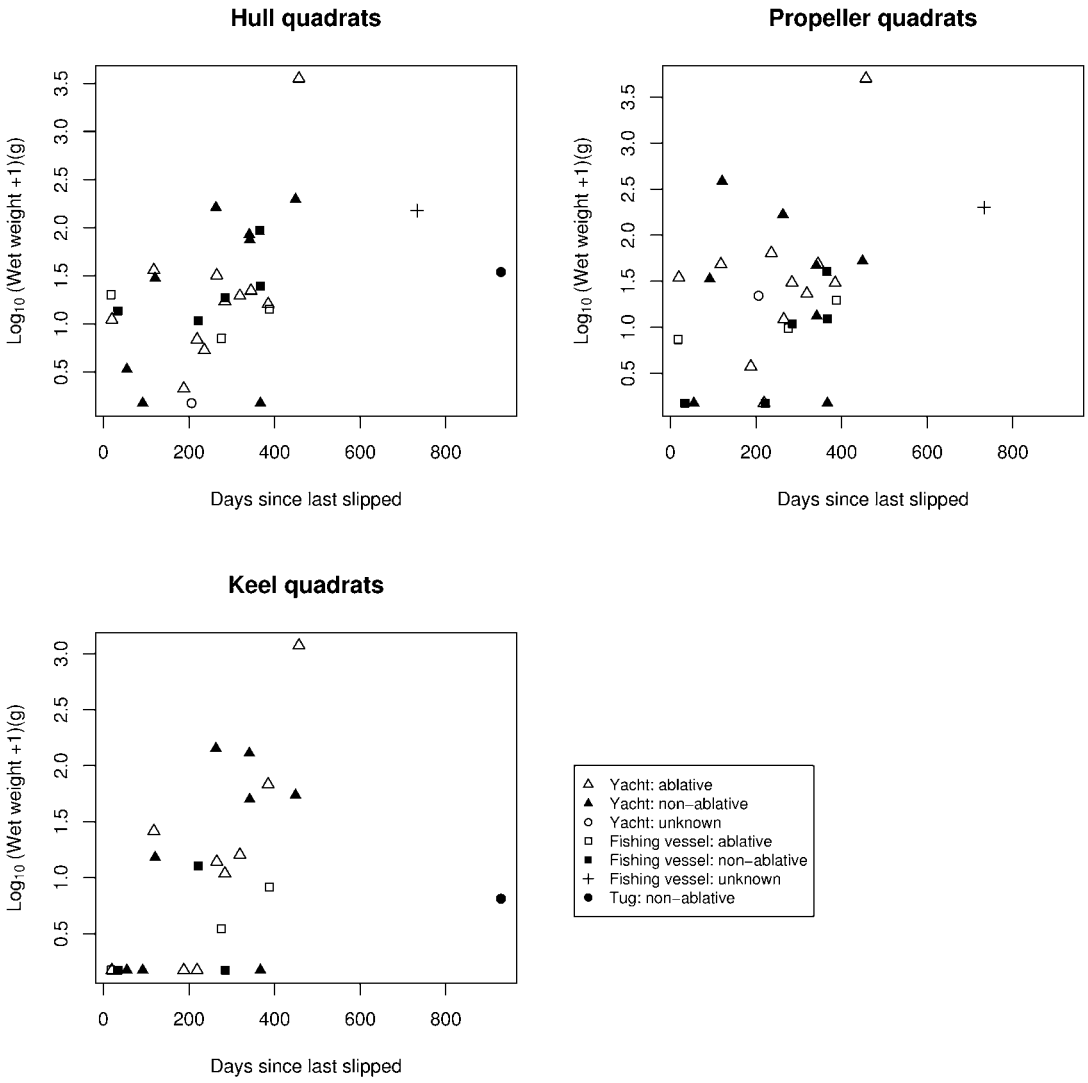
Both terms in the this model are significant different from zero at the $p = 0.05$ level. The adjusted $r^2$ value, however, is low (0.23) indicating that there is substantial variation about this line. This model suggests that it takes approximately 440 days to achieve a mean wet-weight of 50 grams, at which point the level of fouling at boot-top may transit from weed to calcareous fouling (see section 4.1). It is important to emphasise, however, that these are very preliminary investigations. These models, the delta model and the influence of co-variates on it parameters will be developed further in the second stage of the project.

## 4.5 Aquaculture equipment

Initial consultation with finfish and oyster farmers has indicated the fouling is prevalent on marine farming equipment. In summer for example, fish cage nets sometimes need to be changed every three weeks to manage the fouling problem. This is of concern particularly when fish cages are transported between fish farms. Data for the frequency of fish cage movements for two fish farms in south eastern Tasmania are summarised in Figure 9. While very preliminary, the map below demonstrates the potential for transport of marine pests via aquaculture equipment. In addition, the oyster farmer we spoke to said that he occasionally received ‘dirty’ seed, which contained other organisms besides oysters, e.g. small crabs.

A comprehensive survey for all marine farms is required to fully quantify this risk. In addition site visits and inspection of marine farm equipment are important to determine the likelihood of aquaculture equipment (e.g. fish cages and hatchery seed and spat) transporting marine pests. The marine farmers we spoke with were very cooperative and indicated their willingness to allow site visits, sample collection on leases and cooperation in future experimental trials (e.g. grow out trials for seed and spat from hatcheries).

**Figure 8 The relationship between the mean transformed wet weight of quadrat samples and the date since the vessel was last slipped**



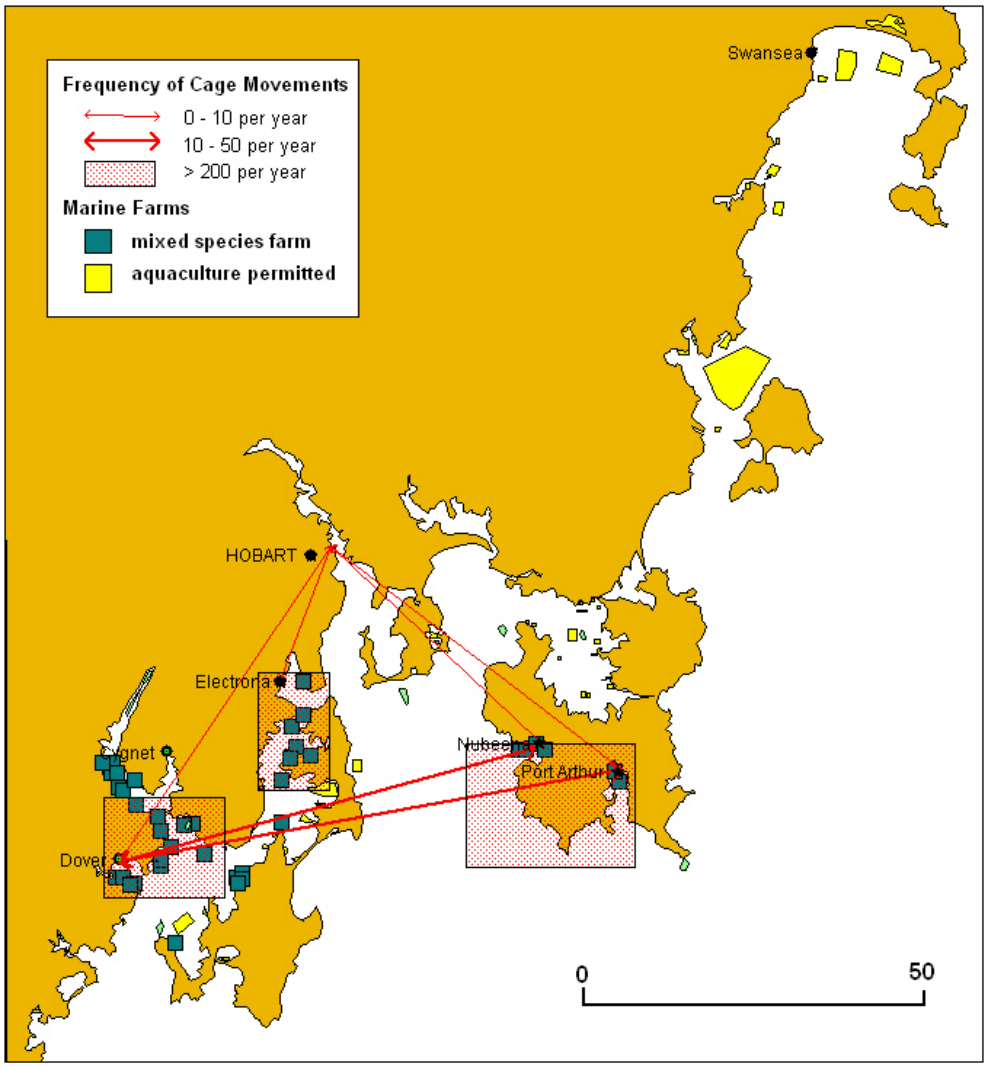



Figure 9 The location of aquaculture facilities in south eastern Tasmania, including the frequency of cage movements for selected fish farms. Map modified with permission from the Department of Fisheries and Forestry Australia (Larcombe *et al.*, 2002)

# 5 SUMMARY AND DISCUSSION

The overall aim of the empirical validation project is to gather quantitative information that supports the development and implementation of the new national protocols designed to minimise the introduction and translocation risks associated with small craft, fishing vessels and aquaculture equipment. The project also aims to investigate the translocation potential of small craft for specific pest species such as *Asterias amurensis* and *Undaria pinnatifida* and ultimately aims to identify quantitative risk factors for small craft bio-fouling that would enable managers to predict significantly fouled vessels for quarantine and compliance purposes.

The current draft assessment protocol for recreational craft and apprehended vessels relies heavily on a qualitative inspection of the degree of fouling present on the hull around the waterline. The initial results from the first year of this project suggest that this approach may have poor resolution at relatively low fouling levels (e.g. mean wet weight of hull fouling less than 50 grams), but good resolution thereafter, and more importantly may not reflect the level of fouling in internal spaces.

It is important to note that most of the vessels sampled during the first year of this project are relatively well maintained. As such there was a high proportion of clean vessels – over a quarter of the hull quadrats sampled weighed less than 1.5 grams – in the sample. There were, however, rare occurrences of very heavily fouled vessels. This pattern gives rise to zero inflated, positively skewed data that may be well described by a delta distribution. On of the key aims of the second stage of the project is to model the effect of potential explanatory co-variates (such as anti-fouling paint age and type, and vessel activity patterns) on the parameters of this distribution. A preliminary analysis of the results, however, suggests that significant hull fouling (greater than 50 grams per $0.5m^2$) is likely to develop on anti-fouled vessels after approximately 400 days. Fouling may be greater in other niche areas.

The sample locations associated with the hull, propeller, rudder and anchor, internal spaces, deck and fishing gear, were identified by an inductive hazard analysis (Infection Modes and Effects Analysis) completed two years ago by CMR and the Victorian DNRE. The wet weight of the samples was used to verify the environmental suitability and occurrence scores allocated to these locations by participants in the hazard analysis workshops. Wet weight, however, is not a good surrogate for the bio-invasion hazard associated with microscopic organisms such as diatoms and dinoflagellates, and did not therefore support the IMEA predictions associated with water retained on board small craft.

By and large the results developed in the document supported most of the IMEA predictions. Notable exceptions include the garboard plank (which had much higher levels of fouling than suggested by the IMEA), the block spaces and other small niche areas on the hull (which had much lower levels of fouling than suggested by the IMEA) and the rudder surface (which had higher levels of fouling than predicted). These results will be re-assessed at the end of the second stage of the project by which time the higher sample size should provide a better level of resolution. The level of fouling associated with fishing gear was also significantly different from that predicted by the IMEA. The results reported here are not an accurate reflection of the translocation risks associated with fishing gear because this gear was washed prior to the vessels being slipped and inspected by the survey team.

The first year's field work was deliberately timed to coincide with the peak period of *Asterias amurensis* larval production in the Derwent estuary, and to continue on beyond this in order to check for the possible settlement of juvenile starfish on the biofouling of small craft. The presence of *A. amurensis* was confirmed using genetic probes (for microscopic life-stages) and morphological identification (for macroscopic adult life-stages). The genetic detection methods used in this project build on those developed for the National Heritage Trust funded Port of Hastings study, conducted by Victorian EPA and for which CSIRO.

This project demonstrates that it is possible to process hull fouling samples for probe application, but in many cases this requires extensive pre-probe processing that is costly both in terms of materials and labour. Indeed the time costs associated with the pre-processing, coupled with the large number of samples, led to substantially greater operational and staff costs than anticipated.

The probe results reported here provide the first evidence of settlement of *Asterias amurensis* on small recreational and commercial vessels. Three contiguous vessels returned positive samples outside the planktonic period of *A. amurensis.* The translocation risks associated with this are difficult to determine. The settlement dynamics of *A. amurensis* on mobile surfaces has yet to be investigated and the relatively small proportion of positive samples from each of the positive vessels, however, suggests that the translocation risk is likely to be small compared to that associated with the ballast water large commercial vessels. Nonetheless it is important to note that these results indicate that small vessels may spread *A. amurensis* to new areas.

The plankton samples collected during the project also indicate that the planktonic period of *Asterias amurensis* in the Derwent is longer than previously thought, starting approximately two months earlier than previously reported and one month earlier than recorded in the ballast water Decision Support System databases.

The proportion of positive samples seems to decrease quite dramatically between August and December, which probably reflects the declining presence of *Asterias* larvae and gametes in the sea-water associated with the samples. Positive water samples were collected from the internal spaces of eight vessels. These positive results, however, indicated a potential translocation risk on only one occasion - water from an anchor well that was not self draining. Virtually all of the other internal spaces that are routinely filled with water, on the boats inspected to date, were self draining – particularly live catch circulation tanks and anchor wells. For these reasons, and a lack of prolonged access to a vessel, the project was unable to develop a journey survival model for *Asterias*.

The project team collected limited information on the movement of aquaculture around the south-east of Tasmania in the early stages of the project but thereafter the survey team did not inspect or collect samples from aquaculture equipment during the first stage of the project.

# ACKNOWLEDGEMENTS

We would like to acknowledge the assistance and co-operation of Greg Mannering, Bosun at the Royal Hobart Yacht Club and Wayne Barrens, Bosun at the Domain slip yard. Both Greg and Wayne provided logistical support and initiated contact with vessel owners to ensure that we had permission to sample their vessels. They provided us with their slipping schedules to enable us to schedule our sampling events in advance, and provided us with up to date information in the event of any schedule changes.

The following vessel owners are also to be thanked for allowing us to sample their vessels: Geoff Copson, Trevor Shawman, H.C. Peacock, Robert De Jonge, Ben Latham, Bruce Palmer, Jason Evans, Kim Newstead, Brian Butler, Ron Wells, Mick Daft, David Gough, Rob Rattray, Dave Weir, Peter Bristow, Ian Davenport, Tim Nolisher, Chris Parker, Steve Glover, Des Clark, Rob Giblin, Bob Drysdale, Northwest Shipping Tugs, Fred Binns, Russell Potter, David Bevan, BF Kaye-Hall, Nigel Mayes, Alan Rust and Bob White.

This study was partially funded by the Australian Department of Environment Heritage through the Natural Heritage Trust under the Empirical Validation project – Stage I (CSIRO reference gy40a).

# REFERENCES

Baltz DM (1991) Introduced fishes in marine systems and inland seas. Biological Conservation, 56: 151-177.

Carlton JT (1985) Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. Oceanography and Marine Biology: An Annual Review, 23: 313-371.

Carlton JT (1995) Biological invasions and cryptogenic species. Ecology, 77(6): 1653-1655.

Carlton JT (2001) Introduced Species in US Coastal Waters: Environmental Impacts and M

Deagle, B.E., Bax, N., Hewitt, C.L., Patil, J.G. (2003). Development and evaluation of a PCR-based test for detection of *Asterias* (Echinodermata: Asteroidea) larvae in Australian plankton samples from ballast water. Marine and Freshwater Research 54:709-719.

Garnham, J. (1998). Distribution and impact of *Asterias amurensis* in Victoria. IN: *Proceedings of a meeting on the biology and management of the introduced seastar Asterias amurensis in Australian waters, 19 May 1998*, (Goggin, C.L. Eds) Centre for Research on Introduced Marine Pests Technical Report 15, Hobart, Tasmania, Australia, p.18-21.

Hayes K. R. (2002), Identifying hazards in complex ecological systems – Part II: Infection Modes and Effects Analysis for biological invasions. Bioinvasions

Larcombe J, Brooks K, Charalambou C, Fenton M, Fisher M, Kinloch M, Summerson R. (2002), Marine Matters, Atlas of marine activities and coastal communities n Australian's South East Marine Region. Agriculture, Fisheries and Forestry Australia Report. Pg 161.

Limpert E., Stahel W. A. and Abbt M. (2001), Log-normal Distributions across the Sciences: Keys and Clues. *BioScience*, **51**(5): 341-352.

Patil, J.G., Hayes, K.R., Gunasekera, R.M., Deagle, B.E., McEnnulty, F.R., Bax, N.J. and Hewitt, C.L. (2004). Port of Hastings National Demonstration Project – Verification of the Type II error rate of the Ballast ater Decision Support System (DSS). Final report for Environmental Protection Authority as project managers for the Victorian Government consortium of the Department of Sustainability and Environment, Department of Infrastructure and EPA, and project partners Australian Quarantine and Inspection Service and CSIRO Marine Research.

R Development Core Team (2004), *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ross D. J. (2001), Impact of Northern Pacific Seastar on Soft Sediment Assemblages. PhD Thesis, University of Tasmania, 170 pp.

Talman, S., Bite, J.S., Campbell, S.J., Holloway, M., McArthur, M., Ross, D.J., Storey, M. (1999). Impacts of Introduced Marine Species in Port Phillip Bay. IN: *Marine Biological Invasions of Port Phillip Bay, Victoria*, (Hewitt, C.L., Campbell, M.L., Thresher, R.E., Martin, R.B. Eds) CRIMP Technical Report No. 20, CSIRO Marine Research, Hobart, Tasmania, Australia, p.261-274.

Turner, E. (1992). A northern Pacific seastar, *Asterias amurensis*, in Tasmania. AMSA Bulletin 120(October):18-19.

Thresher RE, Hewitt CL and Campbell ML (1999) Synthesis: Introduced and cryptogenic species. In: Hewitt CL, Campbell ML, Thresher RE and Martin RB (eds) Marine Biological

Invasions of Port Phillip Bay, Victoria. CRIMP Technical Report 20, pp. 283-295. CSIRO Division of Marine Research, Hobart, Australia.

Walford L and Wicklund R (1973) Contribution to a World Wide Inventory of Exotic Marine and Anadromous Organisms. Fisheries Technical Paper No. 121, Food and Agricultural Organisation, Rome, Italy, 49 pp.

Wanzhu T. (2002), Zero-inflated data, pp. 2387-2392 in El-Shaarawi A. H. and Piegorsch W. W. (Eds) *Encyclopedia of Environmetrics*, John Wiley & Sons, Chichester, England.

Ward, R. D. and Andrew, J. (1995). Population genetics of the northern Pacific seastar *Asterias amurensis* (Echinodermata:Asteriidae): allozyme differentiation among Japanese, Russian, and recently introduced Tasmania populations. Marine Biology 124:99-109.

Willan RC, Russel BC, Murfet NB, Moore KL, McEnnulty FR, Horner SK, Hewitt CL, Dally GM, Campbell ML and Bourke ST (2000) Outbreak of *Mytilopsis sallei* (Recluz, 1849) (Bivalvia: Dreissenidae) in Australia. Molluscan Research, **20**(2): 25-30

# APPENDIX A INFORMATION SHEET



**Small Vessel Translocation of Key Threatening Species**

**Background Information**
Humans are transporting organisms and introducing them to new areas at an unprecedented rate. Introductions of non-native species are a serious threat to the diversity of plants and animals as they invade natural areas and replace native species.

Hull fouling of yachts and fishing boats is contributing to the introduction and spread of non-native species to new sites. The 1999 black-striped mussel incursion in Darwin is a recent example. It was first introduced on the hull of an ocean-going yacht, and then spread to nearby marinas by other local yachts.

In southeast Australia, introduced marine pests such as the northern Pacific seastar, *Asterias amurensis* and Japanese kelp, *Undaria pinnatifida* are a serious threat. The northern pacific seastar is an important predator of scallops and mussels, while Japanese kelp fouls aquaculture facilities increasing operation costs and may hinder abalone and urchin divers. These “key threatening species” are spreading around the coastlines of southeast Australia. Recreational vessels may be inadvertently assisting this process.

**What Are We Doing?**
The CSIRO is undertaking a study to assess the spread of these two introduced pests via small vessels from their main population centres in Tasmania and Victoria to other uninfected regions.

Both *Undaria* and *Asterias* have a water borne and settled stage, therefore we are interested in collecting fouling and water samples. We will be taking samples from wet internal surfaces such as bilges and chain lockers, and from external surfaces such as the hull and keel, which will be scrapped using both rubber and metal scrapers while the boat is slipped.

We will be targeting recreational vessels, fishing vessels and aquaculture facilities that operate in areas where the northern Pacific seastar and/or Japanese kelp occur in high densities, such as the Derwent estuary.

**For More Information.**
If you would like to know more about CSIRO Marine Research and introduced species, visit http://crimp.marine.csiro.au/ or contact Keith Hayes on 6232 5260 or Caroline Sutton on 6232 5386 to discuss the current project.

# APPENDIX B SURVEY RECORD



*Empirical validation survey*

## A. VESSEL & OWNER DETAILS

| | |
|---|---|
| Date: | Location: |
| Vessel name: | Vessel type: |
| Vessel registration: | Owner/skipper name: |
| Owner/skipper tel: | Vessel length (m/ft): |
| Vessel draft (m/ft): | Vessel beam (m/ft): |
| Displacement (tonnes): | Length on water line (m/ft): |
| Hull material(s): | Length between perpendiculars (m/ft): |

## B. ACTIVITY DETAILS

| | |
|---|---|
| Location(s) vessel is usually moored: | |
| Max. # of trips per annum: | Min. # trips per annum: |
| Max. trip duration (days): | Min. trip duration (days): |
| Max. trip distance (kms): | Min. trip distance (kms): |
| Max. # of stops per trip: | Min. # of stops per trip: |
| Date of last trip: | Destination: |
| Antifouling name: | Date last antifouled: |
| Date last slipped: | Date last in water hull cleaning: |

**Boat name:** **Date:** **Samplers initials:**

## C. HULL FOULING RATING

| | HULL SECTION | | |
|---|---|---|---|
| | Boot top | Vertical bottom | Flat bottom |
| PORT | | | |
| STARBOARD | | | |

0 A clean, foul-free surface

10 Continuous and gradual gradations of shades of red and green (incipient slime)

20 Slime as dark green patches with yellow and/or brown coloured areas (advanced slime)

30 Weed as filaments up to 3 in. in length, projections up to 1/4 in. in height; or a flat network of filaments, green, yellow, or brown in colour

40 Calcareous fouling on edges, welded seams, corners, or as discrete patches covering flat areas roughly 9 to 10 in. in diameter

50 Random and scattered distribution of fouling by marine animals** on slightly curved or flat surfaces

60 Area distribution of fouling by marine animals 1/4 in. in diameter or less; fouling does not completely cover or blank out surface

70 Fouling by marine animals 1/4 in in diameter or less that completely covers surface in patches exceeding 9 to 10 in. in diameter; with radiating fringes of fouling growth

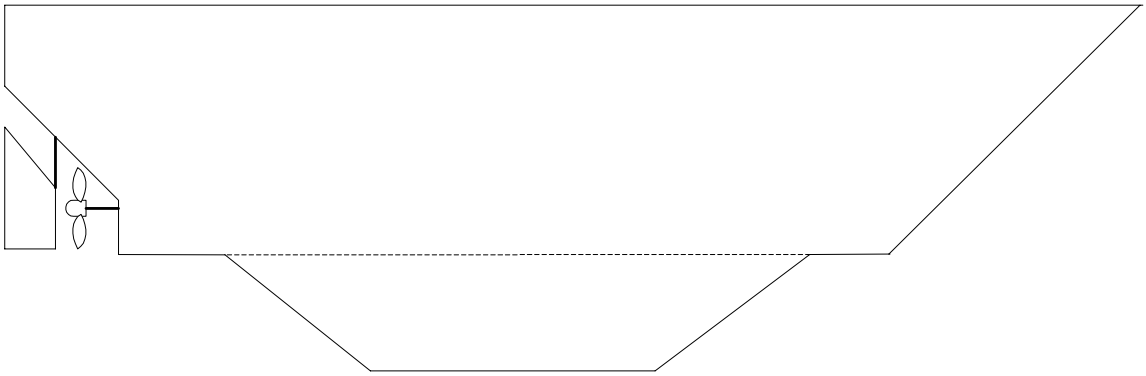
80 Dense fouling by marine animals with upright growth away from hull surface, calcareous shells appear clean or white in colour

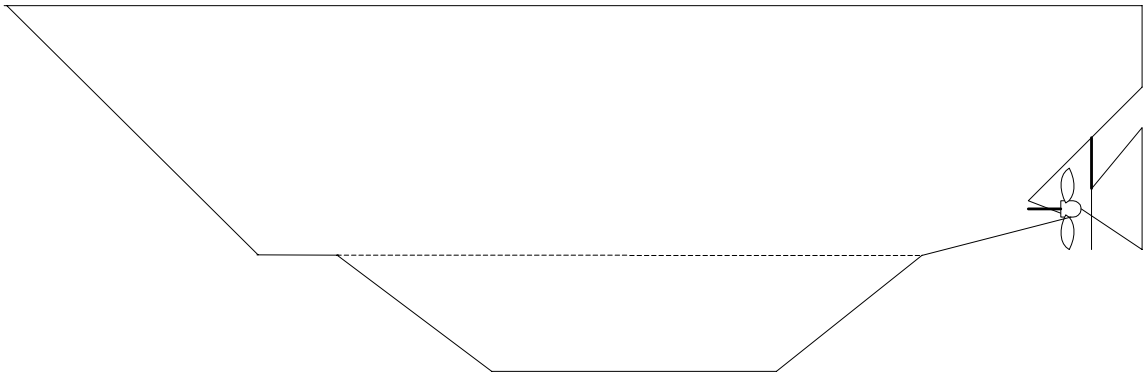
90 Dense fouling by marine animals with upright growth away from hull surface, calcareous shells brown in colour or with slime and/or weed overlay

100 All forms of fouling present particularly soft sedentary animals without calcareous covering

** Fouling marine animals typically include tubeworms, barnacles, bryozoans, hydroids, sea squirts and molluscs.

**Boat name:** **Date:** **Samplers initials:**





**B Boat name: Date: Samplers initials:**

*Vessel survey record – Hull*

| CODE | LOCATION | SAMPLE | PHOTO | NOTES |
|---|---|---|---|---|
| HA | Hull Surface: External | | | |
| HB | Sonar tubes: Internal | | | |
| HC | Skin fittings: External | | | |
| HD | Water inlet/outlet cover plates: External | | | |
| HE | Depth sounder booth: Internal | | | |
| HF | Keel cooling pipes: External | | | |
| HG | Water inlet/outlet cover plates: Internal | | | |
| HH | Depth sounder booth: External | | | |
| HI | Bob-stay fitting: Internal | | | |
| HJ | Paddle wheel and booth: Internal | | | |
| HK | Paddle wheel and booth: External | | | |
| HL | Block space: External | | | |
| HM | Transducer: External | | | |
| HN | Keel - retractable: External | | | |
| HO | Keel - retractable: Internal | | | |
| HP | Keel - fixed: External | | | |
| HQ | False keel: External | | | |
| HR | Stabilisers/trim tabs - folding: Top | | | |
| HS | Stabilisers/trim tabs - folding: Bottom | | | |
| HT | Rolling chock - fixed: Top | | | |
| HU | Rolling chock - fixed: Bottom | | | |
| HV | Head fitting: External | | | |
| HW | Head fitting: Internal | | | |
| HX | Garboard plank: External | | | |
| HY | Bob-stay fitting: External | | | |
| HZ | Marlin board: External | | | |
| HAA | Zinc blocks: Behind | | | |
| HAB | Zinc blocks: Front | | | |
| HAC | Exhaust outlet: External | | | |
| HAD | Exhaust outlet: Internal | | | |
| HAE | Autopilot sensor | | | |
| HAF | Radio earth plate: External | | | |
| HAG | Live catch tank – inlet/outlet: Internal | | | |
| | | | | |
| | | | | |
| | | | | |

**Boat name:** **Date:** **Samplers initials:**

*Vessel Survey Record – Propeller, rudder & anchor*

| CODE | LOCATION | SAMPLE | PHOTO | NOTES |
|---|---|---|---|---|
| PA | Propeller Shaft: External | | | |
| PB | Propeller Surface: External | | | |
| PC | Propeller nozzle: External | | | |
| PD | Outboard sail drive legs: Internal | | | |
| PE | Outboard sail drive legs: External | | | |
| PF | Stern tubes cover/stern gland: Internal | | | |
| PG | Stern tubes cover/stern gland: External | | | |
| PH | Keel extension/Skeg: External | | | |
| PI | Keel extension/Skeg: Internal | | | |
| PJ | Rudder surface: External | | | |
| PK | Tiller flat: External | | | |
| PL | Tiller flat: Internal | | | |
| PM | Anchor Surface: External | | | |
| PN | Chain: External | | | |
| PO | Sea anchors/parachutes: External | | | |
| PP | Sea anchors/parachutes: Water | | | |
| PQ | Anchor buoys: External | | | |
| PR | Anchor buoys: Water | | | |
| PS | Propeller cowling: external | | | |
| PT | Rudder pintel: External | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**Boat name:** **Date:** **Samplers initials:**

*Vessel Survey Record – Internal spaces*

| CODE | LOCATION | SAMPLE | PHOTO | NOTES |
|---|---|---|---|---|
| IA | Seawater/grey-water inlet/outlets: Water | | | |
| IB | Seawater/grey-water inlet/outlets: Internal | | | |
| IC | Sewage holding tank: Water | | | |
| ID | Bilge - closed: Water | | | |
| IE | Bilge – open: Water | | | |
| IF | Engine cooling water filter: Water | | | |
| IG | Engine cooling water filter: Internal | | | |
| IH | Anchor well: Water | | | |
| II | Anchor well: Sediment | | | |
| IJ | Ballast tanks/brine storage tanks: Water | | | |
| IK | Ballast tanks/brine tanks: Sediment | | | |
| IL | Live catch wet well: Water | | | |
| IM | Live catch wet well: Internal | | | |
| IN | Live catch circulation tank: Internal | | | |
| IO | Live catch circulation tank: Water | | | |
| IP | Rudder control room: Water | | | |
| IQ | Rudder control room: Internal | | | |
| IR | Ice makers (sea water): Water | | | |
| IS | Shower holding tank: Water | | | |
| IT | Storage rooms: Water | | | |
| IU | Storage boxes: Water | | | |
| IV | Cockpit/Wheelhouse: Water | | | |
| IW | Cockpit/Wheelhouse: Sediment | | | |
| IX | Dead catch storage – spray room: Water | | | |
| IY | Dead catch storage – insulated: Water | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**Boat name:** **Date:** **Samplers initials:**

*Vessel Survey Record – Fishing gear*

| CODE | LOCATION | SAMPLE | PHOTO | NOTES |
|---|---|---|---|---|
| FA | Net – beach seine: Water | | | |
| FB | Net – beach seine: External | | | |
| FC | Net - purse: Water | | | |
| FD | Net - purse: External | | | |
| FE | Net - gill: Water | | | |
| FF | Net - gill: External | | | |
| FG | Net - trawl: Water | | | |
| FH | Net - trawl: External | | | |
| FI | Net - dip: Water | | | |
| FJ | Net - dip: External | | | |
| FK | Net reels: Water | | | |
| FL | Net reels: External | | | |
| FM | Trawl boards: External | | | |
| FN | Floats - pots: Water | | | |
| FO | Floats - pots: External | | | |
| FP | Trap ropes: External | | | |
| FQ | Traps - octopus: Water | | | |
| FR | Traps - octopus: External | | | |
| FS | Traps - octopus: Internal | | | |
| FT | Traps - Cray/king crab: Water | | | |
| FU | Traps - Cray/king crab: External | | | |
| FV | Traps - Cray/king crab: Internal | | | |
| FW | Traps – crab: Water | | | |
| FX | Traps – crab: External | | | |
| FY | Traps – crab: Internal | | | |
| FZ | Dingy/seine tender boat: Water | | | |
| FAA | Dingy/seine tender boat: External | | | |
| FAB | Dingy/seine tender boat: Internal | | | |
| FAC | Marker buoys: Water | | | |
| FAD | Marker buoys: External | | | |
| FAE | Floats - nets: Water | | | |
| FAF | Floats - nets: External | | | |
| FAG | Scallop harvesters: External | | | |
| FAH | Long lines: External | | | |
| FAI | Jigging machines (squid): External | | | |
| FAJ | Hooker hoses: External | | | |
| FAK | Dive gear: Water | | | |
| FAL | Dive gear: External | | | |
| | | | | |
| | | | | |

**Boat name:** **Date:** **Samplers initials:**

*Vessel Survey Record – Deck*

| CODE | LOCATION | SAMPLE | PHOTO | NOTES |
|---|---|---|---|---|
| DA | Cracks in deck/between plates: Water | | | |
| DB | Hawser pipe: Sediment | | | |
| DC | Gunwale (toe rail): Sediment | | | |
| DD | Hatches: Water | | | |
| DE | Cockpit bins/open storage: Water | | | |
| DF | Winch box: Water | | | |
| DG | Surface: Water | | | |
| DH | Canvas screens: Water | | | |
| DI | Bullwarks: Sediment | | | |
| DJ | Net chute: Sediment | | | |
| DK | Cutting boards: External | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

*Plankton Sample Record*

| CODE | LOCATION | SAMPLE DURATION | NOTES |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |

# APPENDIX C SUCTION SAMPLER

Suction sampler showing the pump and multiple sieve arrangement

Changeable head of the suction sampler

# APPENDIX D R CODE

**#This code analyses the EV I data set**

**#The code is going to read the excel spreadsheets directly using the RODBC library**
**#this library is required, along with MASS and gregmisc**
```
library(RODBC)
library(MASS)
library(gregmisc)
```

**#PART 1: Read in the data and perform initial data exploration**

**#Set the working directory so that it reads to the correct path**
```
setwd("H:/Projects/DEH Empirical Validation I/Reports")
```

**#Make a connection to the excel workbook - note that the spreadsheet names must not include any spaces otherwise the connection cannot read them**
```
evIData <-odbcConnectExcel("H:/Projects/DEH Empirical Validation I/Survey/Survey results - Aug 04.xls")
```

**#Check what tables are held in the connected file**
```
sqlTables(evIData)
```

**#Make two dataFrames using the sqlFetch function**
```
samples <- sqlFetch(evIData, "VesselData")
activity <- sqlFetch(evIData, "Activity")
vessel <- sqlFetch(evIData, “Vessel”)
plankton <- sqlFetch(evIData, “PlanktonData”)
```

**#Beware that R may read empty rows so constrain the dataset!**
```
samples <- samples[!is.na(samples$boatID), ]
activity <- activity[!is.na(activity$vesID), ]
vessel <- vessel[!is.na(vessel$boatID), ]
plankton <- plankton[!is.na(plankton$boatID), ]
```

**#Check what names are held in the data frames**
```
names(samples)
names(activity)
```

**#Summary of the data by major location factor**
```
attach(samples)
by(samples[, 7], list(Location=Location), summary)
```

**#Name and select the data for graphical purposes – note the joining of the**
**#deck samples and fishing gear samples and the ordering of the samples**
```
hull.sam <- samples[Location %in% c(“Hull”),  ]
hull.sam <- hull.sam[order(hull.sam$wetWeight,decreasing=T), ]
int.sam <- samples[Location %in% c(“Internal spaces”), ]
int.sam <- int.sam[order(int.sam$wetWeight, decreasing = T), ]
prop.sam <-samples[Location %in% c(“Propeller rudder & anchor”), ]
prop.sam <- prop.sam[order(prop.sam$wetWeight, decreasing = T), ]
deck.sam<-samples[Location %in% c("Fishing gear", "Deck"), ]
```

**#Size frequency plot of the original data and then the log transformed data**
```
bitmap("Figure1.tif", type="tiffpack", width = 10, height = 10, res =600)
#windows()
par(mfrow=c(2,2))
```

```
truehist(hull.sam$wetWeight, prob=F, xlab = “Wet weight (g)”, ylab = “Count”, main = “Hull”)

truehist(int.sam$wetWeight, prob = F, xlab = “Wet weight (g)”, ylab = “Count”, main = “Internal
spaces”)

truehist(prop.sam$wetWeight, prob = F, xlab = “Wet weight (g)”, ylab = “Count”, main =
“Propeller, rudder & anchor”)
dev.off()

bitmap("Figure2.tif", type = "tiffpack", width = 10, height = 10, res=600)
#windows()
par(mfrow=c(2,2))
truehist(log10(hull.sam$wetWeight+1), prob=F, h = 0.25, xlim=c(0,4), xlab = expression(“Log”
[10]~”(Wet weight +1)(g)”), ylab = “Count”, main = “Hull”)

truehist(log10(int.sam$wetWeight+1), prob = F, h = 0.25, xlim=c(0,4), xlab = expression(“Log”
[10]~”(Wet weight +1)(g)”), ylab = “Count”, main = “Internal spaces”)

truehist(log10(prop.sam$wetWeight+1), prob = F, h= 0.25, xlim=c(0,4), xlab = expression(“Log”
[10]~”(Wet weight +1)(g)”), ylab = “Count”, main = “Propeller, rudder & anchor”)
dev.off()

#Plot boxplots, rotating and setting the axis font to 80% of the default (see ?par)
bitmap("Figure3.tif", type = "tiffpack", width = 10, height = 10, res=600)
#windows()
par(mfrow=c(2,2))

attach(hull.sam)
plot(LocID, log10(wetWeight+1), las = 2, xlab = “Location ID”, ylab = expression(“Log”
[10]~”(Wet weight +1)(g)”), main = ”Hull”, ylim = c(0,4))

attach(int.sam)
plot(LocID, log10(wetWeight+1), las = 2, xlab = “Location ID”, ylab = expression(“Log”
[10]~”(Wet weight +1)(g)”), main = ”Internal spaces”, ylim=c(0,4))

attach(prop.sam)
plot(LocID, log10(wetWeight+1), las = 2, xlab = “Location ID”, ylab = expression(“Log”
[10]~”(Wet weight +1)(g)”), main = ”Propeller, rudder & anchor”, ylim=c(0,4))

attach(deck.sam)
plot(LocID, log10(wetWeight+1), las = 2, xlab = expression(“Log” [10]~”(Wet weight +1)(g)”),
main = ”Deck & fishing gear”, ylim=c(0,4))
dev.off()

#Perform a Wilcoxon (or Mann-Whitney) two sample test – this only assumes that the
#samples come from a common continuous distribution under the null hypothesis
attach(hull.sam)
port.hull.sam<-hull.sam[Side %in% c("Port"), ]
star.hull.sam<-hull.sam[Side %in% c("Starboard"), ]

attach(int.sam)
port.int.sam<-int.sam[Side %in% c("Port"), ]
star.int.sam<-int.sam[Side %in% c("Starboard"), ]

attach(prop.sam)
port.prop.sam<-prop.sam[Side %in% c("Port"), ]
star.prop.sam<-prop.sam[Side %in% c("Starboard"), ]

wilcox.test(port.hull.sam$wetWeight, star.hull.sam$wetWeight)
```

```
wilcox.test(port.int.sam$wetWeight, star.int.sam$wetWeight)
wilcox.test(port.prop.sam$wetWeight, star.prop.sam$wetWeight)

#Plot the ecdf of the data sets – see section 8.3 of an Intro to R.pdf
library(stats)
windows()
par(mfrow=c(2,2))

plot(ecdf(log10(port.hull.sam$wetWeight+1)), do.points=F, verticals=T, xlim=c(0,4), main =
"Hull", xlab = "Log10 (Wet weight+1) (g)", cex.main = 0.8, cex.lab = 0.8, cex.axis = 0.8)
plot(ecdf(log10(star.hull.sam$wetWeight+1)), do.points=F, verticals=T, add=T, lty = 3,
xlim=c(0,4))

plot(ecdf(log10(port.int.sam$wetWeight+1)), do.points=F, verticals=T, xlim=c(0,4), main =
"Internal spaces", xlab = "Log10 (Wet weight+1) (g)", cex.main = 0.8, cex.lab = 0.8, cex.axis =
0.8)
plot(ecdf(log10(star.int.sam$wetWeight+1)), do.points=F, verticals=T, add=T, lty = 3,
xlim=c(0,4))

plot(ecdf(log10(port.prop.sam$wetWeight+1)), do.points=F, verticals=T, xlim=c(0,4), main =
"Propeller, rudder & anchor", xlab = "Log10 (Wet weight+1) (g)", cex.main = 0.8, cex.lab = 0.8,
cex.axis = 0.8)
plot(ecdf(log10(star.prop.sam$wetWeight+1)), do.points=F, verticals=T, add=T, lty = 3,
xlim=c(0,4))

par(xpd = NA)
legend(6,0.25,  c("Port samples", "Starboard samples"), lty=c(1,3), cex=0.8)

#Clean up
rm(star.prop.sam, port.prop.sam, star.hull.sam, port.hull.sam, star.int.sam, port.int.sam)
rm(hull.sam, int.sam, prop.sam, deck.sam)

#Part2: Compare the data with the IMEA
#Create a data frame by location id with the mean of the data for comparison with the
#IMEA analysis – note the use of the order function to re-order by the mean and the two
#different approaches to creating the data frame – the second allows LocID to be a
named
#column – the first does not and needs the additional "data.frame" command
#wet.mean <- tapply(samples$wetWeight, list(samples$LocID), mean)
#wet.count <- tapply(samples$wetWeight, list(samples$LocID), length)
#summ1 <-data.frame(wet.mean, wet.count)
#summ1 <- summ1[order(summ1$wet.mean, decreasing=T), ]

wet.mean <- aggregate(samples$wetWeight, by = list(LocID = samples$LocID, LocCode =
samples$LocCode), mean)
wet.var <- aggregate(samples$wetWeight, by = list(LocID = samples$LocID, LocCode =
samples$LocCode), var)
wet.count <- aggregate(samples$wetWeight, by = list(LocID = samples$LocID), length)

summ1 <- merge(wet.mean, wet.var, by.x = "LocID", by.y="LocID")
summ1 <- merge(summ1, wet.count, by.y = "LocID")
summ1$x.x <- round(summ1$x.x, 2)
summ1$x.y <- round(summ1$x.y, 2)

#Read in the IMEA results
imea <-odbcConnectExcel("H:/Projects/DEH Empirical Validation I/Survey/IMEA1.xls")

#Check what tables are held in the connected file
sqlTables(imea)
```

```
#Make a dataFrame using the sqlFetch function
imea.data<-sqlFetch(imea, "Data")

#Beware that R may read empty rows so constrain the dataset!
imea.data <- imea.data[!is.na(imea.data$LocID), ]

#Convert score into a data frame and merge it with summ1 – beware the order of the first
#two arguments of the merge function
score <- aggregate(imea.data$SOR, by = list(LocID = imea.data$LocID), mean)
score$x <- round(score$x, 2)
summ2 <- merge(score, summ1, by.x = “LocID”, by.y = “LocID”, all.x = T, all.y = T)
summ2$LocCode.x <- as.numeric(summ2$LocCode.x)
summ2$LocCode.x[is.na(summ2$LocCode.x)] <- 0

write.table(summ2, file= “H:/Projects/DEH Empirical Validation I/Reports/Table2.csv”, append =
F, sep = “,”)

#Plot the IMEA score by mean wet weight for the different locations
attach(summ2)
s1 <- summ2[LocCode.x == 1, ]
s1$LocID <- as.character(s1$LocID)
s2 <- summ2[LocCode.x == 2, ]
s2$LocID <- as.character(s2$LocID)
s3 <- summ2[LocCode.x == 3, ]
s3$LocID <- as.character(s3$LocID)
s4 <- summ2[LocCode.x == 4, ]
s4$LocID <- as.character(s4$LocID)

bitmap("Figure6.tif", type = "tiffpack", width = 10, height = 10, res=600)
#windows()
par(mfrow=c(2,2))
par(xpd = NA)

attach(s1)
plot(x.x, x.x.1, pch = LocCode-1, main = “Hull”, xlab = “Severity x Occurrence”, ylab = “Mean
wet weight (g)”, xlim = c(0, 100), ylim = c(0, 500), text(x.x, x.x.1, s1$LocID, pos=3, cex = 0.6))

attach(s2)
plot(x.x, x.x.1, pch = LocCode-1, main = “Propeller rudder & anchor”, xlab = “Severity x
Occurrence”, ylab = “Mean wet weight (g)”, xlim = c(0, 100), ylim = c(0, 500), text(x.x, x.x.1,
s2$LocID, pos=3, cex = 0.6))

attach(s3)
plot(x.x, x.x.1, pch = LocCode-1, main = “Internal spaces”, xlab = “Severity x Occurrence”, ylab
= “Mean wet weight (g)”, xlim = c(0, 100), text(x.x, x.x.1, s3$LocID, pos=3, cex = 0.6))

attach(s4)
plot(x.x, x.x.1, pch = LocCode-1, main = “Deck & fishing gear”, xlab = “Severity x Occurrence”,
ylab = “Mean wet weight (g)”, xlim = c(0, 100), text(x.x, x.x.1, s4$LocID, pos=3, cex = 0.6))
dev.off()

#Clean up
rm(s1, s2, s3, s4)
rm(score, summ2, imea.data)
rm(wet.mean, wet.count, wet.var, summ1, imea)
```

**#Part 3: Plot the incidence of positive Asterias samples**
**#Construct two new data frames showing the proportion of infected samples by location #category**

```
ast1.sum <- aggregate(samples$Ast, by = list(boatID = samples$boatID, LocCode = samples$LocCode), sum, na.rm = T)
ast1.length <- aggregate(samples$Ast, by = list(boatID = samples$boatID, LocCode = samples$LocCode), length)
ast1.prop <- ast1.sum$x/ast1.length$x
ast1.sum <- data.frame(ast1.sum, ast1.prop)
ast1.sum$LocCode <- as.numeric(ast1.sum$LocCode)

ast2.sum <- aggregate(plankton$Ast, by = list(boatID = plankton$boatID), sum, na.rm = T)
ast2.length <- aggregate(plankton$Ast, by = list(boatID = plankton$boatID), length)
ast2.prop <- ast2.sum$x/ast2.length$x

attach(vessel)
sam.loc <- vessel[c("samCode", "boatID")]
ast1.sum <- merge(sam.loc, ast1.sum, by.x = "boatID")

attach(ast1.sum)
a1 <- ast1.sum[LocCode ==1, ]
#a1$samCode <- as.numeric(a1$samCode)
a2 <- ast1.sum[LocCode ==2, ]
#a2$samCode <- as.numeric(a2$samCode)
a3 <- ast1.sum[LocCode ==3, ]
#a3$samCode <- as.numeric(a3$samCode)

bitmap("Figure7.tif", type = "tiffpack", width = 10, height = 10, res=600)
#windows()
par(mfrow=c(2,2))

plot(a1$boatID, a1$ast1.prop, pch = a1$samCode, main = "Hull", xlim = c(0, 30), ylim = c(0,1), xlab = "", ylab = "")

plot(a2$boatID, a2$ast1.prop, pch = a2$samCode, main = "Propeller rudder & anchor", xlim = c(0, 30), ylim = c(0,1), xlab = "", ylab = "")

plot(a3$boatID, a3$ast1.prop, pch = a3$samCode, main = "Internal spaces", xlim = c(0, 30), ylim = c(0,1), xlab = "Boat reference", ylab = "Proportion of Asterias positive samples")

plot(a1$boatID, ast2.prop, pch = a2$samCode, main = "Plankton samples", xlim = c(0, 30), ylim = c(0,1), xlab = "", ylab = "")

par(xpd = NA)
legend(-10,1.5,  c("Royal Yacht Club", "Domain Slipyard"), pch=c(1,2), cex=0.8)
dev.off()
```

**#Make a new data frame showing the proportion of positive samples by location and print #this out as a table**

```
ast3.sum <- aggregate(samples$Ast, by = list(boatID = samples$boatID, LocID = samples$LocID), sum, na.rm = T)

ast3.length <- aggregate(samples$Ast, by = list(boatID = samples$boatID, LocID = samples$LocID), length)

ast3.prop <- ast3.sum$x/ast3.length$x
ast3.sum <- data.frame(ast3.sum, ast3.length, ast3.prop)
ast3.sum <- ast3.sum[ast3.prop !=0, ]
write.table(ast3.sum, file= "H:/Projects/DEH Empirical Validation I/Reports/Table3.csv", append
```

= F, sep = “,”)

**#Clean up**
rm(a1, a2, a3, ast1.sum, ast1.length, ast1.prop, sam.loc)
rm(ast2.prop, ast2.sum, ast2.length)
rm(ast3.sum, ast3.length, ast3.prop)

**#Part 4: Explore the relationship between the 3 potential predictors of hull fouling: 1. Paint #type; 2. Age of paint; and, 3. Activity of the vessel**
**#Read in the sample date (sT), the last trip date (lT), the last slip date (lsT) and the last #anti-foul date (afT). Note use of the coercive “as” function because R read the excel #dates as a factor**
attach(activity)
sT<-strptime(as(activity$sampleDate, "character"), "%Y-%m-%d %H:%M:%S")
lT<-strptime(as(activity$lastTripDate, "character"), "%Y-%m-%d %H:%M:%S")
lsT<-strptime(as(activity$lastSlipDate, "character"), "%Y-%m-%d %H:%M:%S")
afT<-strptime(as(activity$lastAFDate, "character"), "%Y-%m-%d %H:%M:%S")

**#Remove the NA’s from the last slip and anti-foul date replace with with an earlier date**
lsT[is.na(lsT)] <- strptime("1980-01-01", "%Y-%m-%d")
afT[is.na(afT)] <- strptime("1980-01-01", "%Y-%m-%d")

**#Calculate the time between the sample date and the last trip date. Note the change of #class from #POSXt to numeric**
interval1<-round(as((sT-lT)/(60*60*24), "numeric"),0)

**#Assign interval2 the same elements and class as afT – note the same class is important #for subsequent manipulations**
interval2<-afT

**#The vector approach to find the earlier of the times afT or lfT**
interval2[lsT > afT] <- lsT[lsT > afT]

**#The same command as above but with a for loop – runs slower**
for(i in 1:30){
 if(lsT[i] > afT[i]) interval2[i] <- lsT[i]
}

**#Calculate the time between the sample date and the day the vessel was last cleaned #and/or antifouled. Note the change of class from POSXt to numeric**
interval2 <-as.numeric(round((sT-interval2), 0))

**#Find the average the hull fouling indices across the port and starboard**
hfiBT<-(HFIPBT+HFISBT)/2
hfiVB<-(HFIPVB+HFISVB)/2
hfiFB<-(HFIPFB+HFISFB)/2

**#Find the median trip activity per annum**
midTrips<-minTrips+(maxTrips-minTrips)/2

**#Make two new data frames – one for the mean transformed wet weight and one for the #hull fouling indices, allowing comparison with paint type, intervals 1 & 2 and trip activity. #Allow comparison for each vessel across the different fouled locations, by vessel type**
myData1 <- log10(tapply(samples$wetWeight+1,list(samples$boatID,
samples$Location),mean))
myData1<-data.frame(myData1, interval1, interval2, midTrips, boatCode)
myData2 <- data.frame(myData1, hfiBT, hfiVB, hfiFB)

```
myData1<-myData1[ ,3:9]
windows()
plot(myData1, pch=boatCode)
myData2<-myData2[ ,6:12]
windows()
plot(myData2, pch=boatCode)

#Plot up the interesting relationships:
#1. Correlation between the mean wet weight from different locations
bitmap("Figure4.tif", type = "tiffpack", width = 10, height = 10, res=600)
#windows()
par(mfrow=c(2,2))

plot(myData1$Hull, myData1$Internal.spaces, pch = boatCode, xlim=c(0, 4), ylim = c(0,4), main
= “Hull v Internal spaces”, xlab = expression(“Mean Log” [10]~”(Wet weight +1)(g)”), ylab =
expression(“Mean Log” [10]~”(Wet weight +1)(g)”))

plot(myData1$Hull, myData1$ Propeller.rudder...anchor, pch = boatCode, xlim=c(0, 4), ylim =
c(0,4), main = “Hull v Propeller, rudder & anchor”, xlab = expression(“Mean Log” [10]~”(Wet
weight +1)(g)”), ylab = expression(“Mean Log” [10]~”(Wet weight +1)(g)”))

plot(myData1$ Propeller.rudder...anchor, myData1$Internal.spaces, pch = boatCode, xlim=c(0,
4), ylim = c(0,4), main = “Propeller, rudder & anchor v Internal spaces”, xlab =
expression(“Mean Log” [10]~”(Wet weight +1)(g)”), ylab = expression(“Mean Log” [10]~”(Wet
weight +1)(g)”))

par(xpd = NA)
legend(5, 3, c(“Yacht: ablative”, “Yacht: non-ablative”, “Yacht: unknown”, “Fishing vessel:
ablative”, “Fishing vessel: non-ablative”, “Fishing vessel: unknown”, “Tug: non-ablative”),
pch=c(2, 17, 1, 22, 15, 3, 16), cex=0.8)
dev.off()

#Test the strength of the correlation using Spearman’s rank correlation coefficient
cor.0 <- cor(myData1$Hull, myData1$Internal.spaces, use = "all.obs", method = "spearman")
cor.1 <- cor(myData1$Hull, myData1$ Propeller.rudder...anchor, use = "all.obs", method =
"spearman")
cor.2 <- cor(myData1$ Propeller.rudder...anchor, myData1$Internal.spaces, use = "all.obs",
method = "spearman")
cor.0
cor.1
cor.2

#2. Correlation between the days since the last trip and the mean wet weight
windows()
par(mfrow=c(2,2))
plot(interval1, myData1$Hull, pch = boatCode, xlim=c(0, 500), ylim = c(0,4), main = “Days since
last trip v Hull”, cex.main = 0.8, xlab = “Days since last trip”, ylab = “Mean log10 (Wet weight +
1)”, cex.axis = 0.8, cex.lab = 0.8)

plot(interval1, myData1$ Propeller.rudder...anchor, pch = boatCode, xlim=c(0, 500), ylim =
c(0,4), main = “Days since last trip v Propeller, rudder & anchor”, cex.main = 0.8, xlab = “Days
since last trip”, ylab = “Mean log10 (Wet weight + 1)”, cex.axis = 0.8, cex.lab = 0.8)

plot(interval1, myData1$Internal.spaces, pch = boatCode, xlim=c(0, 500), ylim = c(0,4), main =
“Days since last trip v Internal spaces”, cex.main = 0.8, xlab = “Days since last trip”, ylab =
“Mean log10 (Wet weight + 1)”, cex.axis = 0.8, cex.lab = 0.8)

par(xpd = NA)
legend(600, 3, c(“Yacht: ablative”, “Yacht: non-ablative”, “Yacht: unknown”, “Fishing vessel:
```

```
ablative", "Fishing vessel: non-ablative", "Fishing vessel: unknown", "Tug: non-ablative"),
pch=c(2, 17, 1, 22, 15, 3, 16), cex=0.8)

#3. Correlation between the days since the last slip and the mean wet weight
windows()
par(mfrow=c(2,2))
plot(interval2, myData1$Hull, pch = boatCode, xlim=c(0, 1000), ylim = c(0,4), main = "Days
since last slip v Hull", cex.main = 0.8, xlab = "Days since last slip", ylab = "Mean log10 (Wet
weight + 1)", cex.axis = 0.8, cex.lab = 0.8)

plot(interval2, myData1$ Propeller.rudder...anchor, pch = boatCode, xlim=c(0, 1000), ylim =
c(0,4), main = "Days since last slip v Propeller, rudder & anchor", cex.main = 0.8, xlab = "Days
since last slip", ylab = "Mean log10 (Wet weight + 1)", cex.axis = 0.8, cex.lab = 0.8)

plot(interval2, myData1$Internal.spaces, pch = boatCode, xlim=c(0, 1000), ylim = c(0,4), main =
"Days since last slip v Internal spaces", cex.main = 0.8, xlab = "Days since last slip", ylab =
"Mean log10 (Wet weight + 1)", cex.axis = 0.8, cex.lab = 0.8)

par(xpd = NA)
legend(1500, 3, c("Yacht: ablative", "Yacht: non-ablative", "Yacht: unknown", "Fishing vessel:
ablative", "Fishing vessel: non-ablative", "Fishing vessel: unknown", "Tug: non-ablative"),
pch=c(2, 17, 1, 22, 15, 3, 16), cex=0.8)

#4. Correlation between mid-trips and the mean wet weight
windows()
par(mfrow=c(2,2))
plot(midTrips, myData1$Hull, pch = boatCode, xlim=c(0, 400), ylim = c(0,4), main = "Median
trips v Hull", cex.main = 0.8, xlab = "Median trips per annum", ylab = "Mean log10 (Wet weight +
1)", cex.axis = 0.8, cex.lab = 0.8)

plot(midTrips, myData1$ Propeller.rudder...anchor, pch = boatCode, xlim=c(0, 400), ylim =
c(0,4), main = "Median trips v Propeller, rudder & anchor", cex.main = 0.8, xlab = "Median trips
per annum", ylab = "Mean log10 (Wet weight + 1)", cex.axis = 0.8, cex.lab = 0.8)

plot(midTrips, myData1$Internal.spaces, pch = boatCode, xlim=c(0, 400), ylim = c(0,4), main =
"Median trips v Internal spaces", cex.main = 0.8, xlab = "Median trips per annum", ylab = "Mean
log10 (Wet weight + 1)", cex.axis = 0.8, cex.lab = 0.8)

par(xpd = NA)
legend(500, 3, c("Yacht: ablative", "Yacht: non-ablative", "Yacht: unknown", "Fishing vessel:
ablative", "Fishing vessel: non-ablative", "Fishing vessel: unknown", "Tug: non-ablative"),
pch=c(2, 17, 1, 22, 15, 3, 16), cex=0.8)

#5. Hull fouling indices versus each other
windows()
par(mfrow=c(2,2))
plot(myData2$hfiBT, myData2$hfiVB, pch = boatCode, xlim=c(0, 100), ylim = c(0,100), main =
"Boot top v Vertical bottom", cex.main = 0.8, xlab = "Mean boot top fouling index", ylab = "Mean
vertical bottom hull fouling index", cex.axis = 0.8, cex.lab = 0.8)

plot(myData2$hfiBT, myData2$hfiFB, pch = boatCode, xlim=c(0, 100), ylim = c(0,100), main =
"Boot top v Flat bottom", cex.main = 0.8, xlab = "Mean boot top hull fouling index", ylab = "Mean
flat bottom hull fouling index", cex.axis = 0.8, cex.lab = 0.8)

plot(myData2$hfiFB, myData2$hfiVB, pch = boatCode, xlim=c(0, 100), ylim = c(0,100), main =
"Flat bottom v Vertical bottom", cex.main = 0.8, xlab = "Mean flat bottom hull fouling index", ylab
= "Mean vertical bottom hull fouling index", cex.axis = 0.8, cex.lab = 0.8)

par(xpd = NA)
```

```
legend(140, 80, c("Yacht: ablative", "Yacht: non-ablative", "Yacht: unknown", "Fishing vessel: ablative", "Fishing vessel: non-ablative", "Fishing vessel: unknown", "Tug: non-ablative"), pch=c(2, 17, 1, 22, 15, 3, 16), cex=0.8)

#6. Days since last slip versus hull fouling index
windows()
par(mfrow=c(2,2))
plot(interval2, myData2$hfiBT, pch = boatCode, xlim=c(0, 1000), ylim = c(0,100), main = "Days since last slip v Boot top", cex.main = 0.8, xlab = "Days since last slip", ylab = "Mean boot top hull fouling index", cex.axis = 0.8, cex.lab = 0.8)

plot(interval2, myData2$hfiVB, pch = boatCode, xlim=c(0, 1000), ylim = c(0,100), main = "Days since last slip v Vertical bottom", cex.main = 0.8, xlab = "Days since last slip", ylab = "Mean vertical bottom hull fouling index", cex.axis = 0.8, cex.lab = 0.8)

plot(interval2, myData2$hfiFB, pch = boatCode, xlim=c(0, 1000), ylim = c(0,100), main = "Days since last slip v Flat bottom", cex.main = 0.8, xlab = "Days since last slip", ylab = "Mean flat bottom hull fouling index", cex.axis = 0.8, cex.lab = 0.8)

par(xpd = NA)
legend(1500, 80, c("Yacht: ablative", "Yacht: non-ablative", "Yacht: unknown", "Fishing vessel: ablative", "Fishing vessel: non-ablative", "Fishing vessel: unknown", "Tug: non-ablative"), pch=c(2, 17, 1, 22, 15, 3, 16), cex=0.8)

#7. Hull mean wet weight versus hull fouling index
bitmap("Figure5.tif", type = "tiffpack", width = 10, height = 10, res=600)
#windows()
par(mfrow=c(2,2))

plot(myData1$Hull, myData2$hfiBT, pch = boatCode, xlim=c(0, 4), ylim = c(0,100), main = "Hull v Boot top", xlab = expression("Mean Log" [10]~"(Wet weight +1)(g)"), ylab = "Mean boot top hull fouling index")

plot(myData1$Hull, myData2$hfiVB, pch = boatCode, xlim=c(0, 4), ylim = c(0,100), main = "Hull v Vertical bottom", xlab = expression("Mean Log" [10]~"(Wet weight +1)(g)"), ylab = "Mean vertical bottom hull fouling index")

plot(myData1$Hull, myData2$hfiFB, pch = boatCode, xlim=c(0, 4), ylim = c(0,100), main = "Hull v Flat bottom", xlab = expression("Mean Log" [10]~"(Wet weight +1)(g)"), ylab = "Mean flat bottom hull fouling index")

par(xpd = NA)
legend(5, 80,  c("Yacht: ablative", "Yacht: non-ablative", "Yacht: unknown", "Fishing vessel: ablative", "Fishing vessel: non-ablative", "Fishing vessel: unknown", "Tug: non-ablative"), pch=c(2, 17, 1, 22, 15, 3, 16), cex=0.8)
dev.off()

#Clean up
rm(hfiBT, hfiVB, hfiFB, sT, lT, lsT, afT, i)
rm(cor.0, cor.1, cor.2)

#Part 5: Develop a linear model that explains the level of fouling on the hull by paint type, #activity and ship type
#Create two new data frames based on the mean and variance of the quadrats
myData3 <- log10(tapply(samples$wetWeight+1, list(samples$boatID,samples$LocID),mean))
myData4 <- log10(tapply(samples$wetWeight+1, list(samples$boatID,samples$LocID),var))
myData3<-data.frame(myData3, interval1, interval2, midTrips, boatCode)
myData4<-data.frame(myData4, interval1, interval2, midTrips, boatCode)
quads.mean <- myData3[ , c("HA", "PJ", "HP","interval1", "interval2", "midTrips", "boatCode")]
```

```
quads.var <- myData4[ , c("HA", "PJ", "HP","interval1", "interval2", "midTrips", "boatCode")]

#Plot the relationship between the quadrat mean and date since last slipped
bitmap("Figure8.tif", type = "tiffpack", width = 10, height = 10, res=600)
#windows()
par(mfrow=c(2,2))
plot(quads.mean$interval2, quads.mean$HA, ylab = expression("Log" [10]~"(Wet weight
+1)(g)"), xlab = "Days since last slipped", main = "Hull quadrats", pch = boatCode)

plot(quads.mean$interval2, quads.mean$PJ, ylab = expression("Log" [10]~"(Wet weight
+1)(g)"), xlab = "Days since last slipped", main = "Propeller quadrats", pch = boatCode)

plot(quads.mean$interval2, quads.mean$HP, ylab = expression("Log" [10]~"(Wet weight
+1)(g)"), xlab = "Days since last slipped", main = "Keel quadrats", pch = boatCode)

par(xpd = NA)
legend(1100, 2,  c("Yacht: ablative", "Yacht: non-ablative", "Yacht: unknown", "Fishing vessel:
ablative", "Fishing vessel: non-ablative", "Fishing vessel: unknown", "Tug: non-ablative"),
pch=c(2, 17, 1, 22, 15, 3, 16), cex=0.8)
dev.off()

#Remove the outlying tug from the quads dataset and re-do the analysis
quads.mean <- quads.mean[boatCode !=16, ]
lm.quads.mean <- lm(quads.mean$HA , quads.mean$interval2)
summary(lm.quads.mean)

#Clean up
rm(samples, activity, vessel, plankton, evIData, myData1, myData2, myData3, lm.quads.mean)
rm(interval1, interval2, quads.mean, quads.var, midTrips)

#Save the R workspace
save.image("H:/Projects/DEH Empirical Validation I/Reports/Data analysis - July 04.RData")
```