Vessel biofouling as a vector for the introduction of non-indigenous marine species to New Zealand: Fishing vessels

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Executive Summary

- In February 2009, MAFBNZ commissioned the Cawthron Institute to examine and determine the nature and extent of biofouling on fishing vessels arriving in New Zealand. This study focused on fishing vessels entering New Zealand ports from outside New Zealand's Exclusive Economic Zone (EEZ), including both foreign and domestically flagged vessels.

- The specific objectives of this study were to:
  1. Collect data, on the nature and extent of biofouling, on fishing vessels arriving in New Zealand using a standardised sampling methodology as specified by MAFBNZ.
  2. Describe the composition and patterns of biofouling on fishing vessels.

- Between February and September 2009, Cawthron field teams sampled eight fishing vessels from the ports of Auckland (4), Nelson (2) and Timaru (2). Following discussion with MAFBNZ Project Leaders, data for three previously sampled fishing vessels (surveyed by Golder Associates Ltd between March and September 2005 as part of MAFBNZ Project ZBS2004/03B) were included in the final dataset analysed and presented in this report.

- The standardised sampling protocol used in this project allows for the comparison of fouling patterns between different vessel types surveyed as part of the larger MAFBNZ hull fouling project. Sampling included:
  1. Administration of a vessel questionnaire;
  2. Above water assessment of vessel fouling;
  3. Underwater assessment of hull paint condition and fouling;
  4. Collection of photoquadrats from various hull regions (bow, amidships, stern and niche areas) and sampling strata (surface, deep painted areas, and deep unpainted areas);
  5. The collection and identification of any fouling taxa observed during sampling;
  6. Vertical (waterline to keel) and horizontal (length of vessel) video transects of the hull.

- Fouling samples were collected from eight of the 11 vessels sampled, as three vessels had no visible fouling present. Specimens sampled from vessels belonged to ten phyla, and included predominantly sessile taxa (ascidians, bryozoans, barnacles, bivalves, hydroids, algae and tubeworms). Mobile species sampled included amphipods, crabs and chitons. Of the taxa identified to species level, approximately 54 % were non-indigenous to New Zealand, 40 % were indigenous and 5 % were cryptogenic (i.e. of unknown origins).

- Of the non-indigenous species (NIS) encountered, nine are not currently established within New Zealand. Of these, the tubeworm Hydroides albiceps, and the bryozoans Biflustra reticulate, Celleporaria sibogae, Conopeum papillorum had not previously been recorded in New Zealand waters. The most frequently encountered NIS were the tubeworm Hydroides elegans, the oyster Crassostrea gigas, and the barnacles Amphibalanus amphitrites and A. reticulatus. None of the NIS identified are classified as Unwanted Organisms under the Biosecurity Act 1993.

- Fishing vessels surveyed during this study had generally low-to-moderate levels of fouling. Variation in fouling extent (i.e. biomass, species richness, fouling cover) estimates was generally high reflecting: (1) the small number of vessel available for

sampling, (2) the patchy nature of fouling cover across individual vessels, and (3) insufficient replication (for niche areas in particular).

- With regard to hull regions, fouling was generally greatest in the stern regions of the vessel, followed by amidships and bow. Fouling biomass and species numbers on vessel hull regions were consistently highest on areas with poor or non-existent paint coverage. The majority of vessel niche areas sampled had the greatest overall fouling and species richness relative to hull areas. The major "hotspots" of niche area fouling included dry docking support strips (DDSSs), anodes, rudder and rudder shafts, propeller and propeller shafts, and gratings.

- The previous ports-of-call for fishing vessels surveyed in this study were located in three distinct bioregions: Australia/New Zealand, the Northwest Pacific, and the South Pacific. The two vessels arriving from the Northwest Pacific recorded no fouling, while vessels arriving from Australia and/or operating from New Zealand ports had the highest fouling biomass, cover and species richness relative to other bioregions. Vessels arriving from operation in the South Pacific carried the largest proportion of NIS currently not established in New Zealand. However, it is unlikely that of the majority of these NIS would be able to readily establish in the cooler waters of New Zealand.

- Fishing vessels sampled were classified into four main vessel types: bottom longliner, purse seiner, tuna longliner and trawler. Small sample sizes prevented the application of robust analysis to test for differences in biofouling in relation to vessel type.

- This study found a significant positive relationship between the overall surface-assigned level of fouling (LoF) rank allocated to each vessel, and the corresponding biomass, species richness, number of non-indigenous and cryptogenic (NIS+C) species and fouling cover recorded for that vessel. In addition, there was a strong positive relationship between the LoF assigned to quadrats by divers and the corresponding fouling biomass, cover and species richness. In contrast to the finding for other vessel types surveyed by MAFBNZ, surface LoF ranks were not statistically different to diver-assigned values for unpainted areas of hull (DDSSs) and niche areas. However, surface LoF ranks significantly overestimated the extent of fouling on hull areas with adequate anti-fouling protection.

- A combination of low vessel sample size and the low occurrence of NIS+C species in fouling samples added a considerable amount of uncertainty to the predicted relationships between NIS+C species and quantitative and semi-quantitative variables. Biomass, species richness and fouling cover were positively related to the prevalence of NIS+C species. A similar positive relationship was observed for total vessel species richness and the number of NIS+C per vessel. Positive relationships were also observed between numbers of NIS+C species and the number of unique ports and different countries visited by the vessel since last dry-dock.

- The results indicate that, following operation outside the New Zealand EEZ, fishing vessels that enter New Zealand ports are potentially fouled with NIS, including those species already established in New Zealand. However, the fouling extent on the majority of vessels sampled was low. Of the 11 vessels sampled, 3 had no visible fouling, while a further 3 had ≤ 5 species.

1 Introduction

Marine non-indigenous species (NIS) are considered a major threat to the diversity and health of coastal regions worldwide (Carlton and Geller 1993; Vitousek et al. 1997; Cohen and Carlton 1998; Mack et al. 2000). Human-mediated transport vectors such as shipping, aquaculture and fishing have the potential to act as a continuous source for inoculation of NIS into new regions, with the present rate of species movements between different regions at unprecedented levels (Mack et al. 2000).

Of all the possible translocation pathways for NIS, international and regional shipping has perhaps the greatest potential for species spread. Vessels have the potential to transport a range of sessile and mobile marine species via ballast water, in hull recesses (e.g. sea-chests), and as fouling assemblages attached to hull surfaces and niche areas such as grills, gratings, rudders and keels (Carlton 1985; Minchin and Gollasch 2003; Coutts and Taylor 2004; Coutts and Dodgshun 2007). For many years, research on marine species introductions via shipping have focused on ballast water as the most important transport mechanism (Carlton and Geller 1993; Ruiz et al. 2000). However, more recent studies indicate that, in some ports and regions, hull fouling surpasses ballast water transfer as the primary vector for NIS (Gollasch 2002; Hewitt 2002; Hewitt et al. 2004a; Otani et al. 2007; Pettengill et al. 2007). In New Zealand, hull fouling is believed to have made a significant historic contribution to the non-indigenous component of marine floral and faunal communities (Cranfield et al. 1998; Hewitt et al. 2004b).

MAF Biosecurity New Zealand (MAFBNZ) spends considerable resources addressing biosecurity threats to New Zealand's marine systems (MAFBNZ 2007). New Zealand currently has an import health standard (IHS) for ballast water loaded within the territorial waters of another country (MAFBNZ 2005), whereby, any ballast water intended for discharge in New Zealand waters must be exchanged mid-ocean en route to New Zealand (with the exception of discharge in the event of an emergency). Similarly, improved awareness regarding the importance of effective vessel anti-fouling maintenance (as it relates to unwanted species transfers) has been reasonably effective in reducing biofouling transfers (Floerl and Inglis 2005; Piola et al. 2009). However, despite these efforts, fouled vessels continue to arrive at the New Zealand border (Coutts and Taylor 2004; Hewitt et al. 2004b; Coutts and Dodgshun 2007).

Commercial fishing vessels have been implicated in the transfer and/or introduction of a number of marine NIS, including the Asian Green mussel Perna viridis to Cairns, Australia (Hayes et al. 2005), and the arrival of the black striped mussel Mytilopsis sallei into Darwin harbour, Australia, on the hulls of several itinerant Indonesian fishing vessels (Hutchings et al. 2002). In 1994, a Russian super trawler, the Yefim Gorbenko, was dry-docked in New Zealand following several months of operation inside New Zealand territorial waters. The extent of biofouling discovered on the vessel was unprecedented, with over 90 tonnes (wet weight) of biological material being removed (Hay and Dodgshun 1997). Prior to arrival in New Zealand, the vessel had spent 18 months tied up in a Black Sea port awaiting repairs. Although the majority of the fouling present were mussels (thought to be a mixture of Mytilus galloprovincialis and a non-indigenous species of Perna), barnacles, anemones, algae and sponges were also present (Hay and Dodgshun 1997). While it is almost certain that NIS were present, all the defouled material was transferred to landfill prior to scientific examination.

Figure 1.1. Biofouling on the Russian super trawler *Yefim Gorbenko*, showing (a) the wetted areas of the hull completely covered in mussels and associated taxa, and (b) some of the 90 tonnes of fouling removed from the vessel while in dry-dock in Auckland, New Zealand.

1.1 INTERNATIONAL FISHING VESSEL ARRIVALS TO NEW ZEALAND

New Zealand's commercial fishing industry developed quite slowly and throughout the 19th and early 20th century was limited to the small-scale operations in inshore fishing grounds using lines or set nets. In the 1930s, there was no law prohibiting foreign fishing vessels entering and fishing New Zealand waters, provided they did not fish in areas closed to New Zealand vessels. In 1933, the Australian steam trawler, Alfie Cam, became the first foreign-registered fishing vessel to fish within New Zealand waters (Johnson and Haworth 2004). Over the following years, the Alfie Cam, along with her sister ship, the Olive Cam, regularly fished New Zealand waters, frequently porting in Westport for coal and supplies. The outbreak of World War II, in 1939, drew the Australian and New Zealand steam trawling fleets into naval service, and foreign fishing vessel activity in New Zealand waters ceased (Johnson and Haworth 2004).

In the late 1950s, after depleting fish stocks in their home fishing grounds, Japanese fishing vessels began to appear in New Zealand waters, and from 1959, there were regular reports of Japanese vessels longlining for snapper off the west coast of the North Island (Johnson and Haworth 2004). In 1964 25 Japanese longline vessels made 48 voyages to fish New Zealand waters, while in 1965 the numbers decreased to 15 vessels making 32 voyages (Johnson and Haworth 2004). The large quantities of fish being caught by the Japanese fishing fleet acted as a catalyst for extending the New Zealand fishing zone, and in 1965 New Zealand's Territorial Sea and Fishing Zone Act extended the jurisdictional fishing zone from 3 to 12 miles. By 1970 the Japanese agreed to withdraw their vessels from the 12-mile fishing zone, with trawlers ceasing activity immediately, however, 17 longliners continued to fish up to 6 miles from the west coast of the North and South Islands (Johnson and Haworth 2004).

The composition of the foreign fishing vessel fleet in New Zealand changed rapidly in the early 1970s with Russian trawlers having an increased presence in the region. In 1972 the 335 port calls made by foreign fishing vessels to New Zealand ports were made up of: 290 Japanese, 25 Russian, 14 Korean and 1 Chinese vessel (Johnson and Haworth 2004). By 1974 the Soviet trawling capacity had eclipsed the Japanese, and in 1977 Korean vessels began long-lining and trawling (Johnson & Haworth 2004). In 1977 the establishment of New Zealand's 200 nautical mile EEZ brought an end to unregulated fishing by international vessels around the New Zealand coast. This ushered in an era of joint ventures between local fishing companies and foreign fishing fleets, and by 1981 there were 97 joint-venture vessels fishing within the EEZ (Johnson and Haworth 2004).

In 1982 the New Zealand Government struck foreign vessels and joint-venture operations from the tax-credit list, and by 1987 only 41 Russian fishing vessels and six research vessels visited New Zealand ports, largely for fuel and provisions (Johnson and Haworth 2004). Between 2000 and 2007, there was an average of 89 international fishing vessel arrivals to New Zealand per annum (Table 1.1).

Table 1.1. International fishing vessel arrivals (including repeat visits) to New Zealand ports during 2000-07 (average), 2006 and 2007. Data provided by New Zealand Customs.

Port	2000-07 average	2006	2007
Auckland	28	14	24
Bluff	3	4	10
Port Chalmers/Dunedin	2	3	5
Lyttelton	7	10	9
Napier	2	1	3
Nelson	19	14	19
New Plymouth	1	4	2
Tauranga	7	3	3
Timaru	3	1	4
Wellington	14	13	3
Whangarei/Marsden Pt	3	7	1

1.2 PROJECT OBJECTIVES

MAFBNZ has been evaluating the relative risks associated with vessel biofouling through a systematic sampling of international vessels since 2004 (MAFBNZ Project ZBS2004-03). Substantial information has been gathered on the hull fouling risks associated with commercial, passenger, recreational and slow-moving vessels, however, information on fishing vessels has been lacking. Sampling of fishing vessels initially began in 2005, however, only three vessels were sampled (Stuart 2007). This was primarily due to changes in fishing quotas and regulations, resulting in fewer international fishing vessels entering the New Zealand Exclusive Economic Zone (EEZ) to fish and use port facilities.

In February 2009, MAFBNZ commissioned the Cawthron Institute to undertake a second study to examine and determine the nature and extent of biofouling fishing vessels arriving in New Zealand. This study focused on fishing vessels entering New Zealand ports from outside New Zealand's EEZ, including both foreign and domestically flagged vessels, but not domestic vessels operating solely within the EEZ.

The specific objectives of the present study were to:

1. Collect data, on the nature and extent of biofouling, on fishing vessels arriving in New Zealand using a standardised sampling methodology as specified by MAFBNZ.
2. Describe the composition and patterns of biofouling on fishing vessels.

2 Methods

2.1 SAMPLING LOCATIONS AND VESSEL CRITERIA

Between February and September 2009, the Cawthron Institute was contracted to sample fishing vessels arriving in and/or operating from three selected ports in New Zealand (Auckland, Nelson and Timaru; Figure 2.1). Between 2000 and 2007, these three ports received, on average, 56 % of all international fishing vessel arrivals to New Zealand (Table 1.1). A total of eight fishing vessels were sampled in Auckland (4), Nelson (2) and Timaru (2) by the Cawthron Institute. As sampling was dependant on a number of factors including: adequate arrival numbers to the ports of interest, vessel availability for sampling and the adequate notification of vessel arrivals by border agencies, the final numbers of vessels sampled from each location differed marginally from the original targets set.

Following discussions with MAFBNZ Project Leaders, data for three foreign fishing vessels sampled by Golder Associates Ltd (formerly Golder Kingett Mitchell) between March and September 2005 as part of MAFBNZ Project ZBS2004/03B (Stuart 2007) were included in the final dataset analysed and presented in this report. These additional fishing vessels were sampled from the ports of Auckland (2) and Bluff (1), using the same sampling protocol used in the current project, and from here on will be analysed and discussed as vessels sampled "as part of the present study."

Figure 2.1. Ports of interest for the targeted sampling of international fishing vessels arriving in New Zealand.

2.2 SURVEY METHODOLOGY

A standardised vessel sampling protocol (detailed below) was adopted to provide consistency and comparability with previous MAFBNZ biofouling surveys of other vessel classes.

Field sampling entailed:
1. Administration of a vessel questionnaire;
2. Above water biofouling assessment;
3. Underwater biofouling assessment, including:
    i. Quadrat sampling of the hull area;
    ii. Opportunistic sampling of niche areas;
    iii. Waterline to keel video transects.

Each vessel was divided into three horizontal "regions" (Figure 2.2a):
1. Bow (BO);
2. Amidships (AM);
3. Stern (ST).

and into three vertical "strata" (Figure 2.2a):
1. Within 0.5 m of the surface/waterline (referred to as "s-strata");
2. Deeper areas of the hull that were coated with anti-fouling paint (referred to as "n-strata");
3. Deeper areas of the hull where anti-fouling paint was absent or in very poor condition (referred to as "d-strata"). Numerous vessels did not contain areas of hull that were completely devoid of paint, however all vessels had dry-docking support strips (as described by Coutts and Taylor 2004) where paint was either absent or in very poor condition. As such, this stratum consistently occurred within dry-docking support strip (DDSS) areas.

In total, there were nine possible combinations of vessel regions (BO, AM, ST) and locations (s, n, d) available for general hull area sampling (excluding niche areas).

2.2.1 Vessel questionnaire

For each vessel sampled, a questionnaire was completed by the ship's master or representative vessel agent. The purpose of this questionnaire was to obtain general information on the vessel, and its maintenance and voyage histories (Appendix A). These data were then used to assess the reliability of numerous variables (e.g. vessel age, number and location off ports visited, wetted surface area) in predicting fouling patterns and composition.

2.2.2 Above water biofouling assessment

Visibility under water is approximately 1 m in most port and marina environments. This, and the fact that boat and ship hulls tend to curve inwards, make it impossible to see hull fouling assemblages in detail from the surface, except for areas immediately below the waterline (< 0.75 m depth). The above water biofouling assessments performed were based on a semi-quantitative rank scale developed by Floerl et al. (2005) for evaluating fouling levels (Level of Fouling, LoF) on international yachts entering New Zealand. The surface LoF scale ranged from 0 (no fouling or biofilm/slime layer present) to 5 (> 41 % macrofouling cover; Appendix B). Surface LoF observations were made from the dock or in a boat next to the vessel of interest, and allocated to areas of interest, including: (i) bow; (ii) waterline; (iii) hull area below the waterline; (iv) stern and rudder; (v) entire vessel hull.

2.2.3 Underwater biofouling assessment

2.2.3.1 Quadrat sampling - hull area

Vessels were divided into three regions (bow, amidships and stern) with three sampling locations (strata) within each region: (i) the surface within 0.5 m of the waterline (s-strata), (ii) deeper areas of the hull coated in anti-fouling paint (n-strata), and (iii) deeper areas of the hull lacking anti-fouling paint (d-strata) (Figure 2.2a). In each of the nine region × location combinations, three haphazardly placed 0.04 m^2 (20 cm × 20 cm) quadrat samples were collected. Divers collected the following information from each quadrat:

1. A LoF rank for the area enclosed by each quadrat (as per Appendix C), based on the semi-quantitative rank scale developed by Floerl et al. (2005);
2. A qualitative assessment of anti-fouling paint condition (good, average, poor, absent);
3. A digital image of the hull area inside the quadrat using a Canon EOS400 digital camera;
4. Collection of fouling organisms encountered in the quadrat using a paint scraper and plastic zip-lock bag.

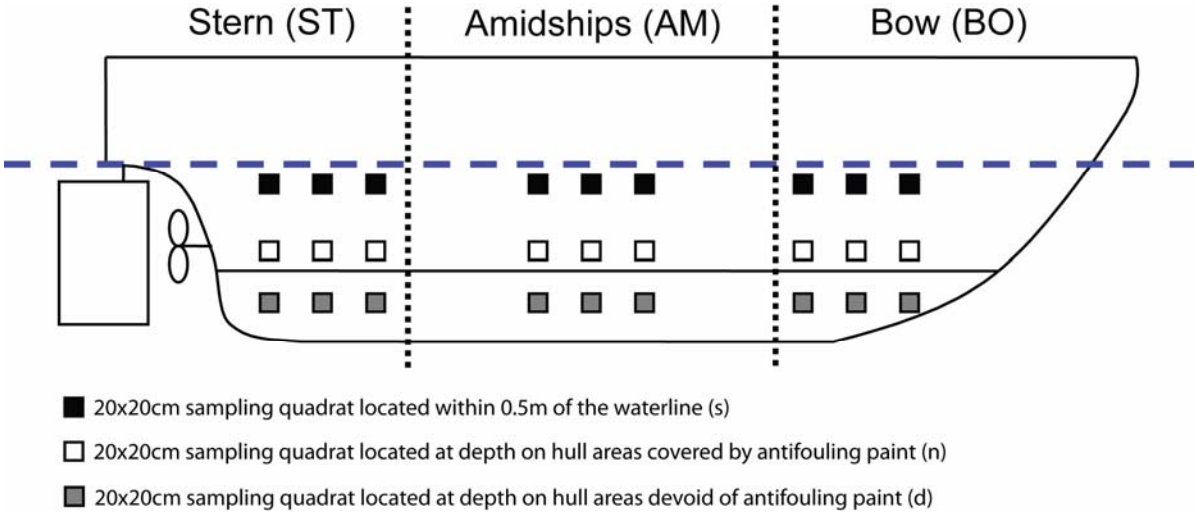
2.2.3.2 Opportunistic sampling - niche areas

The abundance and distribution of fouling organisms varies greatly among the various 'niches' across a ship hull (Figure 2.2b). Previous hull fouling studies have indicated that some areas of vessel hulls have particularly high levels of fouling, including within dry docking support strips, gratings associated with thrusters and sea-chests, bilge keels, keels, rudders, propellers and rope guards and the stern (Coutts and Taylor 2004; Stuart 2007; Davidson et al. 2009; Hopkins and Forrest 2009). During and after quadrat sampling of the vessel hull area, divers also conducted a visual search of the entire submerged part of the vessel to examine fouling in niche areas. Niche areas targeted included:

1. Bow (BO);
2. Bow thruster (BT);
3. Hull area below the waterline (BW);
4. Waterline (WA);
5. Flat bottom keel (KE);
6. Inside of the dry-docking support strips (DDSS);
7. Bilge keels (BK);
8. Sea-chest gratings (GR);
9. Stern (ST);
10. Propeller and its shaft (PS);
11. Rudder and rudder shaft (RS);
12. Anodes (AN).

Each niche area sampled was assigned a LoF rank and a qualitative assessment was made of the anti-fouling paint condition (good, average, poor, absent). Any fouling organisms encountered were photographed and collected. Wherever possible, photographs included the placement of a 0.04 m^2 quadrat, so that quantitative measures of fouling (e.g. percent cover) could be obtained and compared with samples collected from the vessel hull area (Section 2.2.3.1).

(a) Quantitative quadrat sampling of vessel hull areas



(b) Vessel niche areas targeted during opportunistic sampling

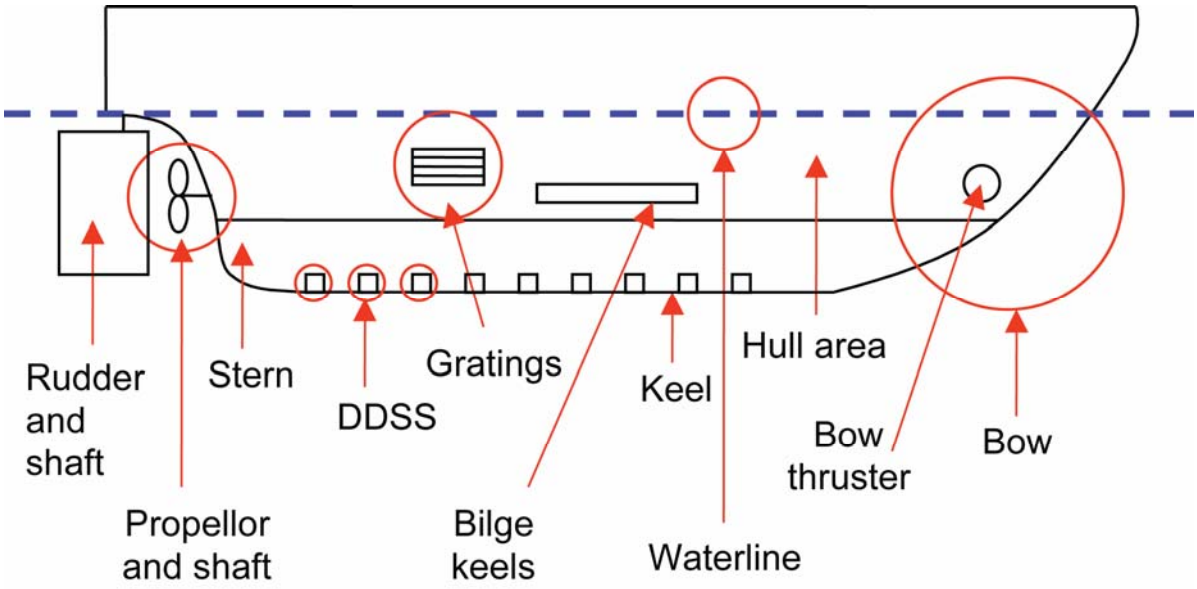


Figure 2.2. Protocols for (a) sampling of quantitative quadrat sampling of vessel hull areas, and (b) areas examined by divers for opportunistic sampling. Note that actual quadrat locations in (a) were selected haphazardly by divers.

2.2.3.3 Waterline to keel video transects

In each of the three regions of the vessel (bow, amidships and stern), a vertical surface-to-keel video transect was recorded using a Sony DCR-VX2000 digital video camera and underwater housing. Wherever possible, gratings, sea-chests, thrusters, and fouling aggregations were included in the footage.

2.3 SPECIES PROCESSING, IDENTIFICATION AND CLASSIFICATION OF STATUS

Fouling samples collected from hull areas and opportunistically sampled niche areas were labelled *in situ* then transferred to the surface for processing. During processing, samples were filtered through a 1 mm mesh sieve, blotted dry, weighed, and sorted into broad taxonomic groups (e.g. ascidians, bryozoans, barnacles, etc.). Each taxonomic group from each sample was preserved in ethanol, or an ethanol-glyoxal mix where necessary, and sent to NIWA's Marine Invasives Taxonomic Service (MITS) for formal identification. The current biosecurity status of each species identified was also assigned, with classification being either non-indigenous to New Zealand, indigenous (native), cryptogenic (of unknown geographic origin) or indeterminate (i.e. insufficient taxonomic resolution to determine origin).

2.4 DATA ANALYSIS

2.4.1 Data management

Assorted data collected during this project, include:
1. Image data (quantitative quadrats);
2. Biomass data (quantitative quadrats);
3. Taxonomic data (sample identification from MITS);
4. Vessel fouling composition data (derived from quantitative quadrats and MITS IDs);
5. LoF ranks (assigned from surface observations and divers assessing quantitative quadrats and opportunistic areas);
6. Vessel dimensions and characteristics (questionnaire);
7. Vessel maintenance history (questionnaire);
8. Vessel voyage history (questionnaire).

Fouling cover (derived from photoquadrats images) and vessel questionnaire data were stored within Microsoft Access databases. All other data (in addition to summaries of fouling cover and vessel questionnaire data) were entered into Microsoft Excel spreadsheets for manipulation, investigation and statistical analysis.

2.4.2 Statistical analysis

Fouling data were often presented and analysed in different forms. For example, biomass data were calculated as mean biomass per sample unit area (i.e. quadrat), as well as overall biomass collected from the vessel. Similarly, fouling cover was assessed as both the percent cover per sampling unit area (quadrat) and the proportion of vessel hull area quantitatively sampled (with quadrats) that was covered in fouling (pseudo whole-vessel percent cover). Finally, the numbers of species present (including subsets, such as, numbers of non-indigenous and cryptogenic (NIS+C) species present) were assessed as both the total number of species collected from the vessel(s) and the number recorded per unit sample area (quadrat).

Non-metric multi-dimensional scaling (nMDS) procedures, based on the Bray-Curtis similarity measure of presence/absence fouling data, were used to determine differences in taxa composition on vessels. These analyses were carried out using PRIMER Version 6 (PRIMER-E Ltd, Lutton, Ivybridge, UK). Similarity percentage analyses (SIMPER) were used to identify specific taxa explaining trends evident in nMDS plots (Clarke and Warwick 1994). Investigations into the factors having an influence on the observed vessel distributions were carried out using the BIOENV procedure (Clarke and Warwick 1994), using the following variables: total biomass, total richness, days since last dry dock, total days spent in port since last dry dock, number of unique ports visited since last dry dock, total number of port visits since last dry dock, number of unique countries visited since last dry dock, age of vessel, estimated wetted surface area (WSA) of vessel and average speed of vessel. Outputs were constrained to the combinations of ≤ 4 variables that provided the highest correlation to the patterns observed in the nMDS data.

Univariate analyses were carried out using the software package Statistica Version 8 (StatSoft Inc., Tulsa, OK, USA). Linear regression was used to investigate relationships between different measures of biofouling extent and composition (e.g. biomass, species richness, cover) and variables such as surface- and diver-assigned LoF ranks. Linear regression was also used to assess the utility of various biofouling parameters and vessel characteristics for predicting the occurrence of NIS+C species on fishing vessel hulls. All data were explored for normality and homogeneity of variance using frequency histograms and plots of residuals versus predicted values, respectively. Where required, log(x+1) transformations were applied. Where transformation did not improve normality, data were analysed untransformed. Values with a Cook's Distance > 1.0 were classified as outliers in the data, and were removed from the analysis prior to the linear regression model being refitted.

Comparisons of surface- versus diver-assigned LoF ranks were undertaken using non-parametric Wilcoxin Match-Pairs test, where surface-assigned LoF ranks for the bow, amidships and stern of each vessel were compared against diver-assigned LoF ranks at the surface (test 1), deep painted areas (test 2) and deep unpainted areas (test 3). Similarly, for each vessel, the overall LoF values assigned were compared to the highest niche area LoF values.

Analysis of variance (ANOVA) was used to test for differences in fouling cover among different vessel hull regions and sampling strata. Two-way ANOVA was used to compare measures of fouling (biomass, species richness and cover) across vessel hull regions (bow, amidships and stern) and sampling strata (surface, deep painted and deep unpainted). One-way ANOVA was used to test for differences in fouling indices between different vessel types, and between vessels from different bioregions. Data were tested for homogeneity and normality using frequency histograms and plots of residuals versus predicted values. Dependant variables were log(x+1) transformed where required.

3 Results

3.1 VESSEL QUESTIONNAIRE INFORMATION

The 11 fishing vessels arrived in New Zealand from operation and/or berthage in four IUCN bioregions (based on Kelleher et al. 1995): Australia/New Zealand, the South Pacific, the Northwest Pacific and Antarctica (Table 3.1). Six vessels were registered to New Zealand, although their areas of operation were outside the New Zealand EEZ (i.e. Antarctica, Australia, and the South Pacific). The remaining vessels were registered to ports in Japan (Table 3.1).

Vessels ranged in length from 44–80 m (beam = 8–13 m, respectively), with estimated wetted surface areas (WSA) ranging from 345–1258 m^2 (Table 3.1). The age of the vessels at time of sampling ranged from 9–31 years, with average operating speeds from 8-13 kn (Table 3.1).

Among vessels surveyed the time since last dry-docking varied from 3 weeks to over 3 years (Table 3.1). The average time since last dry-dock (and application of anti-fouling paint) was 420 ± 105 d (mean ± SE). All vessels surveyed had steel hulls, and high pressure water-blasting appeared to most common hull treatment prior to anti-fouling. The type and manufacturer of anti-fouling coating varied among vessels. Only two of the vessels surveyed, CAWFV07 and KML018, had active treatment systems in place to prevent fouling of sea-chests (comprising ion generation and chemical treatment, respectively). The numbers of different countries visited by vessels since last dry-dock varied from 1–6, with the number of unique ports visited ranging from 1–8 (Table 3.1). The total amount of time spent by vessels in port environments since last dry-dock ranged from 4–292 d (mean ± SE = 70 ± 24 d).

Table 3.1. Summary information (registration, specification, maintenance and voyage history) of the fishing vessels surveyed in this study.

Vessel ID	Port of registration	Vessel type [b]	Estimated WSA (m^2) [c]	Age at time of survey (years)	Average speed (kn)	Time since last dry-dock (d)	Countries visits since last dry-dock	Unique ports visited since last dry-dock	Total port visits since last dry-dock	Total time spent in port(s) since last dry-dock (d)	Bioregions of operation since last dry-dock [d] (Numbers in parentheses represent bioregion numbers)
CAWFV01	NZ	BL	509	17	8.0	572	1	2	9	71	Antarctic (1); Aust. & NZ (18)
CAWFV02	NZ	BL	792	9	10.0	101	1	2	2	14	Antarctic (1); Aust. & NZ (18)
CAWFV03	NZ	PS	691	30	13.0	285	5	5	11	73	South Pacific (14); Aust. & NZ (18)
CAWFV04	Japan	TL	345	19	11.0	144	1	1	1	20	South Pacific (14); Northwest Pacific (16)
CAWFV05	Japan	TL	415	19	11.0	569	6	8	10	47	South Pacific (14); Northwest Pacific (16); Aust. & NZ (18)
CAWFV06	Japan	TL	397	22	10.5	21	1	1	1	4	South Pacific (14); Aust. & NZ (18)
CAWFV07	NZ	TR	1110	12	11.5	910	2	8	13	15	Aust. & NZ (18)
CAWFV08	Japan	TL	409	17	11.5	356	2	3	6	109	South Pacific (14); Northwest Pacific (16)
KML013 [a]	NZ	PS	606	31	10.0	243	4	4	6	54	South Pacific (14); Aust. & NZ (18)
KML014 [a]	NZ	PS	827	18	10.0	1138	5	5	21	292	South Pacific (14); Aust. & NZ (18)
KML018 [a]	Japan	TL	1258	11	12.5	286	3	4	9	75	Northwest Pacific (16); Aust. & NZ (18)

[a] Vessels sampled by Golder Associates Ltd as part of MAFBNZ Project ZBS2004/03b (Stuart 2007)
[b] Vessel type: Bottom longliner (BL); Purse seiner (PS); Tuna longliner (TL); Trawler (TR)
[c] Calculated as: (2 x Length) + (Beam x Draft)
[d] Based on ICUN bioregions presented in Kelleher et al. (1995).

3.2 PAINT CONDITION

Vessel anti-fouling paint condition varied across hull regions and sampling strata (Figure 3.1 and Figure 3.2). Overall, 45 % of all hull areas sampled had good paint condition, while 16 % of hull area was ranked as average, and 38 % as poor (Figure 3.1). The bow and amidships regions of the hull received a higher percentage of good rankings (29 % each) relative to the stern and niche areas (22 and 20 %, respectively; Figure 3.1). In contrast, more average paint conditions rankings were assigned to stern regions (30 %) and niches areas (33 %) relative to bow and stern areas (17 and 20 %, respectively). Poor paint condition rankings were allocated uniformly across all hull regions (23–27 %; Figure 3.1).

No single hull region recorded a ranking of good paint condition across all of the vessels sampled (Figure 3.2). However, all d-strata areas sampled (primarily comprising DDSS) had poor paint condition, which is consistent with a lack of paint being applied during the previous dry-docking event (Figure 3.2). This contrasted markedly with surface (s-strata) and deep painted (n-strata) areas of hull, which consistently recorded good paint condition rankings, particularly on the bow and amidships regions (Figure 3.2). Areas of average paint condition were recorded on all surface and deep painted areas, although rankings were marginally higher on the stern regions of the vessels (Figure 3.2).

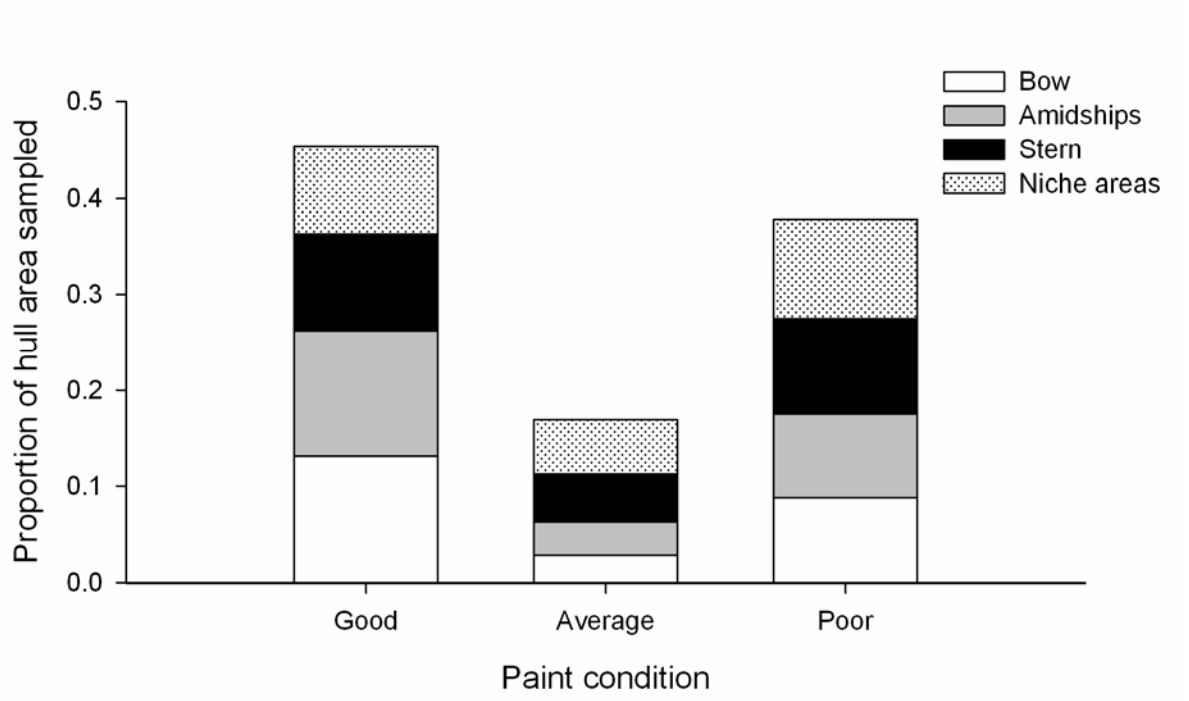


Figure 3.1. Summary of diver-assigned rankings of hull paint condition across all vessels sampled (made during qualitative and quantitative hull sampling). Data are presented as the proportion of sampled hull areas ranked as having good, average or poor paint condition, and the contribution of different hull areas (bow, amidships, stern, niche) to each ranking.

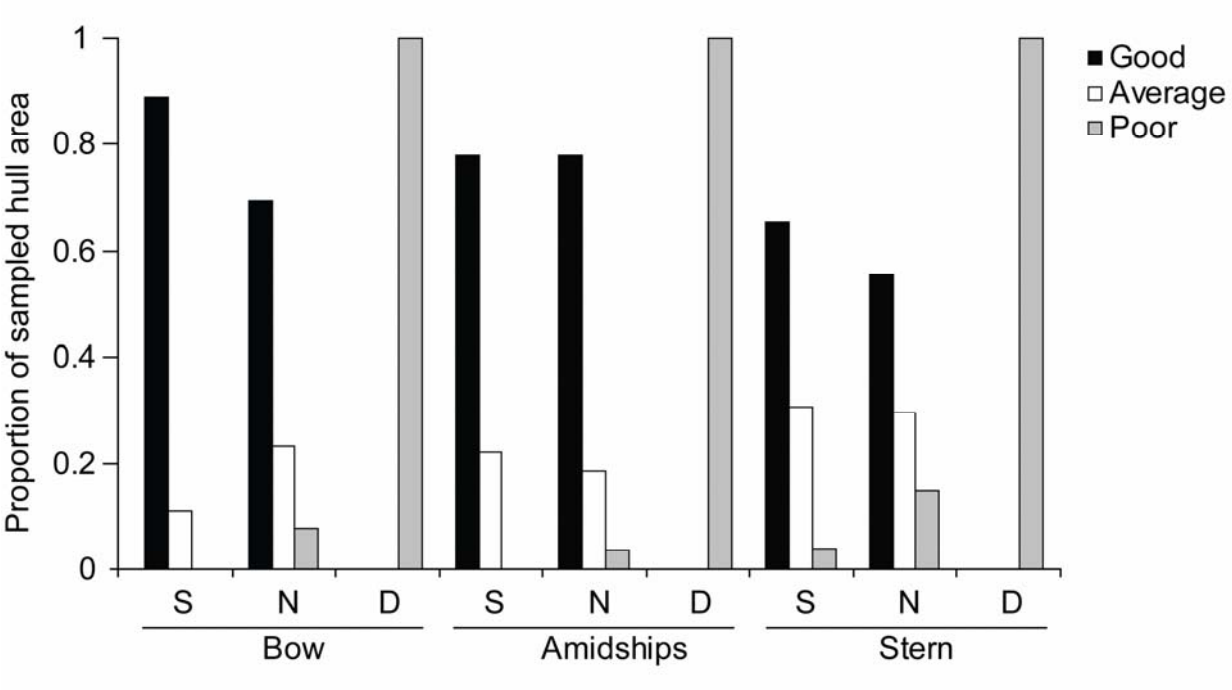


Figure 3.2. Summary of diver-assigned rankings of hull paint condition across vessel hull regions (bow, amidships, stern) and sampling strata (S = surface, N = painted (deep), D = dry-dock support strips). Data are presented as the proportion of sampled hull areas ranked as having good, average or poor paint condition.

3.3 IDENTITY, STATUS AND PATTERNS OF BIOFOULING

3.3.1 Identity and status

Fouling samples were collected from 8 of the 11 vessels sampled, as 3 vessels had no visible fouling present. A total of 102 samples were collected, comprising 187 specimen-per-sample records. A total of 10 phyla were represented in the samples: Arthropoda, Mollusca, Ochrophyta, Annelida, Bryozoa, Chlorophyta, Chordata, Cnidaria, Rhodophyta, and Cyanobacteria (Figure 3.3). Arthropods (mainly crustaceans), molluscs, ochrophyte algae, bryozoa and annelid worms accounted for 81 % of all organisms collected (Figure 3.3). Overall, 37 taxa were identified to species level, while a further 22 taxa were unable to be identified beyond phylum, order, family or genus. These latter taxa were classified as "indeterminate species", in general, due to specimen damage during collection or a lack of sufficient material (particularly for algae) (Figure 3.3). When arranged into broad taxonomic groups, barnacles, algae, bivalves, tubeworms and bryozoans were the most abundant taxa, representing 28 %, 20 %, 12 %, 10 % and 9 % of all taxa, respectively (Figure 3.4).

The biosecurity status of the 37 taxa identified to species level included: 20 non-indigenous species (NIS), 2 cryptogenic species, and 15 indigenous species (Figure 3.3 and Figure 3.4). The numbers of non-indigenous, cryptogenic, indigenous, and indeterminate taxa collected from each vessel is presented in Table 3.2.

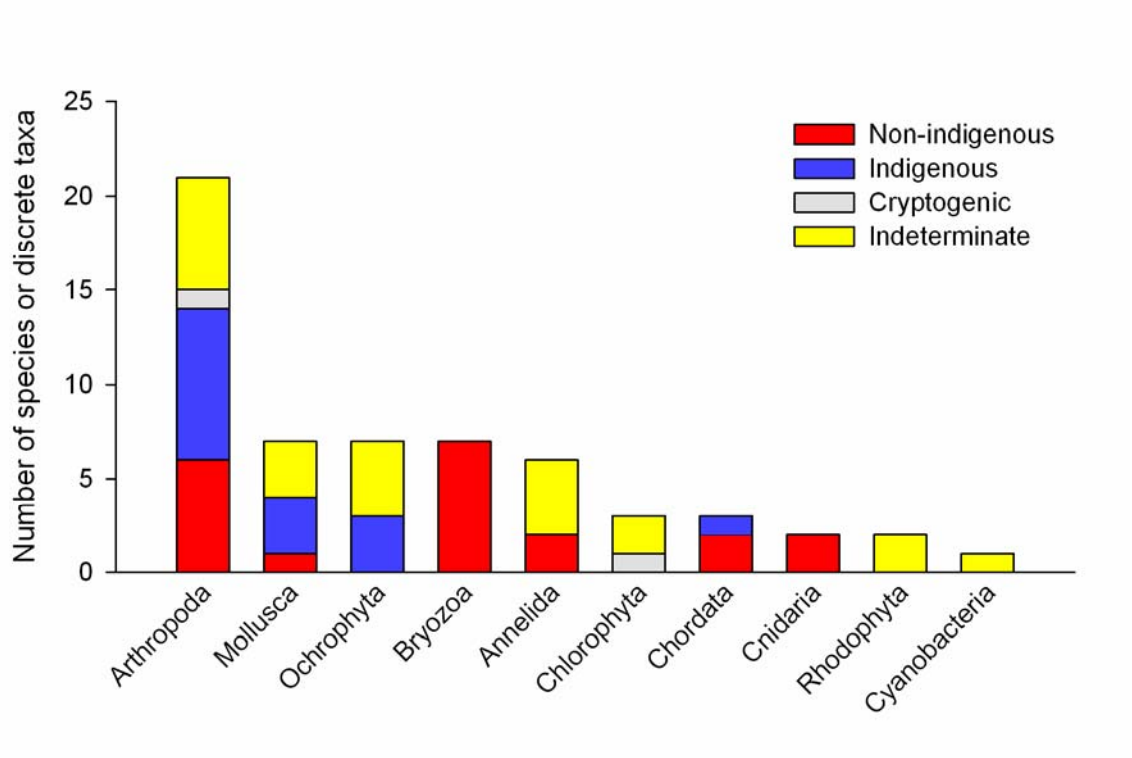


Figure 3.3. Relative abundance of species within each phylum and their associated biosecurity status.

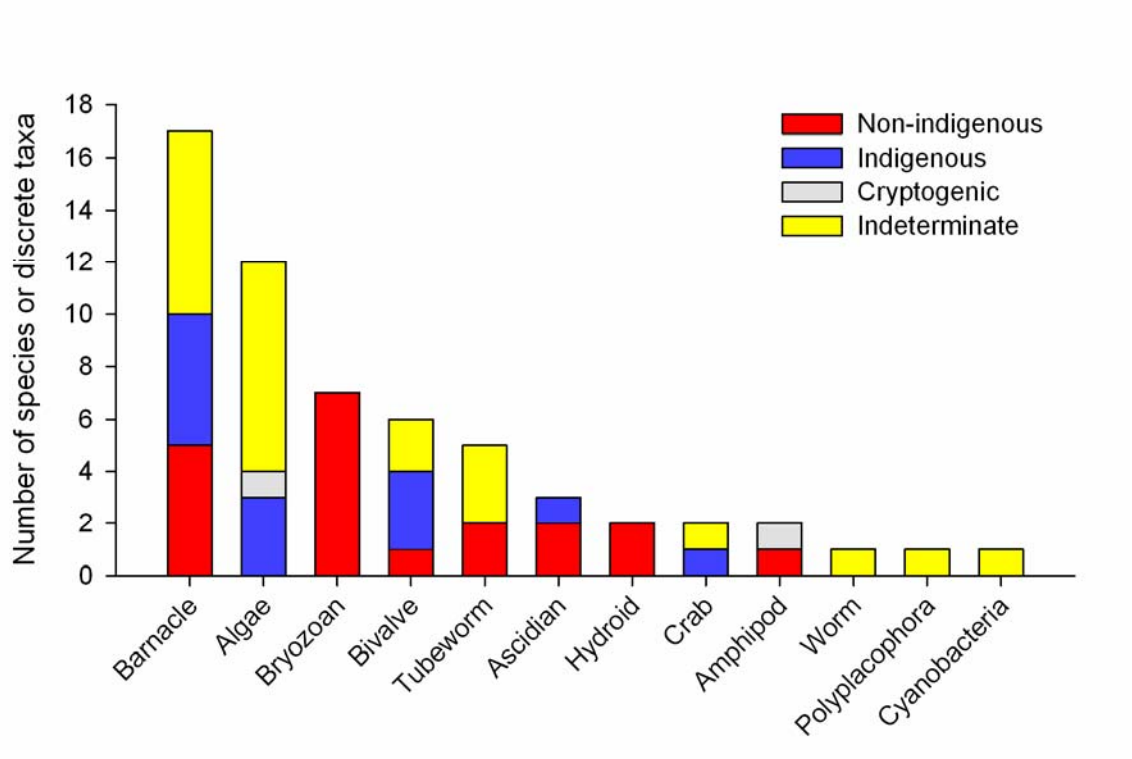


Figure 3.4. Relative abundance of species within each broad taxonomic group and their associated biosecurity status.

Table 3.2. Relative abundance of species, within each biosecurity status category, sampled from 11 fishing vessels hulls.

Vessel ID	Number of species or discrete taxa				
	NIS	Cryptogenic	Indigenous	Indeterminate	Total
CAWFV01	0	0	2	0	2
CAWFV02	0	0	0	0	0
CAWFV03	6	0	2	2	10
CAWFV04	0	0	0	0	0
CAWFV05	1	0	2	0	3
CAWFV06	0	0	0	0	0
CAWFV07	2	1	13	4	20
CAWFV08	1	0	3	1	5
KML013	8	1	0	10	19
KML014	3	1	0	15	19
KML018	3	1	5	6	15

3.3.1.1 Non-indigenous species

Of the 37 taxa identified from hull samples, 20 (54 %) were species non-indigenous to New Zealand. This included polychaete worms (2 species), crustaceans (6), bryozoans (7), ascidians (2), hydroids (2) and bivalves (1) (

Table 3.3). Nine (45 %) of the NIS encountered are not currently established within New Zealand, and of these, the tubeworm *Hydroides albiceps,* and the bryozoans *Biflustra reticulata, Celleporaria sibogae* and *Conopeum papillorum* had not previously been recorded in New Zealand waters. None of the NIS identified are classified as Unwanted Organisms under the Biosecurity Act 1993.

Across all the phyla recorded bryozoans, cnidarians, chordates, annelid worms and arthropod crustaceans contained the greatest proportions of NIS at 100 %, 100 %, 67 %, 33 % and 29 % of species within each phylum, respectively (Figure 3.3). Only one of the NIS encountered was a mobile organism, the amphipod *Jassa staudei*. The most frequently encountered NIS each occurred on at least two vessels. These were the tubeworm *Hydroides elegans*, the barnacles *Amphibalanus amphitrites* and *A. reticulatus*, and the oyster *Crassostrea gigas* (Table 3.3).

Table 3.3. Identification, establishment status and frequency of non-indigenous species encountered on the 11 fishing vessel hulls. Species in bold represent first records in New Zealand.

Phylum, Class	Order	Family	Taxon	Established	No. of Vessels
Annelida					
Polychaeta	Sabellida	Serpulidae	***Hydroides albiceps*[h]**	No	1
Polychaeta	Sabellida	Serpulidae	*Hydroides elegans*	Yes	2
Arthropoda					
Malacostraca	Amphipoda	Ischyroceridae	*Jassa staudei*[n]	No	1
Maxillopoda	Pedunculata	Lepadidae	*Lepas anserifera*	Yes	1
Maxillopoda	Sessilia	Balanidae	*Amphibalanus amphitrite*	Yes	2
Maxillopoda	Sessilia	Balanidae	*Amphibalanus improvisus*[h]	Yes	1
Maxillopoda	Sessilia	Balanidae	*Amphibalanus reticulatus*	Yes	2
Maxillopoda	Sessilia	Tetraclitidae	*Tesseropora wireni*[n]	No	1
Bryozoa					
Gymnolaemata	Cheilostomata	Bugulidae	*Bugula neritina*[h]	Yes	1
Gymnolaemata	Cheilostomata	Membraniporidae	***Biflustra reticulata*[h]**	No	1
Gymnolaemata	Cheilostomata	Lepraliellidae	***Celleporaria sibogae*[h]**	No	1
Gymnolaemata	Cheilostomata	Electridae	***Conopeum papillorum*[h]**	No	1
Gymnolaemata	Cheilostomata	Hippopodinidae	*Hippopodina feegeensis*[h]	No	1
Gymnolaemata	Cheilostomata	Watersiporidae	*Watersipora subtorquata*	Yes	1
Gymnolaemata	Ctenosomata	Vesiculariidae	*Bowerbankia gracilis*[h]	Yes	1
Chordata					
Ascidiacea	Pleurogona	Styelidae	*Styela canopus*[h]	No	1
Ascidiacea	Pleurogona	Pyuridae	*Pyura elongata*[h]	No	1
Cnidaria					
Hydrozoa	Anthoathecata	Tubulariidae	*Ectopleura crocea*[n]	Yes	1
Hydrozoa	Leptothecata	Campanulariidae	*Obelia longissima*[n]	Yes	1
Mollusca					
Bivalvia	Ostreoida	Ostreidae	*Crassostrea gigas*	Yes	2

[h] Species collected exclusively from hull areas
[n] Species collected exclusively from niche areas

3.3.1.2 Cryptogenic species

Two species sampled on vessel hulls were classified as cryptogenic. These were the amphipod, *Jassa marmorata*, and the chlorophyte alga, *Cladophoropsis herpestica* (Table 3.4). The amphipod was encountered on the hull and/or niche areas of two vessels, while the alga occurred exclusively on the hull sections of three vessels (Table 3.4).

Table 3.4. Identification, establishment status and frequency of cryptogenic species encountered on the 11 fishing vessel hulls

Phylum, Class	Order	Family	Taxon	Established	No. of Vessels
Arthropoda					
Malacostraca	Amphipoda	Ischyroceridae	*Jassa marmorata*	Yes	2
Chlorophyta					
Ulvophyceae	Cladophorales	Cladophoraceae	*Cladophoropsis herpestica* [h]	Yes	3

[h] Species collected exclusively from hull areas

3.3.1.3 Indigenous species

The 15 indigenous species collected represent 43 % of all species identified. They included crabs (1 species), barnacles (7), solitary ascidians (1), bivalves (3) and algae (3;Table 3.5). Indigenous species occurred on both domestic- and foreign-flagged vessels, with barnacles being the most frequently encountered indigenous taxa (Table 3.5).

Table 3.5. Identification and frequency of indigenous species encountered on the 11 fishing vessel hulls.

Phylum, Class	Order	Family	Taxon	No. of Vessels
Arthropoda				
Malacostraca	Decapoda	Ocypodidae	*Macrophthalmus hirtipes* [h]	1
Maxillopoda	Pedunculata	Lepadidae	*Conchoderma auritum*	3
Maxillopoda	Pedunculata	Lepadidae	*Conchoderma virgatum*	3
Maxillopoda	Pedunculata	Lepadidae	*Lepas anatifera*	5
Maxillopoda	Pedunculata	Lepadidae	*Lepas australis* [h]	2
Maxillopoda	Sessilia	Archaeobalanidae	*Austrominius modestus*	3
Maxillopoda	Sessilia	Balanidae	*Notomegabalanus campbelli* [h]	1
Maxillopoda	Sessilia	Balanidae	*Notomegabalanus decorus* [h]	1
Chordata				
Ascidiacea	Pleurogona	Pyuridae	*Pyura pachydermatina* [h]	1
Mollusca				
Bivalvia	Mytiloida	Mytilidae	*Aulacomya maoriana* [n]	1
Bivalvia	Mytiloida	Mytilidae	*Mytilus galloprovincialis*	2
Bivalvia	Mytiloida	Mytilidae	*Perna canaliculus* [n]	1
Ochrophyta				
Phaeophyceae	Ectocarpales	Chordariaceae	*Scytosiphon lomentaria* [a]	1
Phaeophyceae	Ectocarpales	Ectocarpaceae	*Hincksia granulosa* [a]	1
Phaeophyceae	Ectocarpales	Scytosiphonaceae	*Petalonia fascia* [a]	1

h Species collected exclusively from hull areas
n Species collected exclusively from niche areas
a Species that were collected during opportunistic sampling but were not considered in any of the hull region and quadrat-based biomass or diversity calculations given they overlapped with hull sections already sampled by qualitative quadrats. These areas included: hull area (HA), bow (BO), below waterline (BW), waterline (WA) and stern (ST). These species were still included in the total-vessel biomass and/or diversity calculations.

3.3.1.4 Indeterminate species

Of the total 59 discrete taxa sampled from the hull of fishing vessels 22 (37 %) were unable to be classified to species level (Table 3.6). Indeterminate taxa included annelid worms (4), crustaceans (6), algae (8), cyanobacteria (1), bivalves (2) and chitons (1). Only indeterminate taxa identified to genus level were included in any of the statistical analysis, unless a sample also contained an organism identified to species level within the same genus (in which case the genus-level indeterminate was excluded).

Table 3.6. Identification of indeterminate species encountered on the 11 fishing vessel hulls.

Phylum, Class	Order	Family	Taxon
Annelida			
Polychaeta	Phyllodocida	Syllidae	indet.
Polychaeta	Sabellida	Serpulidae	indet.
Polychaeta	Sabellida	Serpulidae	*Hydroides* sp.
Polychaeta	Sabellida	Serpulidae	*Spirorbis* sp.
Arthropoda			
Malacostraca	Decapoda	Plagusiidae	*Plagusia* sp.
Maxillopoda	indet.	indet.	indet.
Maxillopoda	Pedunculata	Lepadidae	*Lepas* sp.
Maxillopoda	Sessilia	indet.	indet.
Maxillopoda	Sessilia	Balanidae	*Megabalanus* sp.
Maxillopoda	Sessilia	Tetraclitidae	*Tetraclitella* sp.
Chlorophyta			
Ulvophyceae	Cladophorales	Cladophoraceae	*Cladophora* sp.
Ulvophyceae	Ulvales	Ulvaceae	*Ulva* sp.
Cyanobacteria			
Cyanophyceae	indet.	indet.	indet.
Mollusca			
Bivalvia	Mytiloida	Mytilidae	*Mytilus* sp.
Bivalvia	Pterioida	Ostreidae	indet.
Polyplacophora	Ischnochitonina	Chitonidae	*Chiton* sp.
Ochrophyta			
indet.	indet.	indet.	indet.
Phaeophyceae	Ectocarpales	Ectocarpaceae	*Ectocarpus* sp.
Phaeophyceae	Ectocarpales	indet.	indet.
Phaeophyceae	indet.	indet.	indet.
Rhodophyta			
Bangiophyceae	Bangiales	Bangiaceae	*Bangia* sp.
Florideophyceae	Ceramiales	Rhodomelaceae	*Polysiphonia* sp.

3.3.2 Biofouling biomass, percent cover and species richness

Total per-vessel fouling biomasses recorded across all vessel regions (general hull area + niche area sampling) ranged from 0–14.7 $kg.m^{-2}$ (mean ± SE = 3.5 ± 1.6 $kg.m^{-2}$). Biomass within individual hull area photoquadrats (0.04 m^2) ranged from 0 – 149 g (3.0 ± 0.83 g). Percent cover within quantitative quadrats ranged from 0–100 % cover (6.0 ± 1.3 %) and was positively correlated with per-quadrat biomass (Pearson's $r = 0.623$, $P < 0.001$). The overall number of species (i.e. richness) sampled from each vessel ranged from 0–20 (6.6 ± 1.9 species). Three vessels had no species recorded on their hulls, while 5 vessels had ≥ 9 taxa present. Understandably, there was a positive relationship between the number of non-indigenous and cryptogenic species (NIS+C) per vessel and the total species richness ($r = 0.680$, $P < 0.05$), with a maximum of 9 NIS+C species collected from a single vessel (vessel KML013). With respect to individual hull regions (excluding niche areas), the average

measured fouling cover and species richness was greatest in stern regions (11.1 % cover, 0.64 species) compared to bow (5.0 % cover, 0.23 species) and amidships (8.3 % cover, 0.39 species). In contrast, average biomass was greatest in bow samples (0.11 $kg.m^{-2}$) relative to samples taken from amidships (0.04 $kg.m^{-2}$) and stern (0.07 $kg.m^{-2}$). However, this trend was driven by one vessel, KML018, which had an unusually high fouling biomass on d-strata areas of the bow (2.58 ± 0.75 $kg.m^{-2}$). If these anomalous observations are excluded, then biomass was greatest at the stern region (0.07 ± 0.03 $kg.m^{-2}$), followed by the amidships (0.04 ± 0.03 $kg.m^{-2}$) then the bow (0.03 ± 0.05 $kg.m^{-2}$).

Multivariate analysis was conducted on presence/absence fouling data, however, three vessels (CAWFV02, CAWFV04 and CAWFV06) were omitted due to the total absence of fouling. Non-metric multi-dimensional (nMDS) plots show four groupings of vessels (Groups 1–4) based on ≥ 25 % Bray-Curtis similarity (Figure 3.5). Vessels in Group 1 had a combined total of seven separate species, with barnacles being the major taxa (71 %). A total of 26 discrete species were collected from the vessels in Group 2, with the dominant taxonomic groups being barnacles and algae (31 % each), bivalves (15 %), and hydroids and ascidians (8 % each). Vessels in Group 3 contained 19 distinct species, with barnacles again being the most dominant taxonomic group (37 %), followed by bryozoans and serpulids (21 % each), and algae (16 %). Group 4 comprised only one vessel (CAWFV03) with 9 unique species that were dominated by bryozoans (33 %). SIMPER analyses did not discern any specific taxa responsible for the observed vessel groupings. Rather, patterns were determined by the relatively small contributions (≤ 9 %) of many species across numerous taxonomic groups.

The BIOENV procedure identified "total species richness–total number of port visits–vessel age–average vessel speed" as the variable combination that 'best' explained the vessel patterns observed in Figure 3.5 (Spearman $\rho = 0.46$; Table 3.7). The correlations for the next two most successful combination (both containing three variables) was slightly less (Spearman $\rho = 0.42$ and 0.41) and similarly consisted of arrangements of these four factors (Table 3.7). The single variables that best explained observed patterns in the nMDS were age of the vessel and total species richness (Spearman $\rho = 0.29$ and 0.26, respectively).

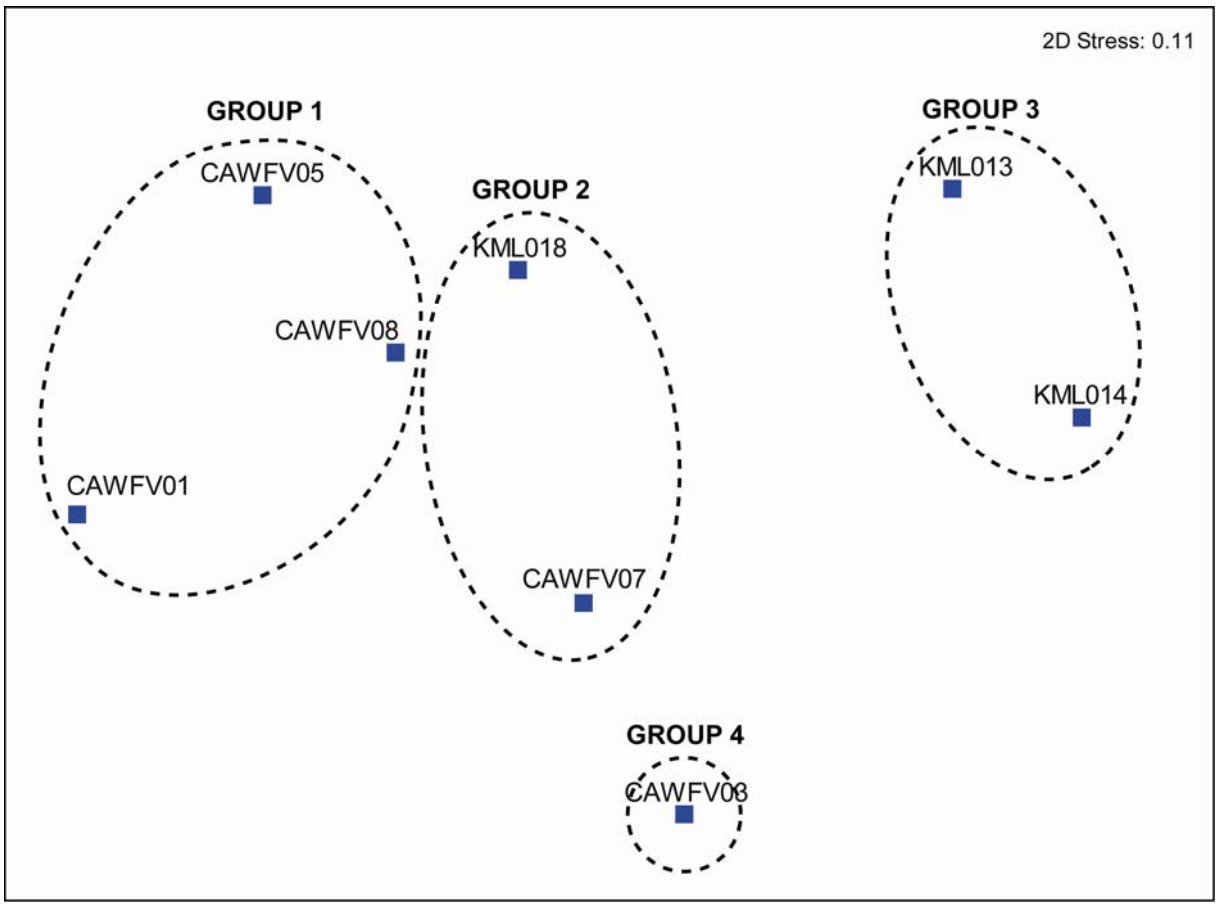


Figure 3.5. nMDS plots of the composition of fouling taxa on fishing vessel hulls . Dotted lines represent ≥ 25 % Bray-Curtis similarity, resulting in four discrete vessel groupings (Groups 1–4).

Table 3.7. Summary of BIOENV analysis in relation to non-metric multi-dimensional (nMDS) vessel distributions and explanatory variables comprising measures of fouling extent, vessel operating parameters and physical characteristics.

Number of variables	Best variable combination	Correlation (ρ)
4	Total richness–Total number of port visits–Vessel age–Avg. vessel speed	0.46
3	Total number of port visits–Vessel age–Avg. vessel speed	0.42
3	Total richness–Vessel age–Avg. vessel speed	0.41
4	Total number of port visits–Unique countries visited–Vessel age–Avg. vessel speed	0.40
3	Total richness–Total number of port visits–Vessel age	0.40
4	Total biomass–Total richness–Total number of port visits–Vessel age	0.38
4	Total biomass–Total richness–Vessel age–Avg. vessel speed	0.38
3	Total biomass–Total richness–Vessel age	0.37
4	Total richness–Total number of port visits–Unique countries visited–Vessel age	0.36
2	Total number of port visits–Vessel age	0.36

3.3.2.1 Utility and accuracy of LoF ranks

Prior to quantitative underwater sampling, surface observations of LoF were allocated to the overall vessel and specific regions of the hull (bow, amidships, stern). The overall surface LoF rank allocated to each vessel was positively correlated to: (1) the recorded biomass collected from the vessel ($r = 0.680$, $P < 0.05$), (2) the species richness recorded for the vessel ($r = 0.868$, $P < 0.001$), (3) the number of NIS+C taxa collected ($r = 0.665$, $P < 0.05$) and (4) the percent cover of fouling ($r = 0.870$, $P < 0.01$; Figure 3.6). Fishing vessels with surface LoF ranks of 2–3 had a total biomass of 1.5 ± 1.1 kg.m^{-2}, total species numbers of 3.8 ± 1.5 and 1.6 ± 1.1 NIS+C species (mean ± SE; Figure 3.6). By comparison, vessels assigned surface LoF ranks of 4–5 had over four times the amount of biomass (7.9 ± 3.3 kg.m^{-2}), species richness (13.3 ± 2.5) and numbers of NIS+C taxa (5.0 ± 1.4; Figure 3.6).

While some positive relationships were observed between the surface LoF ranks assigned to different sections of the vessel (bow, amidships, stern) and fouling extent, relationships were inconsistent across hull regions. The amidships region was the only hull region to show a relationship between LoF rank and biomass ($r = 0.688$, $P < 0.05$), though this trend was weak (Figure 3.7b). There was a stronger relationship between LoF and total species richness and number of NIS+C in the bow ($r = 0.768$ and 0.948, respectively, $P < 0.01$; Figure 3.7d, g) and amidships regions ($r = 0.837$ and 0.968, respectively, $P < 0.001$; Figure 3.7e, h). The amidships and stern regions both showed a positive relationship between LoF and the percent cover of fouling ($r = 0.902$ and 0.785, respectively; $P \leq 0.01$; Figure 3.7k, l). It should be noted, however, that due to the patchy distribution and low numbers of LoF ranks assigned to each hull region, the interpretation of any observed relationships should be done with a degree of caution.

Significant positive correlations were observed between diver allocated LoF ranks and biomass, species richness and percent cover within each quadrat ($r = 0.513$, 0.700 and 0.843, respectively, $P \leq 0.001$;

Figure 3.8). Three individual quadrats ranked by divers as having LoF 0–1 were misclassified, with biomass and percent cover being recorded. This observer error resulted in the average fouling biomass and percent cover of LoF ranks 0–1 ($n = 191$) being 0.001 ± 0.0008 kg.m^{-2} and 0.09 ± 0.13 %, respectively (mean ± SE). Quadrats assigned LoF ranks 2–3 recorded an average fouling biomass of 0.05 ± 0.01 kg.m^{-2} that covered 5.5 ± 1.1 % of the quadrat area and contained 1.0 ± 0.16 species. Not surprisingly, fouling biomass (0.6 ± 0.2 kg.m^{-2}), cover (52.7 ± 6.3 %) and richness (2.0 ± 0.3) was greatest within quadrats allocated LoF ranks of 4–5 (Figure 3.8).

Given the ease by which surface LoF ranks can be obtained compared to underwater LoF assessments, it is useful to compare the accuracy of the two methods. We compared surface LoF assessments of the bow, amidships and stern of each vessel against their corresponding diver-assigned surface (s-strata), deep painted (n-strata) and DDSSs (d-strata) LoF ranks within each hull region. No significant differences were detected between surface LoF scores and diver-assigned waterline (s-strata; Wilcoxon Matched Pairs, $Z = 1.343$, $P = 0.179$) and deep unpainted/poorly painted (d-strata; Wilcoxon Matched Pairs, $Z = 0.440$, $P = 0.660$) scores. In contrast, diver LoF ranks of painted areas (n-strata) of the hull at depth were significantly lower than surface assessments (Wilcoxon Matched Pairs, $Z = 2.641$, $P = 0.008$). The overall vessel surface LoF scores were not significantly different to niche area LoF values assigned by divers (Wilcoxon Matched Pairs, $Z = 0.420$, $P = 0.674$).

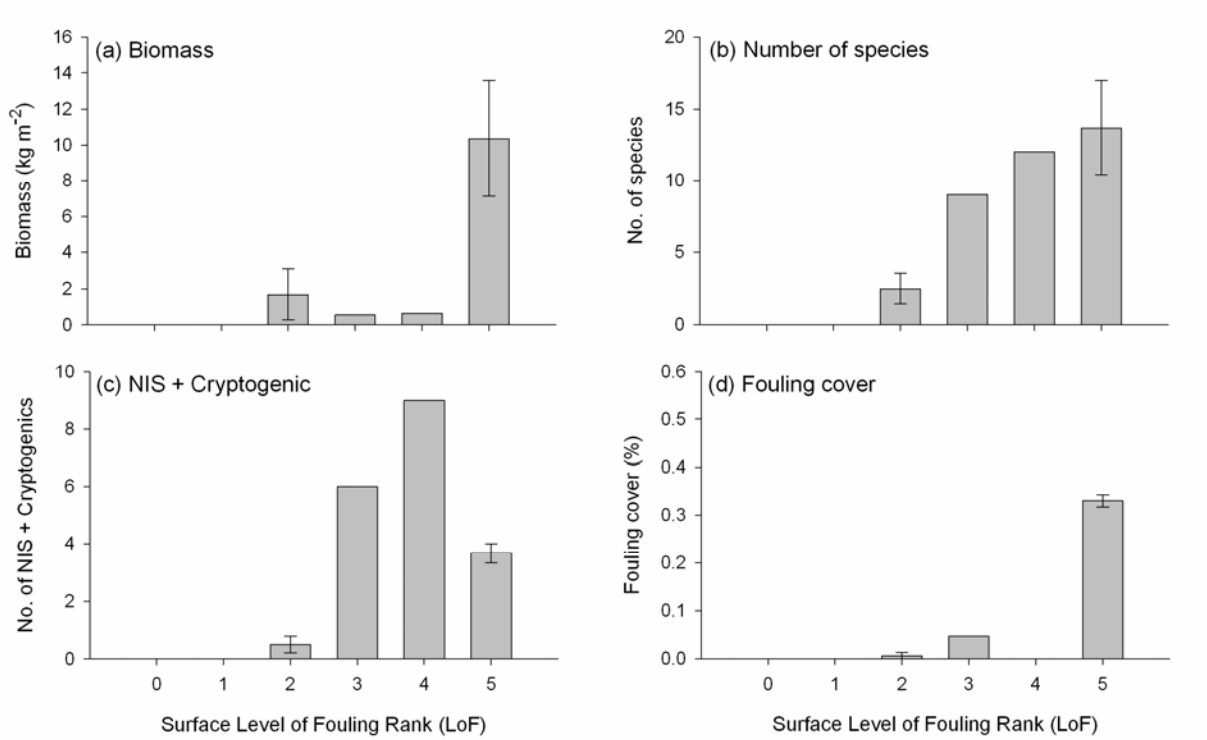


Figure 3.6. Relationship between surface LoF ranks allocated to the entire vessel and the estimates of fouling extent. (a) Total biomass. (b) Species richness. (c) Number of NIS+C species. (d) Percent fouling cover. Bars represent means ± SE.

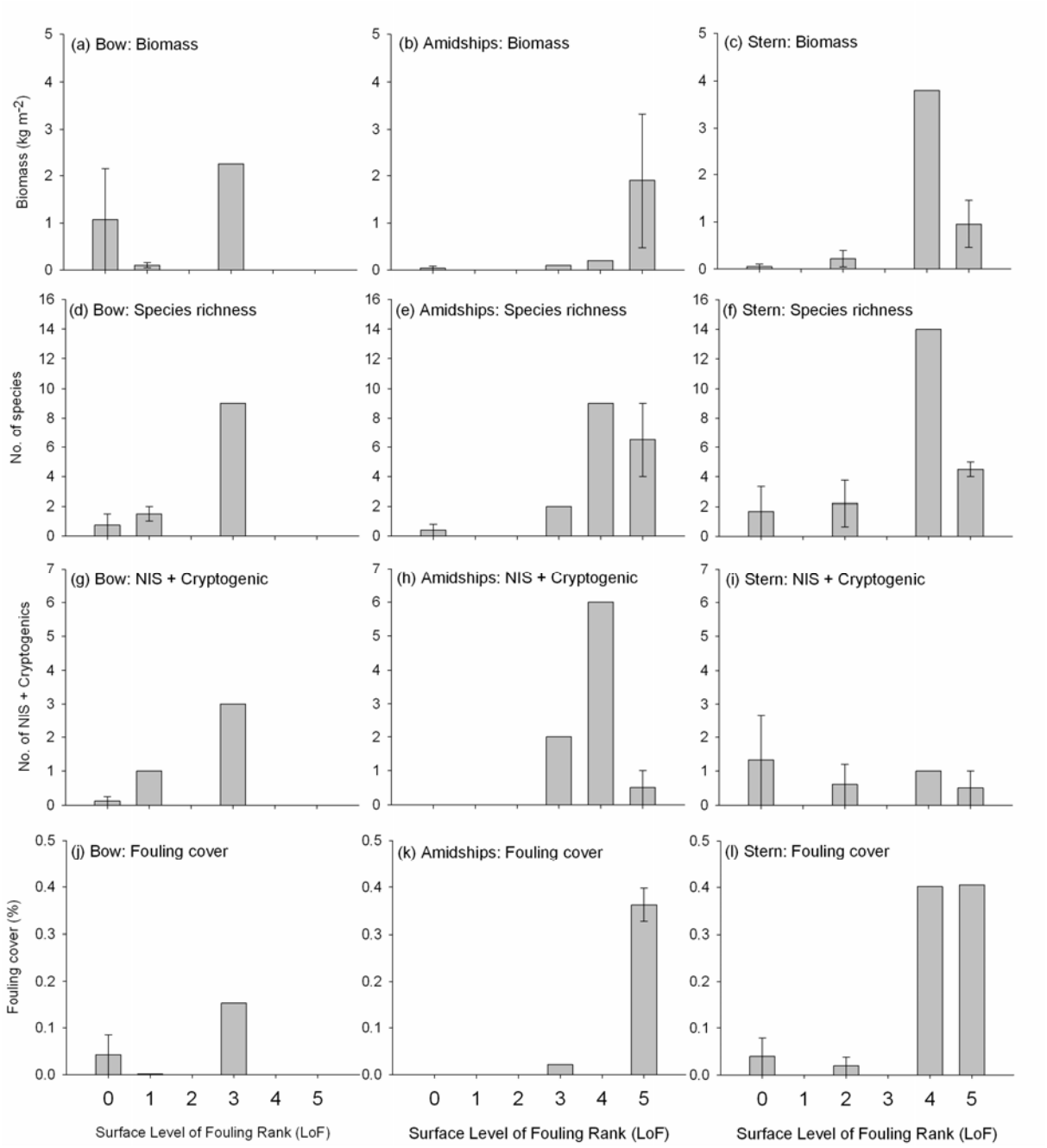


Figure 3.7. Relationship between surface LoF ranks allocated to the bow (column 1), amidships (column 2) and stern (column 3) regions of the vessel and estimates of fouling extent. (a-c) Total biomass. (d-f) Species richness. (g-i) Number of NIS+C. (j-l) percent fouling cover. Bars represent means ± SE.

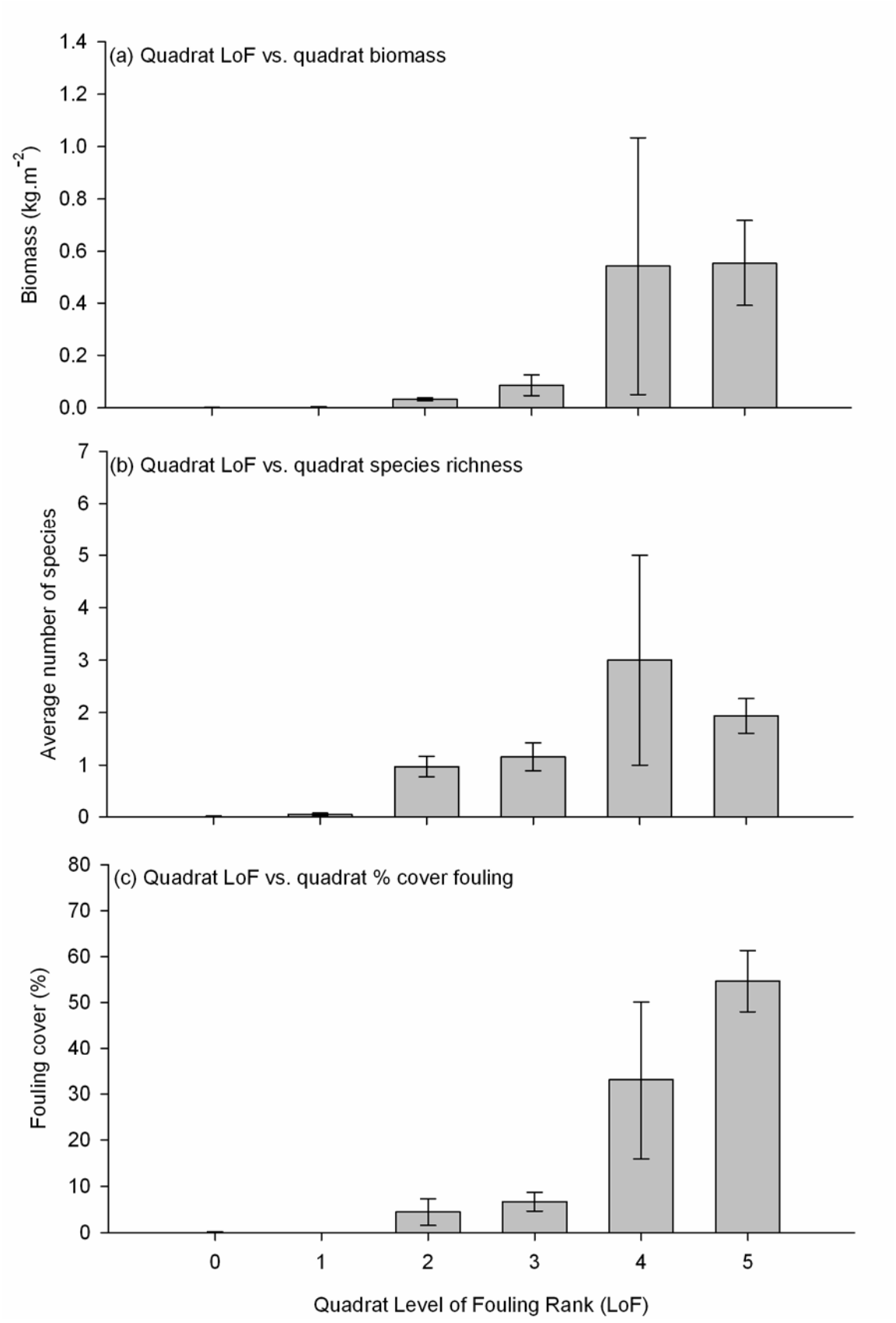


Figure 3.8. Relationship between diver-assigned levels of fouling allocated to each sample quadrat and the estimates of biofouling. (a) Total biomass. (b) Species richness. (c) Percent fouling cover. Bars represent means ± SE.

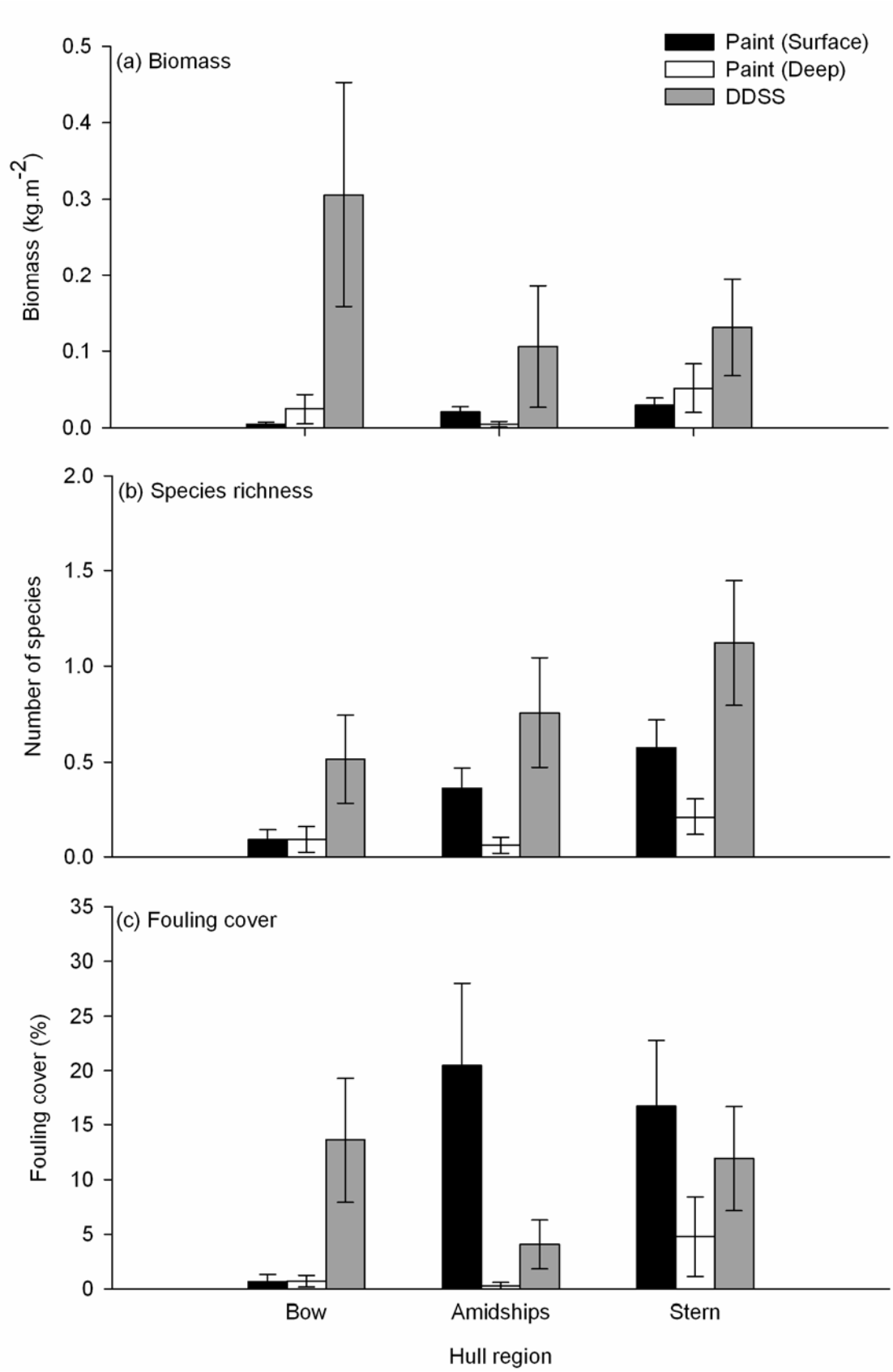
3.3.2.2 Fouling patterns on hull regions and strata

While there were no differences in the patterns of biomass per unit area recorded across the bow, amidships and stern regions of the vessel hulls, there was significant difference in the occurrence of fouling biomass among sampling strata (Table 3.8a; Figure 3.9a). This difference was a result of significantly greater fouling biomass within DDSS areas (0.18 ± 0.06 $kg.m^{-2}$) relative to surface and deep painted areas of hull (0.02 ± 0.004 and 0.03 ± 001 $kg.m^{-2}$, respectively; Tukey's HSD $P < 0.01$). Species richness per quadrat increased along the length of the vessel from bow (0.23 ± 0.08), amidships (0.39 ± 0.1) to stern (0.63 ± 0.12; Table 3.8b, Figure 3.9b), with stern species numbers significantly greater than those found at the bow (Tukey's HSD $P < 0.05$; Figure 3.9b). In addition, DDSS areas contained significantly greater numbers of species than surface and painted areas of hull (Tukey's HSD $P < 0.01$; Table 3.8b, Figure 3.9b). Fouling cover varied across strata, with significantly higher levels found in surface (12.6 ± 3.3 %) and DDSS (9.9 ± 2.6 %) areas compared with deep painted areas (1.9 ± 1.2 %; Tukey's HSD $P < 0.05$; Table 3.8c; Figure 3.9c).

Table 3.8. Summary of ANOVAs examining differences in the fouling biomass, species richness per unit area and percent cover of fouling across different hull regions (bow, amidships, stern) and strata (surface, deep painted, deep DDSS).

Source	d.f.	MS	F	*P*	Significant differences
(a) Biomass ($kg.m^{-2}$)					
Region	2	0.116	0.945	0.390	
Strata	2	0.831	6.765	**0.001**	DDSS > s-strata and n-strata
Region x Strata	4	0.149	1.211	0.306	
Error	288	0.123			
(b) Richness [a]					
Region	2	1.031	5.605	**0.004**	Stern > Bow
Strata	2	1.894	10.295	**<0.001**	DDSS > s-strata and n-strata
Region x Strata	4	0.124	0.672	0.612	
Error	288	0.184			
(c) Percent cover [a]					
Region	2	0.977	2.920	0.056	
Strata	2	1.647	4.921	**0.008**	DDSS and s-strata > n-strata
Region x Strata	4	0.492	1.471	0.212	
Error	234	0.335			

[a] Denotes data that were log(x+1) transformed


Paint (Surface)
Paint (Deep)
DDSS
(a) Biomass
Biomass (kg.m-2)
0.5
0.4
0.3
0.2
0.1
0.0
(b) Species richness
Number of species
2.0
1.5
1.0
0.5
0.0
(c) Fouling cover
Fouling cover (%)
35
30
25
20
15
10
5
0
Bow
Amidships
Stern
Hull region

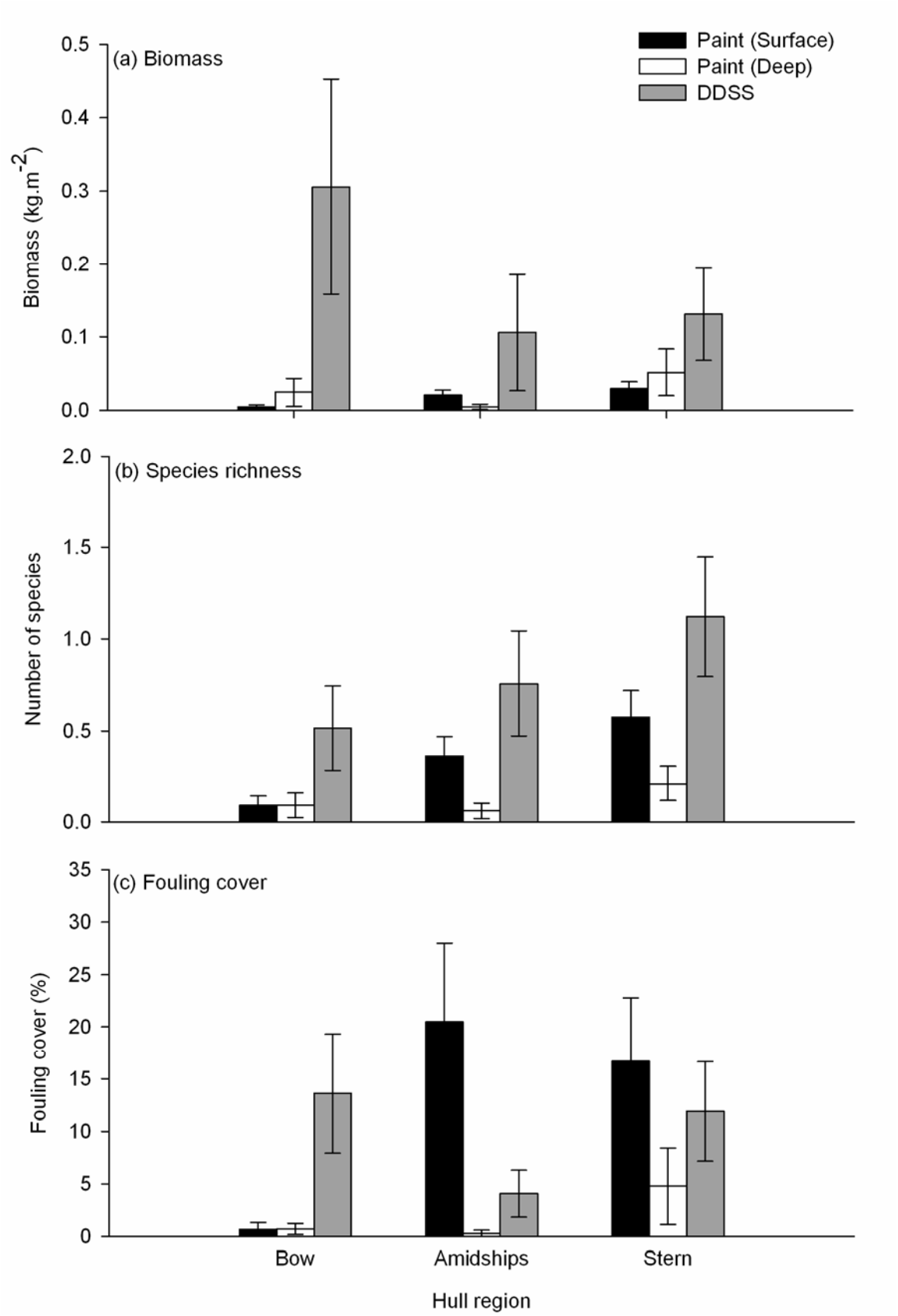


Figure 3.9. Average (a) biomass, (b) species richness, and (c) percent fouling cover recorded per sampling unit (0.04 m^{-2} quadrat) across different hull regions (bow, amidships, stern) and strata (surface, deep painted, deep DDSS). Bars represent mean ± SE.

3.3.2.3 Fouling patterns in niche areas

Despite the "opportunistic" nature of niche area sampling (i.e. unbalanced collection of samples, occurring when and where fouling was observed), an attempt was made to quantify the collection of data in these areas. The same 0.04 m^2 quadrats used during hull area sampling were also employed during niche area sampling, which involved: (1) photographs of niche area fouling within the quadrat, (2) collection of fouling bounded within the quadrat area and (3) measurements of biomass, species richness and percent cover of fouling within quadrat. However, small sample sizes among numerous niche areas and high within-area variability made statistical analysis unfeasible.

A total of eight different niche areas were examined (bow thruster, keel, bilge keel, DDSS, grills/gratings, anodes, propeller and shaft, rudder and shaft). As a result of the opportunistic method of sample collection and often small sample sizes, there was considerable variability in the biomass, species richness and percent cover of fouling among the different niche areas. Average fouling biomass collected from bilge keels (mean ± SE = 1.40 ± 1.35 $kg.m^{-2}$), DDSS (0.67 ± 0.62 $kg.m^{-2}$), anodes (0.61 ± 0.56 $kg.m^{-2}$), propeller and propeller shafts (0.62 ± 0.36 $kg.m^{-2}$) and rudder and rudder shafts (1.16 ± 0.75 $kg.m^{-2}$) were all greater than the largest average biomass recovered for any given hull area (bow DDSS at 0.31 ± 0.15 $kg.m^{-2}$; Figure 3.10a). Similarly, the average species richness recorded in all niche areas (with the exception of keel areas) was greater than the largest number recorded in a hull section (stern DDSS at 1.1 ± 0.32 species; Figure 3.10b). Opportunistic sampling of DDSS yielded the greatest average fouling cover (68.0 ± 7.0 %), followed by grills/gratings (36.2 ± 25.1 %), anodes (34.1 ± 33.0 %) and rudders and rudder shafts (28.3 ± 10.7 %), however, variation was again very high (Figure 3.10c).

Total average niche area biomass (0.64 ± 0.19 $kg.m^{-2}$), richness (2.3 ± 0.30 species) and percent cover (23.4 ± 5.23 %) of fouling in niche areas was 14.4 times, 5 times and 1.6 times greater than those associated with painted areas of hull, respectively (i.e. surface and painted deep strata; Figure 3.9 and Figure 3.10). While differences were generally less, niche areas also recorded greater fouling compared to unpainted/poorly painted hull areas, with 3.5, 3 and 2.4 greater biomass, number of species and percent cover, respectively (Figure 3.9 and Figure 3.10).

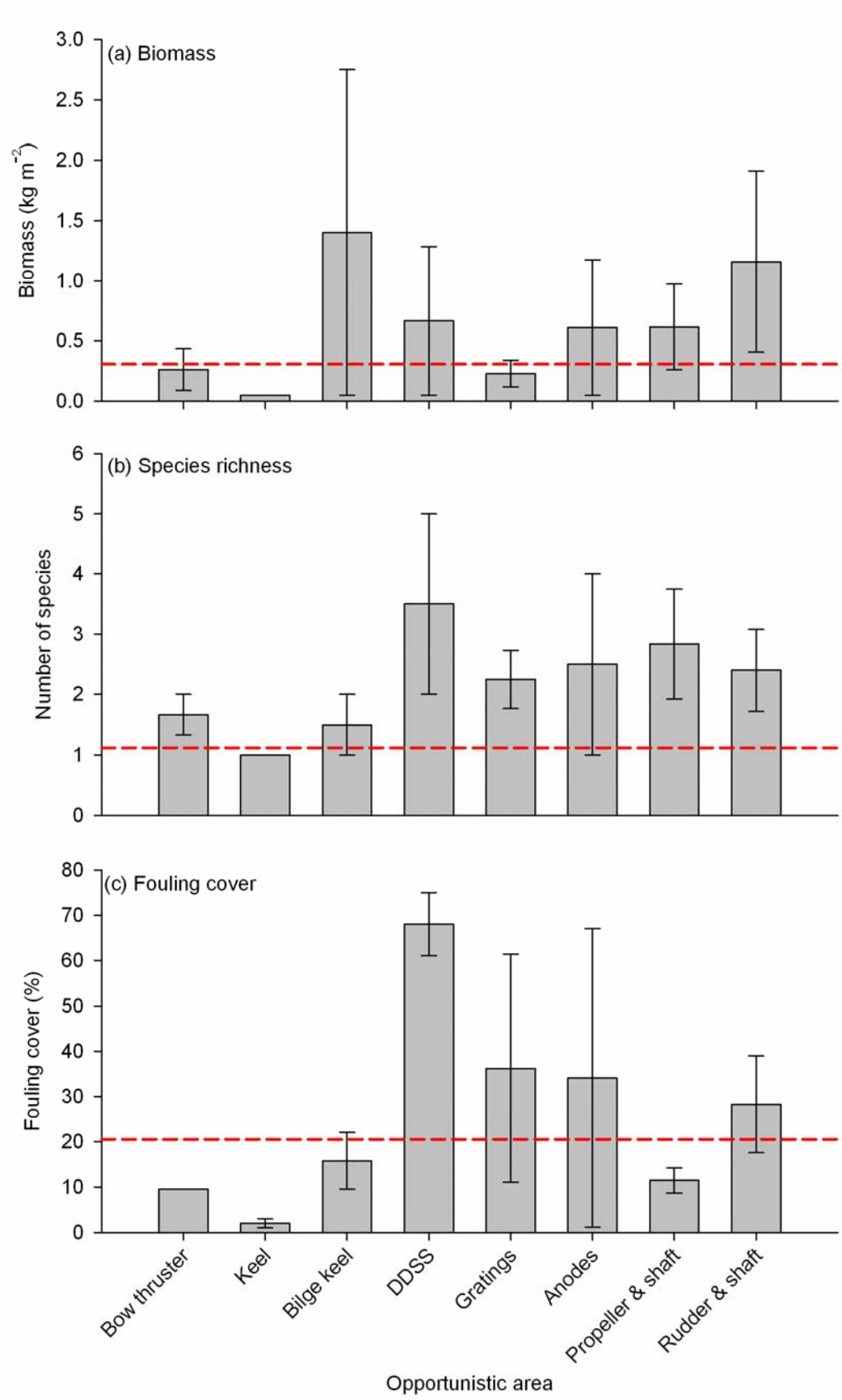


Figure 3.10. Average (a) biomass, (b) species richness, and (c) percent cover of fouling recorded as part of opportunistic sampling of different vessel niche areas. Dotted lines represent the greatest corresponding mean value recorded for hull regions. Bars represent means ± SE.

3.3.2.4 Fouling patterns between fishing vessel types

The fishing vessels surveyed in this study were categorised into four main types, based on their fishing activity and mode of operation: bottom longliner (n = 2), purse seiner (3), tuna longliner (5) and trawler (1). The small sample sizes prevented the application of robust analysis to test for differences in biofouling in relation to vessel type. The trawler surveyed recorded the greatest fouling biomass (mean ± SE = 0.47 ± 0.16 kg.m^{-2}), species richness (1.87 ± 0.41 species) and fouling cover per unit area (33.69 ± 6.61 %; Figure 3.11). In contrast, the two bottom longliners had the least amount of fouling, with one vessel hull completely devoid of fouling, and the second having relatively few barnacles associated with niche areas (keel and anode; Figure 3.11). Biomass and fouling cover varied among vessel types, for example, a high degree of fouling cover was observed on the trawler compared to other vessel types (Figure 3.11a and c). Overall species numbers also varied across vessel types, with the trawler having greater species richness relative to all other vessel types and the purse seiners having more species than the tuna and bottom longliners, respectively (Figure 3.11b).

3.3.2.5 Fouling patterns between bioregions

There were three distinct bioregions (based on Kelleher et al. 1995) in which surveyed fishing vessels operated immediately prior to arrival in New Zealand: Australia and New Zealand (n = 5), the Northwest Pacific (predominantly Japan; n = 2), and the South Pacific (n = 4). No biofouling was recorded on either of the vessels originating from the Northwest Pacific, resulting in this bioregion being omitted from statistical analysis. While the small sample size prevented the application of robust analysis to test for differences in biofouling in relation to vessel type, similar levels of fouling biomass and species richness were observed on vessels from Australia/New Zealand or the South Pacific (Figure 3.12a and b). However, vessels operating from Australia/New Zealand had greater fouling cover (approximately three times) on their hulls relative to vessels arriving from the South Pacific (Figure 3.12c).

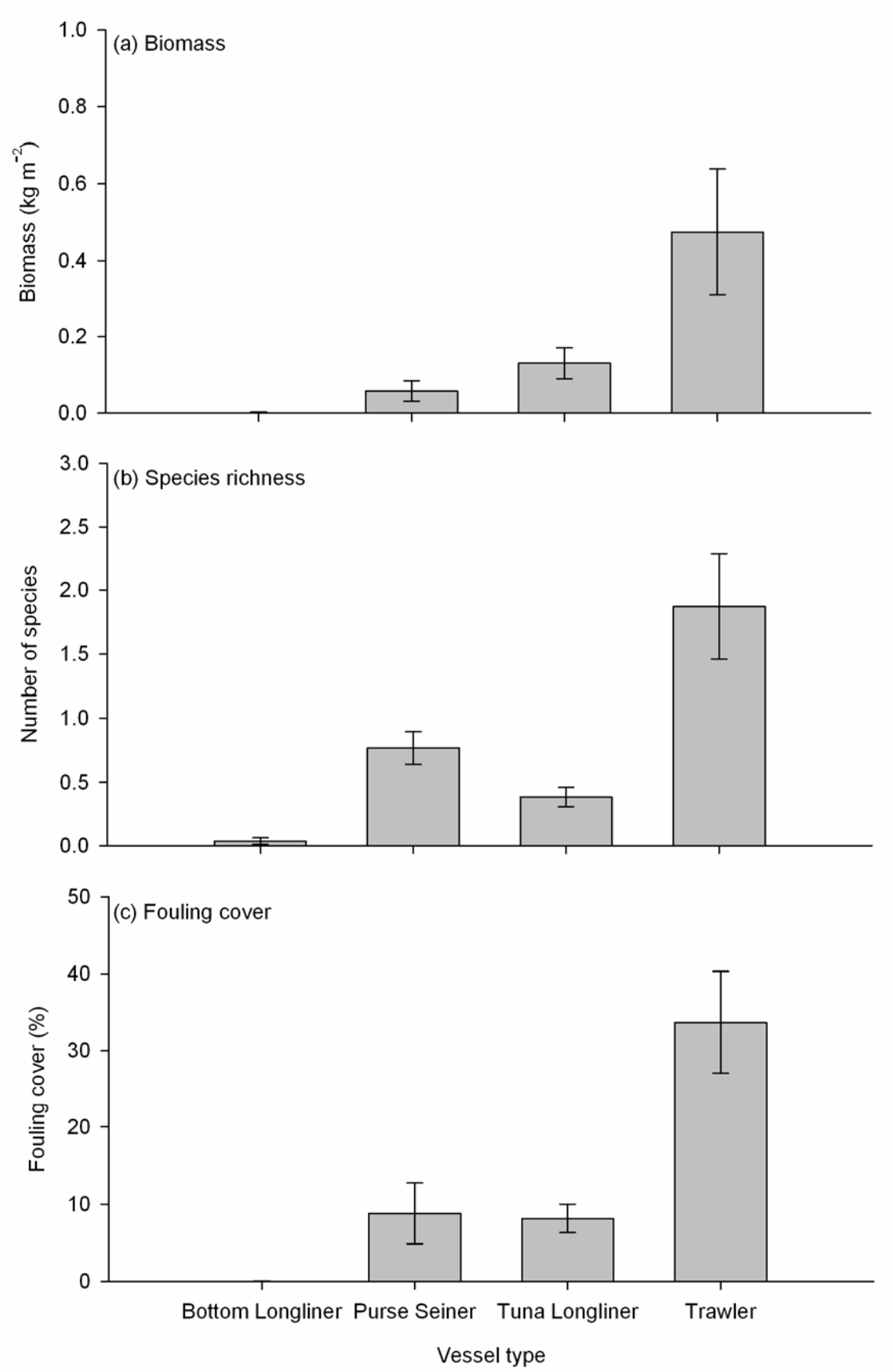


Figure 3.11. Average (a) biomass, (b) species richness, and (c) percent cover of fouling recorded per sampling unit (0.04 m^{-2} quadrat) across different vessel types (vessel sample size per vessel type = 2, 3, 5 and 1 for bottom longliner, purse seiner, tuna longliner and trawler, respectively). Bars represent means ± SE.

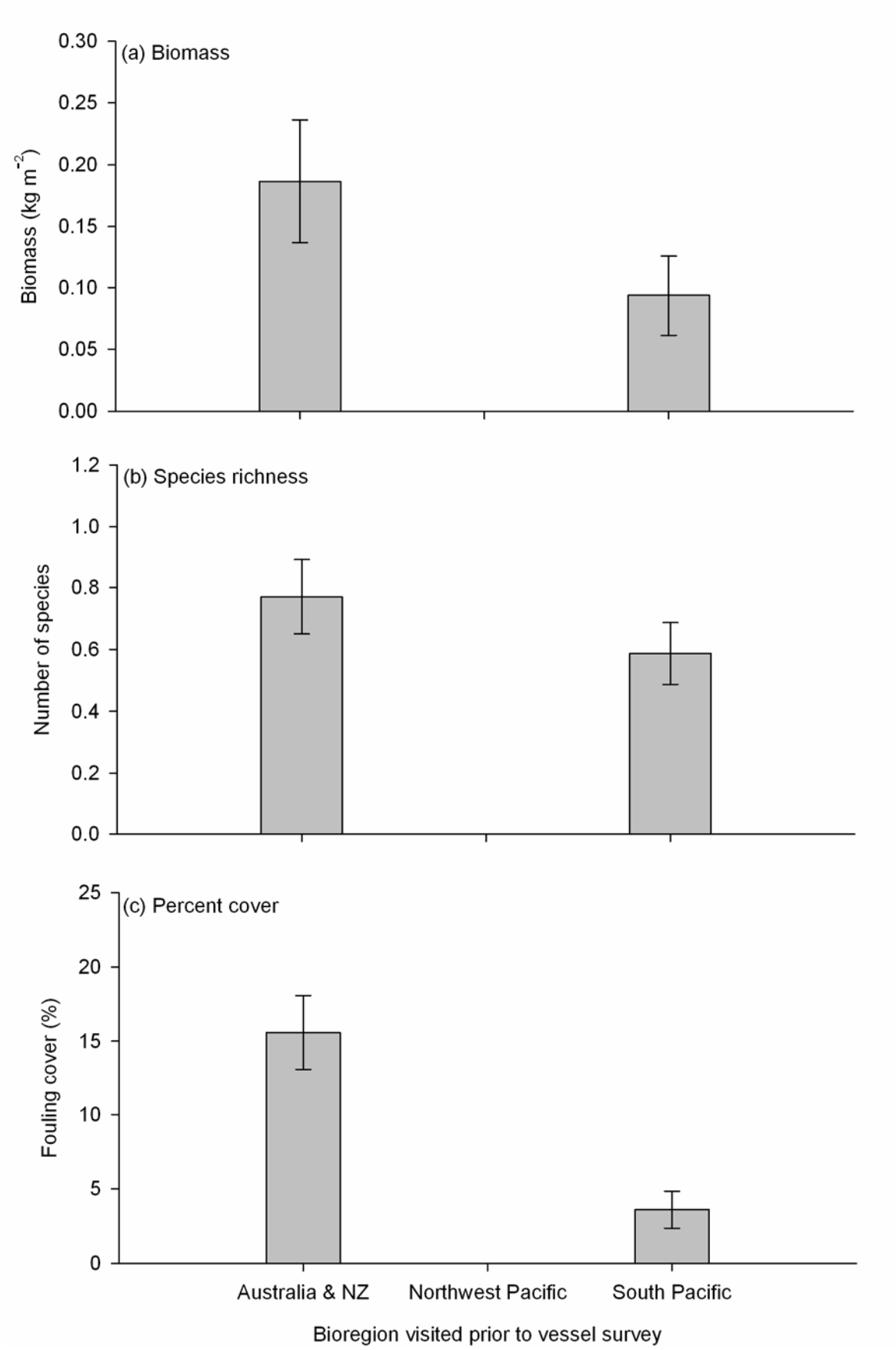


Figure 3.12. Average (a) biomass, (b) species richness, and (c) percent cover of fouling recorded per sampling unit (0.04 m^{-2} quadrat) across different bioregions of vessel operation immediately prior to survey in New Zealand (vessel sample size per bioregion = 5, 2 and 4 for Australia/NZ, Northwest Pacific and South Pacific, respectively). Bars represent means ± SE.

3.4 PREDICTORS OF NON-INDIGENOUS AND CRYPTOGENIC SPECIES (NIS+C)

Fouling extent on fishing vessels was characterised in a number of ways including: biomass (both total measured biomass per vessel, and biomass per sampling unit area), spatial cover (proportion of sampled hull area that was covered in fouling growth, and percent cover of fouling per sampling unit area), and as LoF ranks (allocated by surface observers and divers). As previously discussed in Section 3.4.2.1, there was a significant positive relationship between the overall vessel LoF rank allocated by surface observers and the number of NIS+C species observed ($r = 0.665$, $P < 0.05$; Figure 3.6c). The average number of NIS+C species collected per sampling unit area (i.e. quadrats sampled on vessel hull areas) also increased significantly with increasing diver-assigned LoF ranks ($F_{5,291} = 11.090$, $P < 0.001$; Figure 3.13), however, the increase was not uniform. Rather, quadrats assigned LoF ranks 2 and 4 had the highest NIS+C species numbers (0.5 ± 0.16 and 0.66 ± 0.33, respectively), while quadrats with LoF values of 3 and 5 recorded lower numbers (0.32 ± 0.13 and 0.34 ± 0.15, respectively; Figure 3.13).

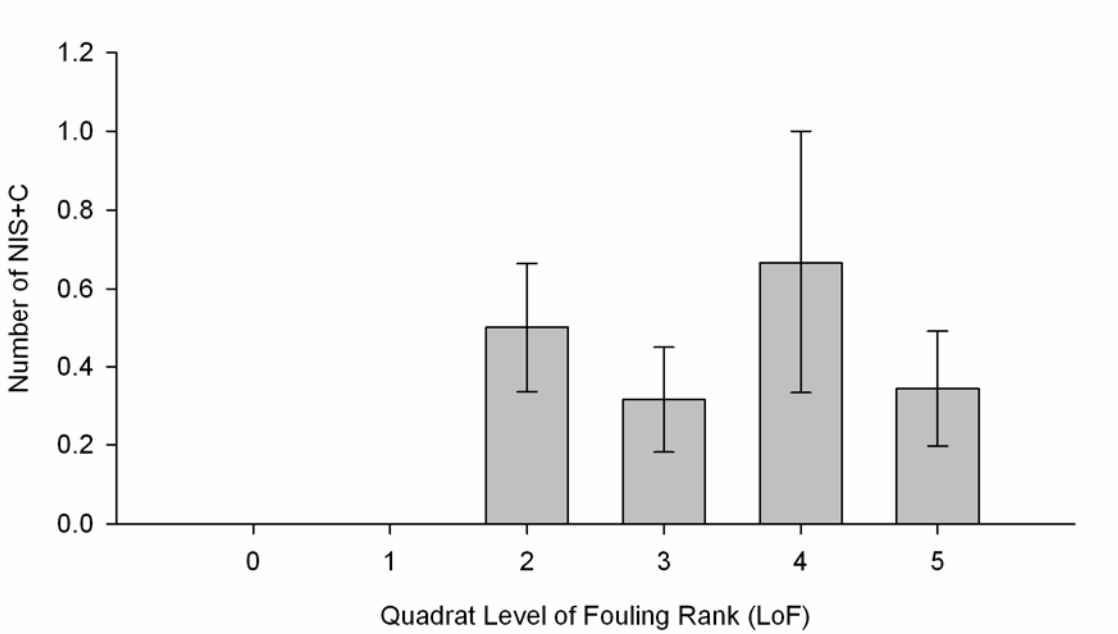


Figure 3.13. Relationship between diver-assigned levels of fouling allocated to each sample quadrat on vessel hull areas and the number of non-indigenous and cryptogenic species recorded for the quadrat. Bars represent means ± SE.

We examined a range of other measured biofouling parameters and vessel characteristics to ascertain their suitability as predictors of the presence of NIS+C species on fishing vessel hulls. Fouling biomass, species richness and percent fouling cover (per quadrat) all showed significant positive relationships with the occurrence of NIS+C species ($P \leq 0.001$; Table 3.9; Figure 3.14a–c). However, the predictive power of these variables was generally poor, with biomass and fouling cover each explaining approximately 17 % of the variation in the number of NIS+C species present, while number of species per quadrat explained 49 % (Table 3.9). When examining predictors on an overall vessel scale, the total number of species collected from each vessel was again only a reasonable predictor, explaining approximately half (46 %) of the variation in NIS+C species numbers for each vessel (Table 3.9, Figure 3.14d). Similarly, percent cover showed a positive relationship with NIS+C ($P = 0.046$; Table 3.9, Figure 3.14e), also explaining 45 % of variation in NIS+C numbers. The only other potential explanatory variables showing a positive relationship with the number of NIS+C present were the total number of port visits since last dry-dock ($P = 0.029$) and the number of different countries visited since last dry-dock ($P = 0.002$; Table 3.9, Figure 3.14f, g). Ultimately, the predictive power of these variables should be interpreted with caution given the overall low number of vessels sampled ($n = 11$), the relatively low number of NIS+C ($n = 18$) and total species ($n = 35$) recorded, and the often low R^2 values returned by many of the tests.

Table 3.9. Linear regression analysis of relationships between the number of NIS+C species (dependant variable) and potential predictive variables, including measures of fouling extent, vessel operating parameters and physical characteristics.

Independent variable	Model equation	*P*	R^2	*n*	No. of outliers removed	Change in R^2 after removing outliers
Per unit of sampled area (quadrat)						
Biomass ($kg.m^{-2}$)	log(NIS+C) = 0.0191+(0.617*log(Biomass))	**0.001**	0.160	296	1	0.041
Species richness	log(NIS+C) = -0.00628+(0.383*log(Species richness))	**<0.001**	0.486	297	0	n/a
Fouling cover (%)	log(NIS+C) = 0.00483+(0.0556*log(Cover))	**<0.001**	0.172	242	1	0.031
Per entire vessel						
Total biomass	log(NIS+C) = 0.239+(0.399*log(Total biomass))	0.128	0.238	11	0	n/a
Total number of species	NIS+C = 0.492+(0.314*Total no. of species)	**0.021**	0.462	11	0	n/a
Percent cover of fouling	log(NIS+C) = 0.173+(4.273 * log(Percent cover of fouling))	**0.046**	0.454	9	0	n/a
Days since last dry-dock	NIS+C = 2.033+(0.00122*Days since last dry-dock)	0.676	0.020	11	0	n/a
Total days spent in port since last dry-dock	log(NIS+C) = -0.241+(0.396*Log(Days spent in port))	0.107	0.262	11	0	n/a
Number of unique ports visited since last dry-dock	NIS+C = -1.592+(6.482 *Log(No. unique ports visited))	0.118	0.250	11	0	n/a
Total number of port visits since last dry-dock	log(NIS+C) =-0.204+(0.714*Log(Total no. port visits))	**0.029**	0.427	11	0	n/a
Number of countries visited since last dry-dock	NIS+C = -1.229+(1.571*No. countries visited since last dry-dock)	**0.002**	0.703	10	1	0.357
Age of vessel	NIS+C = -2.172+(0.253*Age of vessel)	0.052	0.357	11	0	n/a
Estimated wetted surface area (WSA)	NIS+C = -16.700+(6.904*Log(Estimated WSA))	0.177	0.193	11	0	n/a
Average speed	NIS+C = -4.473+(0.649*Average Speed)	0.375	0.088	11	0	n/a

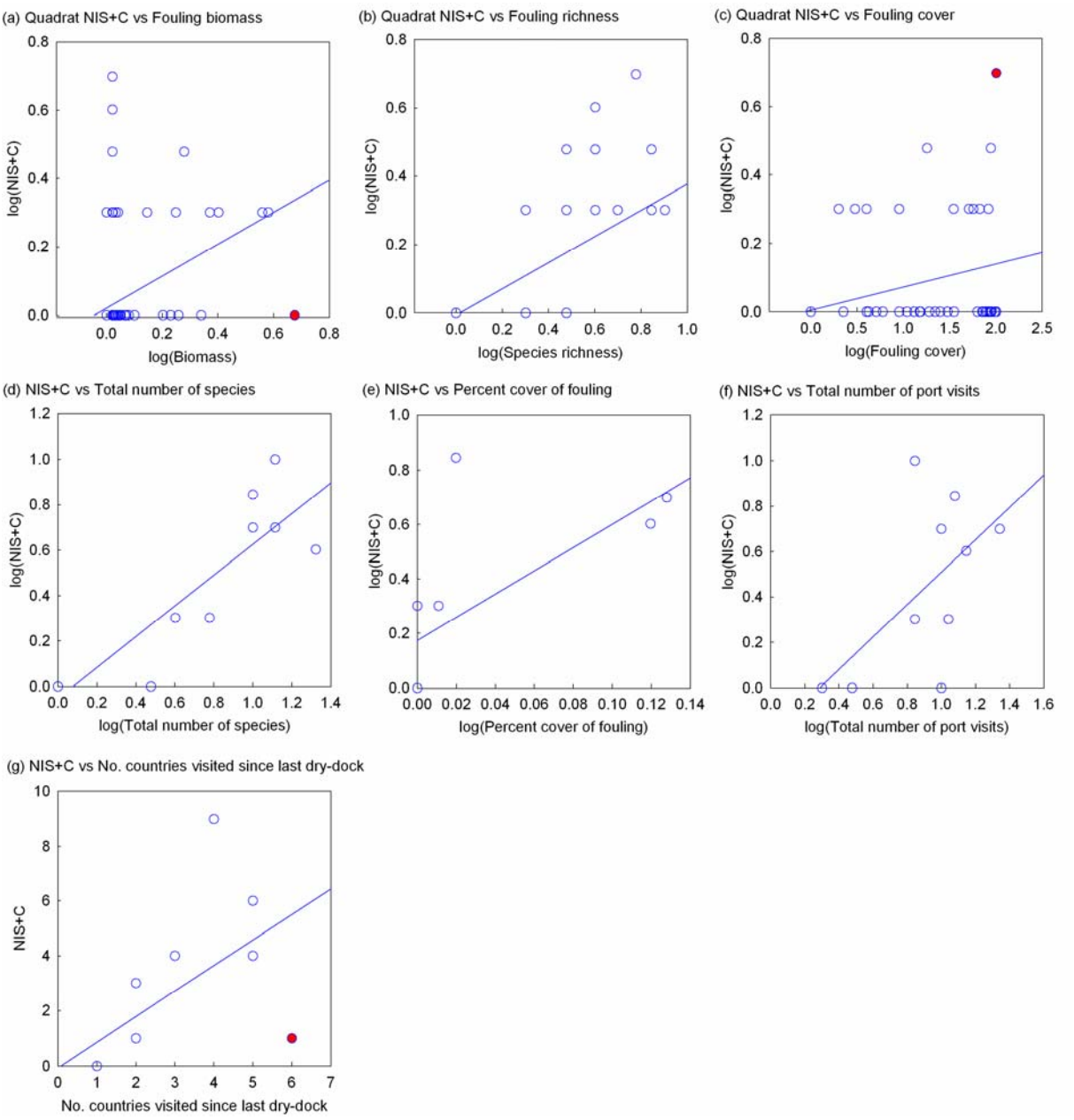


Figure 3.14. Relationships between the number of NIS+C species and potential predictive variables that showed a significant positive relationship in linear regression analysis, including measures of fouling extent (a–e) and a range of vessel operating parameters and physical characteristics (f–h). Data points shown in red (b, c, g) represent outliers that were removed from the final linear regression analysis to improve the goodness of fit.

4 Discussion

Results of this study show that international and domestic fishing vessels operating outside New Zealand's EEZ have the potential to transport fouling communities, including NIS, into New Zealand. Fouling organisms were collected from five vessels in the current study (out of a total of eight), with a further three vessels included in the analysis from a previous project (Stuart 2007). Specimens sampled from the vessels belonged to ten phyla, and included predominantly sessile taxa (ascidians, bryozoans, barnacles, bivalves, hydroids, algae and tubeworms). Mobile species sampled included amphipods, crabs and chitons. The fouling assemblages described in this study appear overall to be less diverse and extensive than other hull fouling communities described on commercial vessels (James and Hayden 2000; Coutts and Taylor 2004; Davidson et al. 2009), passenger liners (Stuart 2007) and slow-moving barges and oil platforms (Hopkins and Forrest 2009), however, the number of vessels sampled in this study was also comparatively less than other studies.

4.1 IDENTITY, STATUS AND EXTENT OF FOULING

Of the 77 taxa collected from the 11 fishing vessels surveyed, 37 were able to be identified to species level. Of these species, 54 % are considered non-indigenous to New Zealand, and to our knowledge, 45 % of these (9 out of 20) are not currently established in New Zealand. Non-indigenous species (NIS) collected included two species of tubiculous polychaete worms, five barnacles, one amphipod, seven bryozoans, two ascidians, two hydroids and one oyster. A further two species (one amphipod and one alga) are considered cryptogenic species (i.e. of unknown geographic origin) and are already established in New Zealand. Of the NIS recorded in this study, none are currently listed as Unwanted Organisms by MAFBNZ.

A number of the NIS collected from fishing vessels are well known cosmopolitan species, of which some have the ability to become nuisance foulers. These include: the tubeworm *Hydroides elegans*, the bryozoans *Bugula neritina*, *Watersipora subtorquata* and *Bowerbankia gracilis*, and the oyster *Crassostrea gigas* – all of which are established in New Zealand. One vessel in particular, CAWFV03, was found to have all of these species present. It should be noted that, despite some of these cosmopolitan species (e.g. *B. neritina*, *W. subtorquata*) demonstrating high tolerances to common anti-fouling biocides (Floerl et al. 2004; Piola and Johnston 2006), almost all instances of their occurrence were on hull or niche areas with poorly maintained and/or absent anti-fouling coverage. The only exception was the occurrence of *W. subtorquata* colonies growing along a weld line on the painted hull section of one vessel. The most frequently encountered NIS (*H. elegans*, *C. gigas*, *Amphibalanus amphitrites* and *A. reticulatus*) were found on two vessels each, highlighting the fact that the overall occurrence of NIS during this study was low.

Four NIS (the tubiculous polychaete, *H. albiceps*, and the bryozoans *Biflustra reticulata, Celleporaria sibogae* and *Conopeum papillorum*) collected on vessel KML013 (Stuart 2007) represented the first known record of these species in New Zealand waters. *H. albiceps* has a relatively wide distribution, occurring along most of the east and northeast coast of Australia, as well as Panama, Sri Lanka, India, Indonesia, Japan and numerous South Pacific Islands[1]. Unlike its congener *Hydroides elegans*, *H. albiceps* is not considered a prominent fouler (G. Read, pers. comm.). The holotype specimens for both *B. reticulata* and *C. papillorum* are

[1] Australian Fauna Directory, Department of the Environment, Water, Heritage and the Arts, Australian Government. Accessed 15 January, 2010. http://www.environment.gov.au/biodiversity/abrs/online-resources/fauna/afd/taxa/Hydroides_albiceps

from Vanuatu, with little described of their distribution beyond this region (Tilbrook et al. 2001). *C. sibogae* was originally described from Indonesia, but has also been recorded from the Solomon Islands and the Philippines (Winston and Heimberg 1986; Tilbrook 2006).

4.2 PATTERNS IN THE EXTENT AND COMPOSITION OF FOULING

The fishing vessels surveyed in this study generally had low-to-moderate levels of fouling present (mean = 0.12 $kg.m^{-2}$ biomass, 0.6 number of species and 9.5 % fouling cover per sample quadrat). Variation in fouling extent (i.e. biomass, species richness, fouling cover) estimates was generally high, reflecting: (1) the small number of vessels available for sampling, (2) the patchy nature of fouling cover across individual vessels, and (3) insufficient replication (for niche areas in particular). Overall, fouling cover and species richness was greatest in the stern regions of the vessel, followed by amidships and bow. That biomass was found to be greatest at the bow was unexpected given that this area of the hull experiences the greatest hydrodynamic forces. Further examination revealed that fouling by large numbers of barnacles on DDSSs at the bow of one vessel, KML018, heavily influenced the analyses. When these data were removed, biomass trends reflected the patterns in fouling cover and richness (i.e. stern > amidships > bow).

Fouling biomass and species numbers were consistently highest on DDSSs. With the exception of bow regions, fouling cover was greatest in areas of the hull just below the waterline. This trend was largely driven by the abundant algal growth in this near-surface zone and the propensity for algal species (e.g. *Ulva* sp., *Chladophora* sp., *Scytosiphon lomentaria*) to rapidly colonise large areas while constituting little biomass. Deep areas of hull with well maintained paint coverage (n-strata) generally contained the lowest fouling biomass, cover and species richness.

In comparison to hull regions, the majority of niche areas sampled had the greatest overall fouling and species richness. Major "hotspots" of niche area fouling included DDSSs, anodes, rudder and rudder shafts, propeller and propeller shafts, and gratings. Conservatively, we estimate that niches areas comprise ≤ 10 % of the total submerged hull area of these vessels, yet on average, the species richness and number of NIS+C species encountered on niche areas were up to 5 times greater than those encountered on general hull areas. Similarly, studies on other vessel types have shown niche areas to contain greater species numbers (Coutts and Taylor 2004; Davidson et al. 2009; Hopkins and Forrest 2009).

The small sample size prevented the application of robust analysis to test for differences in biofouling in relation to vessel type. However, several of the trends observed can be linked to operational factors associated with the vessels in question. For example, the only trawler in the study, the most fouled vessel sampled, was also the vessel with the second longest period since last dry-dock (approximately 2.5 years; Table 3.1), which likely explains the heavy degree of fouling present. With respect to the largely non-fouled bottom longliners, their primary area of operation was within the southern oceans (including Antarctica), where the cold-water conditions may inhibit establishment of new fouling and/or detrimentally impact existing fouling via mechanisms such as temperature-induced mortality (but see Lewis et al. 2004). In addition, these vessels often travel through ice sheets that would realistically remove much of the fouling that may be present, possibly with the exception of some protected niche areas (Lewis et al. 2004; Lee and Chown 2009; Figure 4.1).

The previous ports-of-call for fishing vessels surveyed in this project were located in three distinct bioregions: Australia/New Zealand (temperate ports; $n = 5$); the Northwest Pacific

(namely Japan; $n = 2$); and the South Pacific (New Caledonia and American Samoa; $n = 4$). The two vessels arriving from the Northwest Pacific recorded no fouling, which is most likely attributable to the fact that these vessels had both been dry-docked and antifouled less than 5 months earlier. In contrast, vessels arriving from Australia (Tasmania) and/or operating from New Zealand ports had the highest fouling biomass, cover and species richness relative to other bioregions. This may be indicative of the fact that these vessels travel smaller distances prior to arrival in New Zealand ports, therefore maintaining more "intact" hull fouling assemblages. Changes in en route water temperature may also explain observed differences in fouling. Fouling on vessels travelling from the South Pacific to New Zealand would likely experience a considerable drop in temperature en route, which may affect the survival and/or extent of some hull fouling taxa. In contrast, any changes in water temperatures between and within Australia and New Zealand would likely be small, given the similar latitudinal positions of the Australian and New Zealand ports in question. However, the influence of temperature changes on the survival of biofouling, even between ports on similar latitudes, would largely be dependent on the voyage route taken by the vessel between these ports.

Figure 4.1. Evidence of ice scour on the hull of the bottom longliner San Aotea II (CAWFV01) which frequently operates in the Ross Sea (Antarctica).

Vessels arriving from Australia/New Zealand and South Pacific ports carried similar numbers of barnacles, bryozoans, algae and worm species, while ascidian, bivalve and hydroid species occurred more frequently on vessels from Australian and/or New Zealand ports. Five of the seven non-established NIS encountered in this study arrived on vessels originating from South Pacific ports. In addition, the non-established NIS, *Styela canopus*, was sampled from a vessel that had recently frequented numerous South Pacific ports (American Samoa, Fiji, Tuvalu and the Marshall Islands), despite its previous port of departure being Nelson, New Zealand. The other non-established NIS, *Pyura elongata*, was found on a vessel that had previously been operating out of several Tasmanian ports where this species is known to occur[2]. Given their origins in the South Pacific, it is unlikely that the majority of non-established NIS encountered in this study would be able to readily establish in the cooler waters of New Zealand.

[2] Australian Fauna Directory, Department of the Environment, Water, Heritage and the Arts, Australian Government. Accessed 15 January, 2010. http://www.environment.gov.au/biodiversity/abrs/online-resources/fauna/afd/taxa/Pyura_elongata

4.3 UTILITY AND ACCURACY OF LOF RANKS

There was a strong positive relationship between the LoF assigned to quadrats by divers, and the corresponding fouling biomass, cover and species richness. While one would expect there to be a strong correlation between LoF ranks and actual fouling present in situations where areas of interest are directly visible by the assessment personnel (e.g. divers assessing underwater quadrats), the accuracy of LoF ranks in predicting the extent of vessel fouling becomes much more important when considering surface-allocated LoF values. This study found a significant positive relationship between the overall surface-assigned LoF rank assigned to each vessel, and the corresponding biomass, species richness, number of NIS+C species and fouling cover recorded for that vessel. Encouragingly, no vessels assigned an overall LoF rank from the surface of 0 or 1 recorded any fouling. Some positive relationships were also observed between surface LoF ranks and observed fouling within specific vessel regions (i.e. bow, amidships and stern), however confidence in the reliability of these relationships is not strong due to the low sample size of the present study (11 vessels) and the patchiness and low numbers of LoF ranks assigned to each hull region.

No statistical difference was observed between surface-assigned LoF ranks and diver-allocated LoF for near surface (s-strata) and deep unpainted (DDSS, d-strata) hull areas. However, surface LoF ranks did significantly overestimate the extent fouling on areas of hull with adequate anti-fouling protection (n-strata). This is perhaps due to the fact that algal and biofilm cover can be quite prominent within surface areas of the hull (due to increased light), and may bias the surface observers impression of the remainder of the vessel hull. Surprisingly, surface LoF ranks were not statistically different to diver-assigned values for unpainted areas of hull (DDSSs) and niche areas, suggesting vessels appearing clean at the surface had relatively little fouling in these areas, and vice-versa. This finding is contradictory to observations made for other vessel types (e.g. recreational yachts, slow-movers, merchant ships) surveyed as part of the wider MAFBNZ hull fouling programme, where surface LoF ranks were seen to underestimate the extent of fouling in niche areas and hull locations with little or no paint coverage (e.g. Hopkins and Forrest 2009).

4.4 PREDICTORS OF NON-INDIGENOUS AND CRYPTOGENIC SPECIES

A combination of low vessel sample size and the low occurrence of NIS+C in fouling samples added a considerable amount of uncertainty to the predicted relationships between NIS+C species and quantitative and semi-quantitative variables. Linear regression analyses showed that the prevalence of NIS+C species was positively related to biomass, species richness and fouling cover, respectively. However, given the generally low associated R^2 values for these relationships (< 0.2 for biomass and fouling cover), little confidence can be placed in the predictive power of per-quadrat observations. A similar positive relationship was observed for total species richness and the number of NIS+C per vessel, with overall vessel species richness explaining 46 % of the variation in overall vessel NIS+C richness on vessels. Positive relationships were also observed between numbers of NIS+C species and the number of unique ports and different countries visited by the vessel since last dry-dock. This result is intuitive given that the more countries and unique ports a vessel visits, the greater likelihood of that vessel encountering species that are non-native to its home region. The existence of such relationships provides some encouragement that vessel voyage and maintenance histories may be used to predict the occurrence of NIS on visiting vessels.

4.5 CONCLUSIONS

The results indicate that, following operation outside the New Zealand EEZ, fishing vessels that enter New Zealand ports are potentially fouled with NIS, including those species not already established in New Zealand. However, the fouling extent on the majority of vessels sampled was low. Of the 11 vessels sampled, 3 had no visible fouling, while a further 3 had ≤ 5 species. This study indicated there was a positive relationship between surface-assigned LoF ranks and various measures of fouling extent (biomass, cover and species richness) and the presence of NIS+C species. However, low vessel sample size and the low occurrence of NIS+C in fouling samples means these relationships should be interpreted with caution. For example, this study found that LoF ranks allocated by surface observers were not statistically different to diver-assigned LoF ranks in known hull fouling "hotspots", such as niche areas and hull locations with poor anti-fouling paints coverage. This finding runs contrary to other studies examining recreational vessels, slow movers and passenger liners, where surface-assigned LoF ranks consistently underestimated the extent of fouling in these areas. As such, the reliability of surface observer LoF ranks in accurately predicting the extent of vessel hull fouling remains questionable, despite some positive relationships having been observed in this study. We believe that direct assessment of vessel fouling (e.g. diver observations, ROV surveys, dry-dock inspections) remain the most reliable methods for assessing fouling extent. This may be particularly useful given that some predictive relationships were observed between extent of fouling and the presence of NIS+C species.

5 Acknowledgements

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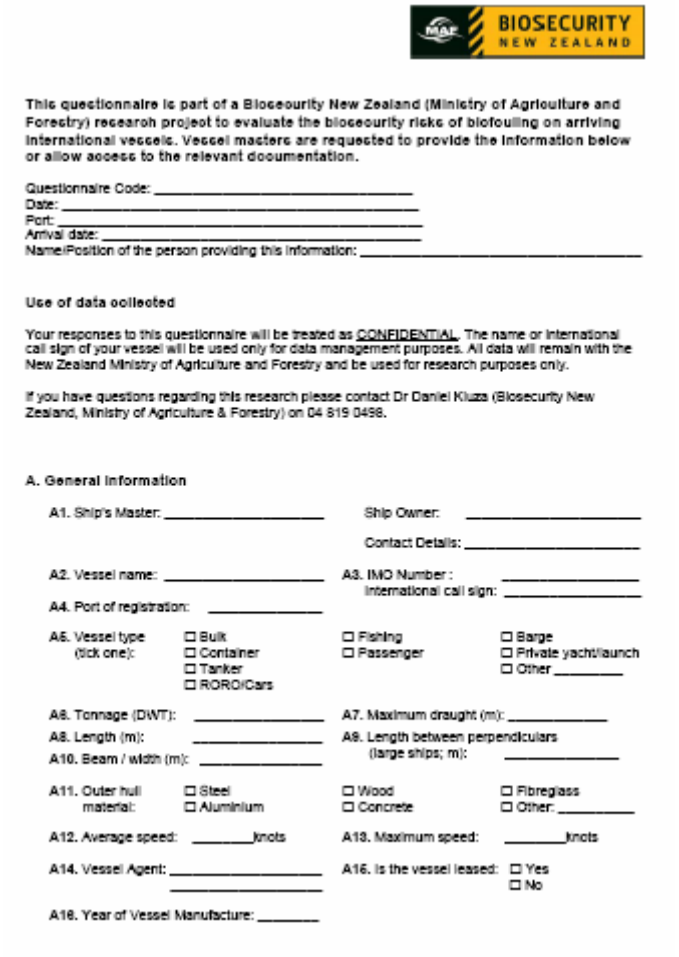
7 Appendices

8 Appendix A: Vessel survey questionnaire



BIOSECURITY NEW ZEALAND

This questionnaire is part of a Biosecurity New Zealand (Ministry of Agriculture and Forestry) research project to evaluate the biosecurity risks of biofouling on arriving international vessels. Vessel masters are requested to provide the information below or allow access to the relevant documentation.

Questionnaire Code: _____
Date: _____
Port: _____
Arrival date: _____
Name/Position of the person providing this information: _____

Use of data collected

Your responses to this questionnaire will be treated as CONFIDENTIAL. The name or international call sign of your vessel will be used only for data management purposes. All data will remain with the New Zealand Ministry of Agriculture and Forestry and be used for research purposes only.

If you have questions regarding this research please contact Dr Daniel Kluza (Biosecurity New Zealand, Ministry of Agriculture & Forestry) on 04 819 0498.

A. General information

A1. Ship's Master: _____ Ship Owner: _____
Contact Details: _____

A2. Vessel name: _____ A3. IMO Number : _____
International call sign: _____

A4. Port of registration: _____

A5. Vessel type (tick one): ☐ Bulk ☐ Container ☐ Tanker ☐ RORO/Cars ☐ Fishing ☐ Passenger ☐ Barge ☐ Private yacht/launch ☐ Other _____

A6. Tonnage (DWT): _____ A7. Maximum draught (m): _____
A8. Length (m): _____ A9. Length between perpendiculars (large ships; m): _____
A10. Beam / width (m): _____

A11. Outer hull material: ☐ Steel ☐ Aluminium ☐ Wood ☐ Concrete ☐ Fibreglass ☐ Other: _____

A12. Average speed: _____knots A13. Maximum speed: _____knots

A14. Vessel Agent: _____ A16. Is the vessel leased: ☐ Yes ☐ No
_____

A18. Year of Vessel Manufacture: _____

B. Maintenance history

B1. Vessel Classification Society or Survey Certification: _____

B2. When was the vessel last dry-docked (large ships) or removed from the sea (small vessels) (dd/mm/yy): _____

B3. Where did this take place? Please indicate country: _____
and port: _____

B4. What type of maintenance was undertaken on the vessel (tick as many options as appropriate): ☐ Removal of fouling ☐ Application of antifouling paint ☐ Repairs ☐ Other: _____

B6. Hull preparation prior to painting: _____

B8. When was the vessel's current antifouling paint applied? (dd/mm/yy): _____

B7. Are multiple paint types currently in use? ☐ Yes....☐ No.

B8. What type of paint was used? Manufacturer/s: _____
Product name/s: _____

B9. Has the vessel's hull been cleaned by scrubbing, brushing or other methods since its latest antifouling paint application? ☐ No ☐ Yes → ☐ While the vessel was removed from the water ☐ While the vessel was in the water
Date (dd/mm/yy): _____
Where did this occur (country and port): _____

B10. What type of cathodic protection is used on your vessel's hull? ☐ Anode ☐ Electrical C3 ☐ Combination ☐ None ☐ Other: _____

B11. Is there any specific treatment that occurs for the seachests? ☐ Hot water ☐ Chemical ☐ Other: _____ ☐ No sea chests on this vessel.

C. Voyage history

We require some important information on this vessel's travel route and port visits since its last antifouling paint renewal (see B6 on previous page). If possible, we would appreciate if you could provide the requested information to both questions C1 and C2.

C1. (a) How many different *ports* has this vessel visited since its most recent antifouling paint application? _____ ports.

(b) How many different *countries* has this vessel visited since its most recent antifouling paint application? _____ countries.

(c) What was the maximum period that the vessel was moored or laid-up in one location? _____ years, _____ months, _____ days.

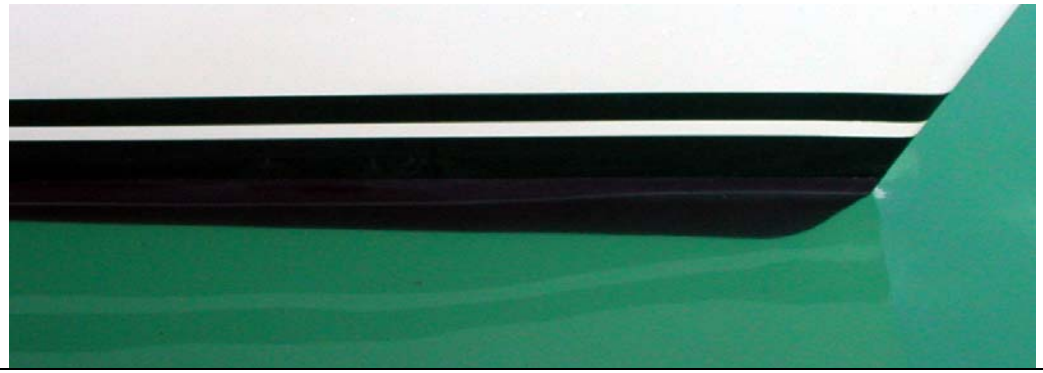
(d) Where and when was this? Please indicate the port (_____), country (_____) and dates (dd/mm/yy to dd/mm/yy: _____ _____).

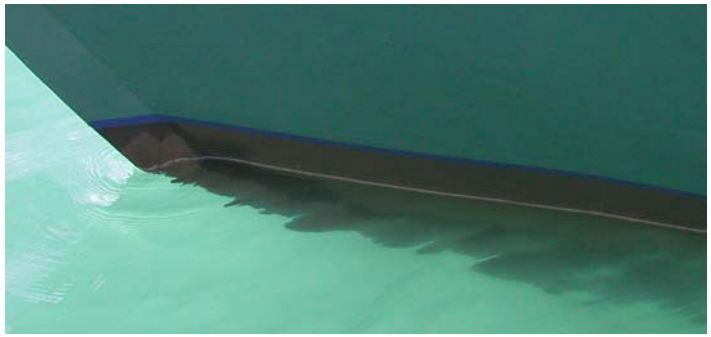
C2. In addition, please supply details from the logbook on the last 20 (or until the last antifouling application) ports of call. *If it is easier for you, please supply an electronic printout of your voyage memo.*

	Port and country	Arrival (dd/mm/yy)	Departure (dd/mm/yy)	
1.				(last port visited prior to current location)
2.				

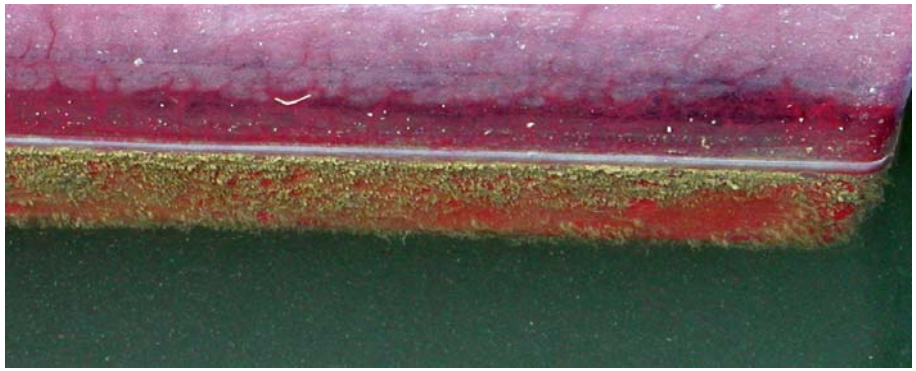
Appendix B: Criteria for allocating level of fouling (LoF) ranks by above-water visual inspection

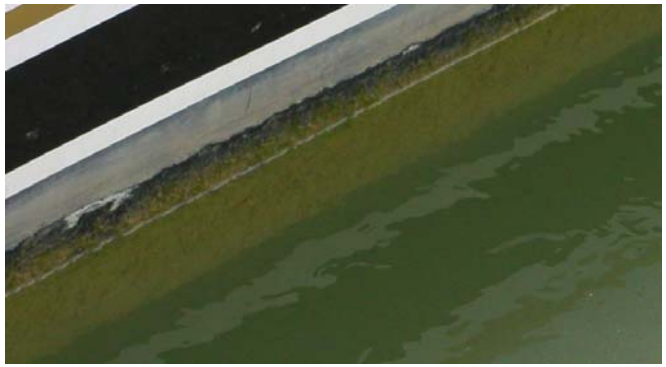
LoF rank	Criteria
0	No visible fouling. Hull entirely clean, no biofilm (slime) on any visible submerged parts of the hull.



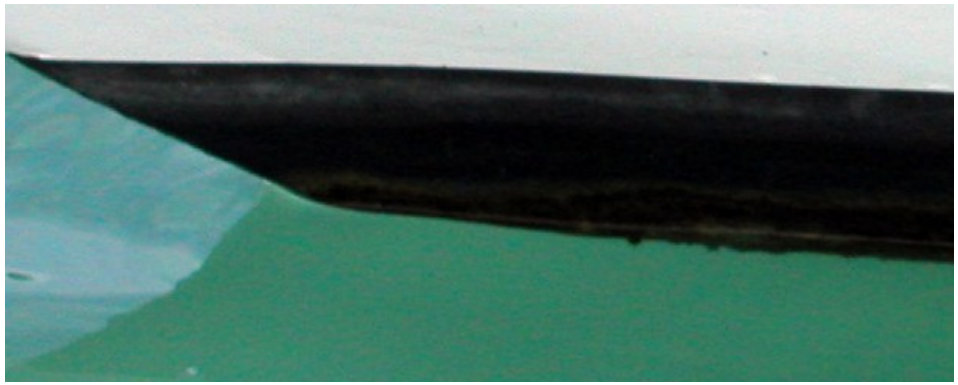


1	Hull partially or completely covered in slime fouling. Absence of any macrofouling.





LoF rank	Criteria
2	Light fouling. 1 – 5 % of visible hull surface covered by macrofouling or filamentous algae. Usually remaining area covered in slime.

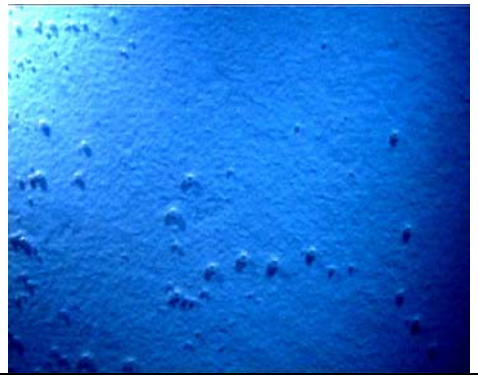
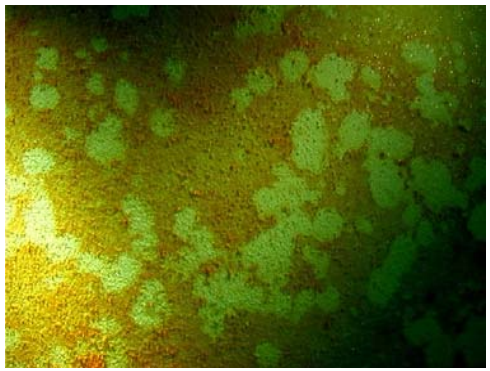


3	Considerable fouling. Macrofouling clearly visible but still patchy. 6 – 15 % of visible hull surface covered by macrofouling or filamentous algae. Usually remaining area covered in slime.

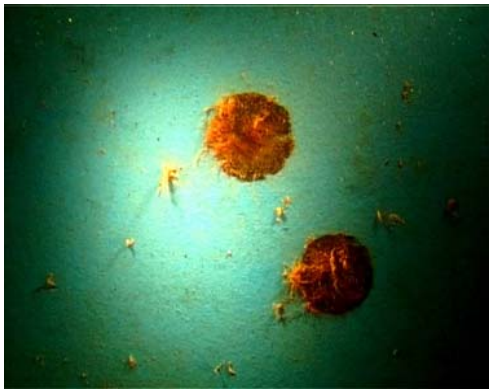
LoF rank	Criteria
4	Extensive fouling. 16 – 40 % of visible hull surface covered by macrofouling or filamentous algae. Usually remaining area covered in slime.

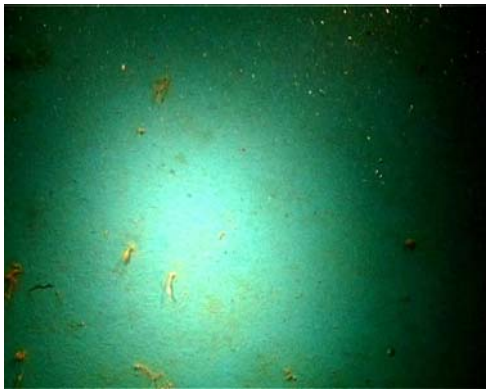
5	Very heavy fouling. 41 – 100 % of visible hull surface covered by macrofouling or filamentous algae. Usually remaining area covered in slime.

9 Appendix C: Criteria for allocating level of fouling (LoF) ranks underwater by divers

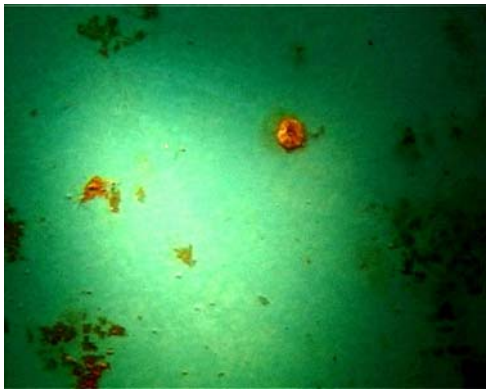
LoF rank	Criteria
0	No visible fouling. Hull entirely clean, no biofilm (slime) on any visible submerged parts of the hull.  
1	Hull partially or completely covered in slime fouling (biofilm). Absence of any macrofouling.  

LoF rank	Criteria
2	Light fouling. 1 – 5 % of visible surface covered by very patchy macrofouling or filamentous algae. Remaining area often covered in slime. Examples below show presence vs. absence of fouling in two adjacent areas of a hull (low LoF overall).





3	Considerable fouling. Macrofouling clearly visible (usually > 1 species) but still patchy. 6 – 15 % of visible hull surface covered by macrofouling or filamentous algae. Remaining area often covered in slime.



LoF rank	Criteria
4	Extensive fouling. 16 – 40 % of visible hull surface covered by macrofouling or filamentous algae. Remaining area often covered in slime.  
5	Very heavy fouling. 41 – 100 % of visible hull surface covered by macrofouling or filamentous algae. Remaining area often covered in slime.