

# The potential for hull-mediated species transfers by obsolete ships on their final voyages

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## ABSTRACT

Shipping has contributed strongly to biological invasions in coastal ecosystems, transferring species in ballast tanks and on exposed underwater surfaces (hulls). A long history exists that documents biota associated with ships' hulls, including some recent analyses of modern ships, but relatively little is known about the associated risks of invasion. In general, the likelihood of invasion is expected to increase with increasing propagule supply, which suggests that high-density transfers on hulls may pose a relatively high invasion risk. Obsolete vessels are expected to be at an extreme end of the spectrum for biofouling, since they sit at anchorage for long periods and are towed at relatively slow speeds when moved, but this remains largely unexplored. In this paper, we quantified the biofouling communities of two obsolete vessels, one stationary for one decade and the other for two decades, before and after their final transit from California to Texas. Pre-departure biofouling surveys across both vessels detected 22 species of macroinvertebrates. The biomass was dominated by the introduced bryozoan *Conopeum chesapeakensis*, which occurred in 98% of samples and created a three-dimensional structure (2–5 cm thick). Mobile species, inhabiting the vertical biofouling matrix, were more numerous than sessile ones. Interestingly, the non-native Asian clam *Corbula amurensis*, not previously associated with hull fouling assemblages, was recorded in 9% of samples. During the 43-day voyage, organisms encountered salinity variation that ranged between zero (Panama Canal) and at least 37 parts per thousand (Brownsville, Texas) and temperatures that varied between 9.9 °C and 31.6 °C. Upon arrival in Texas, we measured an expected decrease in biofouling extent across both vessels but also a surprising increase in species richness (57 species were recorded), with small compositional differences between ships that did not exist prior to departure. Several species were recorded alive upon arrival, including non-natives that are not known to be established in Texas waters. The physiological tolerance and associated risk of colonization have not yet been evaluated for these organisms, or for the broader species pool associated with a standing fleet ( $n > 200$  ships) that may undergo similar movements. Nonetheless, a compelling case exists for vector management based on organism flux alone, to reduce the risk of coastwise and inter-oceanic invasions.

## Keywords

Biological invasions, hull biofouling, inter-oceanic species transfers, non-indigenous species, shipping.

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## INTRODUCTION

In invasion ecology, the quality and quantity of inocula (propagule pressure) are pivotal for the establishment success of organisms (Ruiz *et al.*, 2000; Colautti *et al.*, 2006). Quantitative characteristics of inocula can be defined as density, magnitude, frequency,

duration, and taxonomic diversity. Although the exact relationship between inocula quantity and probability of establishment is not well defined for any species, and likely varies in space and time, there is a strong theoretical and empirical support for increasing invasion success (establishment) with the density and frequency of inocula (Grevstad, 1999a; Drake *et al.*, 2005; Drake & Lodge,

2006). At its most simplistic, establishment and subsequent persistence are more likely as inoculant size and frequency increases (although see also Grevstad, 1999b).

For coastal ecosystems, commercial shipping has been an important source of invasions in many global regions (Cohen & Carlton, 1995; Ruiz *et al.*, 2000; Hewitt *et al.*, 2004; Gollasch, 2006). Ships transfer organisms in their ballast tanks and on their hulls and associated underwater surfaces, creating opportunities for invasion (Allen, 1953; Carlton, 1985; Gollasch, 2002). To reduce associated propagule densities, and thereby invasion risk, ships are being required to treat ballast water before discharge (IMO, 2004; Minton *et al.*, 2005; US Code of Federal Regulations 33 CFR 151). Most ships also maintain prescribed schedules for hull husbandry, including the application of antifouling paints, to reduce the colonization of underwater surfaces. Although the major driver for hull maintenance is ship performance, increasing fuel efficiency through decreased drag (friction), it also has the effect of reducing high density transfers on vessels in routine and constant service.

Given the different motivations for management of ballast water vs. hulls, high-density (and magnitude) transfer events associated with hulls are still possible in some circumstances, as there are no explicit rules or regulations to prohibit these. In particular, specific types of vessel activity are known to affect organism density on ships' submerged surfaces. Port residence times of weeks to months (or greater) have been associated with very dense assemblages of biofouling organisms (Brock *et al.*, 1999; Coutts, 2002; Coutts & Taylor, 2004), far greater than is commonly encountered on most commercial ships that routinely spend ~12–72 h in port. Not surprisingly, it also appears that extent of biofouling decreases with vessel speed and increases with time since dry docking and application of antifouling coatings (Coutts, 1999; Ruiz *et al.*, 2004).

Transfers of high-density hull fouling assemblages have been considered relatively rare events, but an exception may exist for reserve fleets and ships destined for disposal. For example, in the USA, the Maritime Administration (MARAD) retains retired vessels in their Ready Reserve Fleet (RRF), which is divided among three locations on the Atlantic, Pacific, and Gulf coasts. These ships remain at anchorage for years to decades, without hull husbandry. Many of these vessels become obsolete and are removed for dismantling and recycling, being towed at relatively slow speeds to another location for disposal. At present, the RRF consists of more than 200 vessels distributed among the three locations, creating the opportunity for regular high-density transfers of species associated with their hulls.

To date, the biota associated with vessels' hulls in the RRF and the likelihood of species transfers associated with their movement are virtually unexplored. In this study, we measured the biofouling assemblage on the hulls of two RRF vessels in San Francisco Bay, California (before departure), and again in Brownsville, Texas (after arrival), following transit through the Panama Canal from the Pacific to the Atlantic Ocean. We compared the assemblages in space and time to determine (1) the effect of 'voyage' on biofouling extent and species composition; (2) whether variation between ships increased or decreased

under the same treatment (i.e. the voyage); and (3) whether hull locations were important differentiating factors (or hotspots) for fouling assemblages as is the case for 'regular' ships (Coutts & Taylor, 2004). Finally, we considered the potential biogeographical implications and invasion risk to the destination port.

## METHODS

### Vessels and geographical locations

Our study examined the extent and taxonomic composition of biofouling organisms on two RRF vessels, before and after being towed from the Pacific to the Atlantic Ocean. The vessels were initially sampled in Suisun Bay, California, site of the Pacific Coast RRF, and again after arrival to ship breaking facilities in Brownsville, Texas. Suisun Bay extends west of the Carquinez Straits in San Francisco Bay and is flushed by the San Joaquin/Sacramento river delta from the east. The RRF is near the northern shore of the Bay where water conditions are dominated by seasonal freshwater flows from the delta causing salinity fluctuations that range from approximately zero to 22 ppt (MARAD, unpublished data). Peaks in salinity generally occur in September and October followed by lows during freshwater flows in January. The Port of Brownsville is situated on the south-western coast of Texas where salinity is consistently 34 ppt or higher.

Each ship had been resident in the RRF for over a decade, prior to our study. The Florence was a 183.2-m-long oil tanker that was put into service in 1954 and joined the Reserve Fleet in February 1984 having been dry docked just prior to its arrival at Suisun Bay. The Point Loma, a 150-m-long military sea transportation service vessel launched in 1957, joined the Reserve Fleet in October 1993.

Our pre-transit sampling occurred on 8–9 February 2006, and the vessels departed San Francisco Bay on 14 February for Texas. The voyage, a tandem tow of over 5000 nautical miles, took 43 days to complete and crossed at least 32° degrees latitude and 43° degrees longitude. After departing San Francisco Bay, the vessels travelled in a south-easterly direction to the Panama Canal and then north-westerly towards Brownsville. Post-transit sampling was conducted on the days subsequent to each vessel's arrival at docks in Brownsville on 30 March and 1 April 2006.

### Sampling

Vessels were surveyed *in situ* using a commercial diver and surface support team on a dive boat. Sampling was conducted over two separate dives per ship in both California and Texas (eight dives in total). Diving was conducted using surface-supplied air and real-time audio and visual communications with the surface team. Sampling consisted of photo-quadrats and collections of biota, including macroinvertebrates and macroalgae, which was carried out in a stratified random design around the hulls and underwater appendages of both ships. At each vessel location, the diver took a photo-quadrat by fixing the camera against the surface of the ship to record an image. In Suisun Bay, the camera used by the diver was fitted with a 'clear-water box', consisting of

a cylinder of tough transparent Perspex, attached to the camera in front of the lens. This simple device served two important functions; first, it allowed for video footage to be taken in very poor visibility (10–20 cm), and second, it provided a measured surface area in the field-of-view that could be used in the analysis of images to determine organism density or percentage cover. The area of each photo-quadrat was 181.5 cm<sup>2</sup> (6-inch diameter). Photo-quadrats of the same area were taken in Brownsville, but the clear-water box was not required. The diver also collected a biological sample from a random point within a 1-m radius of the photo-quadrat location. Samples were collected using paint scrapers to remove biota from an area of 231 cm<sup>2</sup> (a 6-square-inch area). Samples were placed directly into pre-labelled re-sealable plastic bags, and the bag number was relayed to the surface such that detailed notes could be taken on the locations where each sample was collected. Sample bags were then stored in a mesh dive bag until a batch was returned to the surface. Once returned to the surface, they were transferred to dockside for preliminary sorting within 2 h of each sample being collected.

Overall, 99 samples were collected from both ships in the pre-departure surveys and 92 samples from the post-arrival surveys with corresponding photo-quadrats at each underwater location on the vessels. Sampling was stratified across three depths (immediately below the waterline, mid-depth, and flat bottom) using transverse 'belly line' transects of the hull. Five samples per transverse transect were carried out on the Florence and six per transect for the Point Loma.

In addition to hull sampling, replicate samples were taken from the underwater appendages, such as rudders, propellers, stern tubes, struts, and sea chests. Sea chests are of particular interest in shipping vector studies as they can harbour an organism assemblage that is unlike other fouling assemblages because of microhabitat differences (Coutts *et al.*, 2003). The Point Loma had two open sea chests, one each near the stern and bow. Samples were taken of the sediment from the laterally facing sea chest near the bow and also from the grating of the downward facing sea chest near the stern; no sediment was present at the latter, because this sea chest's grating opened face down on the underside of the hull. Samples were also taken from the rudders, propeller shafts, and propeller-shaft struts on this twin-screw vessel. The Florence had one propeller (and one rudder) from which samples were collected, but its sea chests had been blanked (covered) prior to its arrival in the Reserve Fleet, which denied access to sampling and presumably to aquatic biota.

One temperature logger (HOBO brand, Onset Computer Corp., Bourne, MA, USA) was attached to each ship during the pre-transit survey to determine ship-side water temperature variation throughout the voyage. The temperature was recorded at 5-min intervals for Point Loma and 16-min intervals for Florence. Each data logger was retrieved upon arrival to Brownsville, and data were downloaded for analysis.

### Sample processing, taxonomy, and analysis

At the time of sampling, a preliminary sorting of biological samples was carried out on dockside to assess whether organisms were

alive upon collection. Four ecologists processed the samples by pouring them from re-sealable bags into basins for visual examination and sorting. Examples of each morphospecies that showed evidence of being alive (such as movement or intact, closed valves) were collected and placed in a 'live' jar, and the remaining bulk sample was placed in a separate container. Samples were fixed in formaldehyde solution initially, transferred to 70% ethanol, and subsequently examined in the laboratory. Voucher specimens were put in separate vials and sent to taxonomic specialists for identification and verification. A species presence/absence matrix was constructed and used for univariate and multivariate analysis.

The photo-quadrats were examined by quantifying the percentage cover of 10 readily distinguishable coarse categories in each image: barnacles, dead barnacles, encrusting biota, branching species, paint/hull, tubeworms, amphipods, organism scars, algae, and 'other'. Images were analysed using the point count method to determine percentage cover of each category by superimposing 100 random dots over each photo-quadrat. A matrix of percentage cover for each fouling category was created and used in analysis.

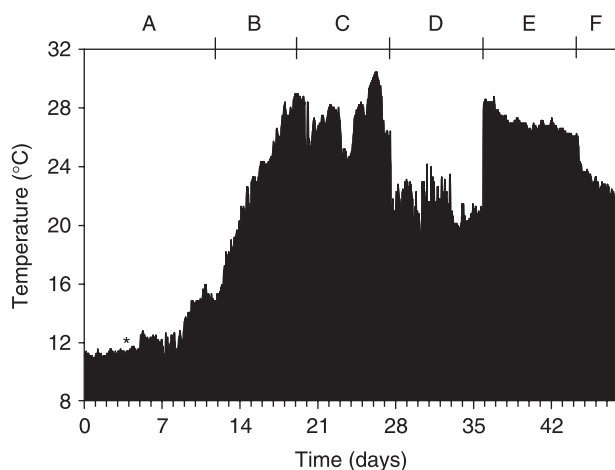
Univariate analysis and multivariate analyses were carried out on species occurrences (biological samples) and percentage cover per photo-quadrat using SPSS (version 13.0) and PRIMER (Clarke & Warwick, 2001 [Primer-E Ltd, Plymouth, UK]). Tests were conducted using transit (two levels; pre and post), ship (two levels; Florence and Point Loma), and hull location (four levels; appendages, waterline, mid-depth, and bottom) as factors. ANOSIM (Analysis of Similarity) was used to test for significant differences in species composition between groups of samples. The test statistic for this technique generally produces a value between zero (no differences between assemblages) to one (totally different). Ordinations were carried out using the multidimensional scaling (MDS) technique, which produces a plot revealing sample similarity; samples close together in the plot are compositionally similar while those far apart are dissimilar. The plots were produced from a Bray–Curtis similarity matrix. SIMPER analysis was used to identify the species (or fouling categories) that contributed most to dissimilarities between groups of samples.

Species rarefaction curves (using 5000 random iterations of sample order) were generated to compare pre- and post-transit species richness for each ship using ECOSIM software (Gotelli & Entsminger, 2006). This was conducted on the biological sample data only and provided 95% confidence limits for each curve such that significant differences can be detected. This approach also allowed for an assessment of the relative completeness of the species inventories for each ship on each sampling occasion.

## RESULTS

### Voyage and temperature variation

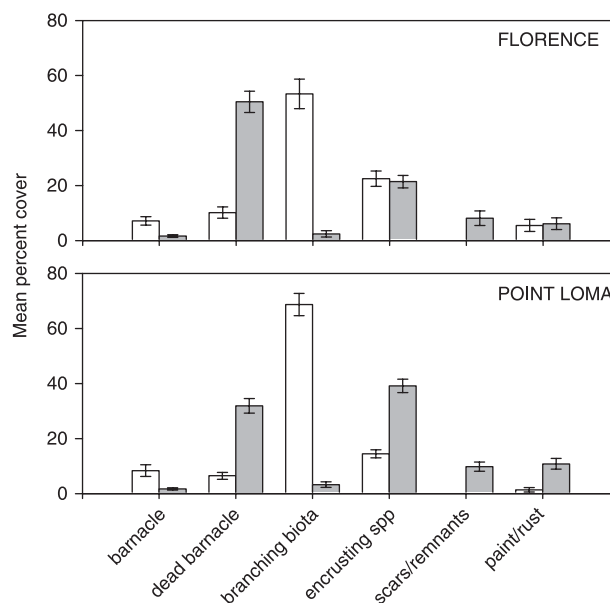
During transit from California to Texas, biofouling assemblages were exposed to considerable changes in physical and chemical conditions. The attached organisms encountered physical disturbance from wave action and swell while at sea as well as sheer



**Figure 1** Ship side temperature along the voyage route. The temperature data were retrieved from a temperature logger that was attached to the hull of the Point Loma. Letters at the top of the plot correspond to stages in the voyage referred to in the text.

forces from the vessels' propulsion. Sea conditions were reported as calm throughout the voyage, and tow speeds were recorded between 4.9 and 7.9 knots (averaging 6.4 knots over 24 reports provided by towing personnel). The temperature signal throughout the voyage reflected several stages of the journey (Fig. 1). During the first 11 days (A in Fig. 1), the temperature remained steady ( $< 12^{\circ}\text{C}$ ) as the vessels were prepared for departure in San Francisco Bay, followed by a moderate increase in temperature as the voyage proceeded southward along the coast of California and Mexico. The subsequent 8 days (B), when the vessels travelled from approximately  $23^{\circ}10' \text{N}$  to  $15^{\circ}30' \text{N}$ , a more rapid temperature increase with diel fluctuations was recorded. This was followed by a period (C) where temperatures varied between  $24^{\circ}\text{C}$  and  $31^{\circ}\text{C}$  as the ships entered the Panama Canal. During the fourth phase of the voyage (D), ship-side temperature was heavily influenced by passage through the Canal locks, with a sharp initial decrease of  $6^{\circ}\text{C}$  to  $7^{\circ}\text{C}$  in less than 2 h upon entry and a subsequent increase of similar magnitude upon exit. The Florence was towed first through the Canal, and was tied up in Limon Bay (Caribbean side) for about 3 days while the tug returned for the Point Loma. Once rejoined for tandem towing, the vessels travelled north through the Caribbean (E) and into the Gulf of Mexico to the Port of Brownsville (F) with a drop in temperature that probably coincided with passage through the Yucatan Channel.

Large changes in salinity were also experienced during transit for organisms associated with the two vessels. Although water salinity was not measured along the cruise track, Suisun Bay had surface salinities as low as 0.2 ppt at the time of departure. The ships spent 21 days in coastwise transit along the Pacific that generally has salinities  $\sim 35$  ppt, prior to entering freshwater of the Panama Canal (at least 7 days) and re-emerging into full strength seawater for the end of the voyage. Surface salinities at Brownsville were measured as 37 ppt at the time of post-transit sampling.



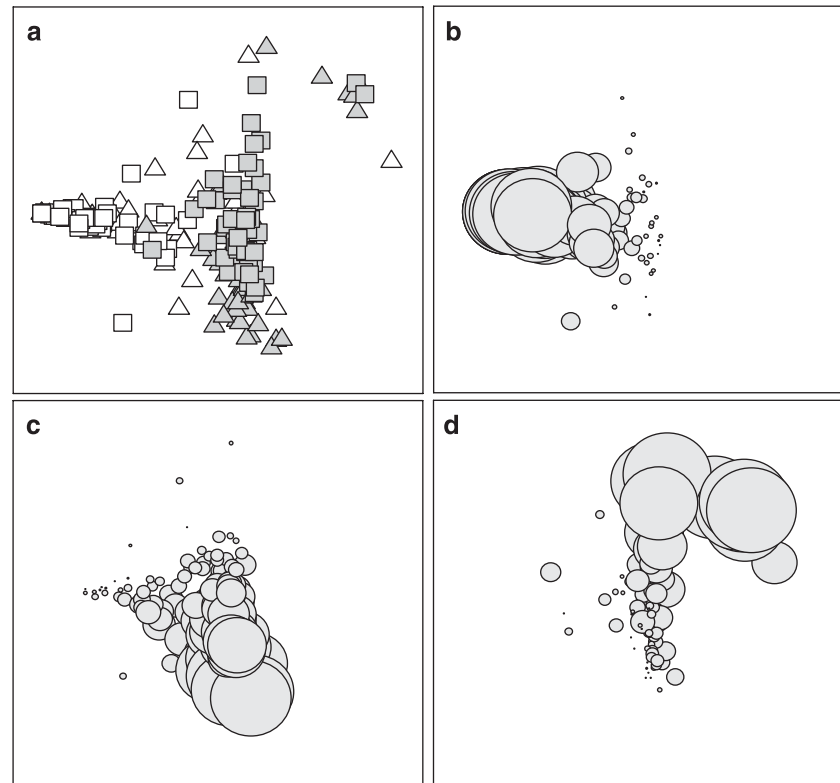
**Figure 2** Comparisons between the mean percentage cover of biofouling on both ships sampled in Suisun Bay and Brownsville. Mean (+ standard error) percentage cover of pre- (white bar) and post- (grey bar) voyage for six biofouling categories is presented. The only non-significant differences between pre- and post-voyage cover occurred for encrusting species and paint/rust on the Florence.

#### Biofouling extent: photo-quadrat data

The pre-transit survey indicated that nearly all of the available underwater surface area of these vessels, an estimated  $8172 \text{ m}^2$  for the two vessels combined, was covered with biofouling organisms; only 14% of photo-quadrats had any bare space within them. In contrast, post-transit surveys revealed that 76% of photo-quadrats had some organism-free space. A MANOVA, using the six most abundant categories from photo-quadrat analysis, revealed a significant interaction effect between pre-/post-transit and ship (Wilks'  $\lambda = 0.760$ ,  $F = 9.94$ ,  $P < 0.0001$ ), indicating that certain biofouling categories did not respond to the effect of voyage consistently between ships. Furthermore, quantitative comparisons among vessels between pre- and post-transit percentage cover estimates of biofouling (Fig. 2) revealed: (1) significant and substantial reductions in percentage cover of branching biota, which was consistent across ships and hull locations; (2) significant decreases in live barnacle cover and increases in dead barnacle cover; (3) a significant increase in encrusting species/biofilm on one vessel only; (4) significant increases for both vessels in non-living remnants of organisms (residual barnacle markings or scars mainly), which were not evident in pre-transit surveys; and (5) a significant increase in paint/hull percentage cover for one vessel only.

The loss of branching species and increase in bare space were due to the effect of the voyage alone. In contrast, the increase in dead barnacles, encrusting species/biofilm, and organism scars may have resulted in part from loss of three-dimensional vertical branching biota, causing increased visibility of a primary fouling

**Figure 3** Comparison of biofouling assemblage organization between pre- and post-transit. (a) MDS plot showing the organization of photo-quadrat samples between pre-(white) and post-(grey) voyage for Florence (triangles) and Point Loma (squares). The stress value for this plot was 0.11. Parts (b) (c) and (d) show the same plot with bubble sizes representing the prevalence of branching species (mainly the bryozoan *Conopeum chesapeakensis*), dead barnacles, and organism-free space, respectively. The three bubble plots reveal differences in percentage cover of biofouling categories across both vessels that help explain the variation between pre- and post-transit (see text).



layer that was previously obscured by a thick mat of biofouling. Certainly, some organisms may also have settled to the underwater surfaces of vessels during the 47 days between the surveys.

An ANOSIM test revealed that there was a substantial and significant difference in biofouling assemblage similarity between pre- and post-transit surveys ( $r = 0.64$ ,  $P < 0.001$ ) as well as a lesser effect of ship ( $r = 0.102$ ,  $P < 0.001$ ; Fig. 3a). SIMPER analysis showed that branching biota, dead barnacles, and organism-free space (paint/rust and scars/remnants categories combined) contributed 38.6%, 23.5%, and 13.1% to the differences between pre- and post-transit biofouling extent, respectively. Branching biota dominated percentage cover across many samples and areas of the ships' hulls in Suisun Bay, particularly at greater depths, but decreased substantially across all hull locations in Brownsville (Fig. 3b). An increase in dead barnacle cover (Fig. 3c) and organism-free space (Fig. 3d) also played an important role in differentiating between pre- and post-transit biofouling assemblages, but not uniformly across vessels or hull locations. In particular, organism-free space was more frequent in quadrats taken near the bows of each vessel. Assemblage dissimilarity between ships was more pronounced at the end-point of the voyage (ANOSIM,  $r = 0.215$ ,  $P < 0.001$ ) compared to pre-departure, where the ships were not at all dissimilar in terms of photo-analysed fouling composition (ANOSIM,  $r = 0.006$ ,  $P > 0.05$ ).

#### Species richness and assemblage composition: pre-transit

There were 22 unique taxa (hereafter referred to as species) recorded on the vessels' hulls prior to departure. They included

representatives of five phyla; Annelida, Arthropoda, Bryozoa, Cnidaria, and Mollusca. There were two clear groups of species based on prevalence across samples; nine frequent species occurred in  $> 40\%$  of samples and the remaining 13 occasional species in  $< 10\%$  (see Appendix S1 in Supplementary Material). Live specimens of 12 species were encountered during initial sample processing, including all nine frequently occurring species. Only six of the 22 species were not recorded from both vessels, with a total of 17 recorded from the Point Loma and 21 from the Florence. A multivariate ordination (nMDS) of the presence/absence data revealed that little difference existed between ships or hull locations in terms of community organization and ANOSIM tests confirmed that the assemblages on both ships were quite similar (all  $r = 0.13$ , all  $P > 0.01$ ). There was also no significant difference in sample species richness between ships but there were significant differences between vessel appendage locations (running gears) and some depths on the hull (two-way ANOVA; ship,  $F = 1.32$ ,  $P > 0.05$ ; hull location,  $F = 4.25$ ,  $P < 0.01$ ). The qualitative sample (grab) of sediment taken from the sea chest of the Point Loma had just one macroorganism species retained in it – the polychaete *Neanthes succinea* (Frey & Leuckart 1847).

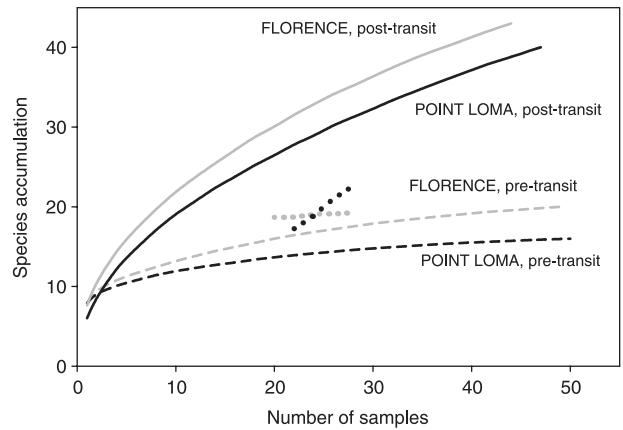
The most widespread pre-transit species was the encrusting bryozoan, *Conopeum chesapeakensis* (Banta *et al.*, 1995), which covered large areas of the hulls of both vessels, occurring in 98% of samples collected and dominated space in the pre-transit photo-quadrats ('branching biota' above). It formed the bulk of the fouling matrix ( $> 5$  cm thick in many areas) that created three-dimensional habitat that other species, particularly mobile species, utilized. It is an encrusting bryozoan but formed unusual

tufts on these vessels with upright strands of zooids, creating colonies that developed vertically out from the hull as well as horizontally across the hull. The hydrozoan, *Turritopsis* sp. (see Miglietta *et al.*, 2006), was the only other sessile fouling species recorded frequently across samples, highlighting how mobile species were widespread and dominated richness measures across both ships. Eight non-native species that are currently absent in Texas waters were recorded (see Appendix S1 in Supplementary Material).

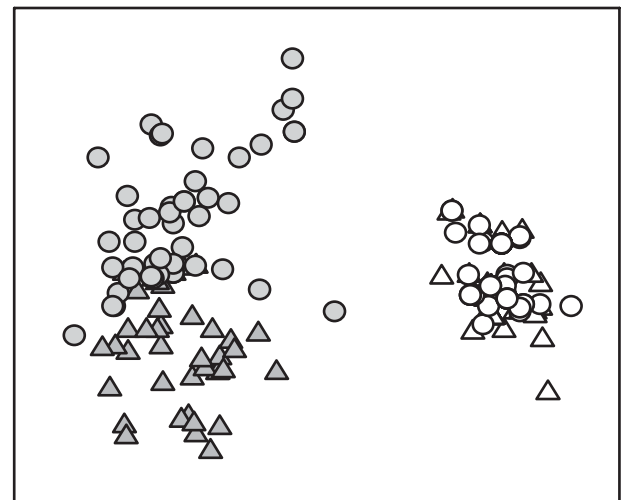
**Species richness and assemblage composition: post-transit**

Surprisingly, there were 57 species recorded from the 92 biological samples collected from both vessels after transit to Brownsville. Live specimens were observed for 22 of the 57 species, including all 11 of the prevalent species (those occurring in greater than 10% of post-transit biological samples). Thirteen species from Suisun Bay sampling were not recorded in Brownsville. By contrast, an additional 48 species were collected from post-transit samples that were not previously recorded on these ships (see Appendix S1 in Supplementary Material). Most of the ‘new’ species to these ships were spatially rare; 60% were singletons or doubletons (occurring in only one or two samples, respectively). On a per sample basis, the Florence had significantly more species on average than the Point Loma when they arrived in Brownsville, but there was no significant difference between depths (two-way ANOVA; ships,  $F = 6.9, P < 0.01$ ; depths,  $F = 0.48, P > 0.05$ ; interaction,  $F = 1.73, P > 0.05$ ). In addition, small dissimilarities in assemblage organization occurred between both vessels but not between hull locations (ANOSIM, between two ships,  $R = 0.27, P < 0.001$ ; between four hull locations,  $R = 0.035, P = 0.13$ ).

The effect of transit on species richness and assemblage organization is highlighted by comparisons of rarefaction curves and ordinations of pre- vs. post-transit biofouling assemblages. The increase in species richness after the voyage, dominated by rare species, was reflected in rarefaction curves where asymptotes for species accumulations were not approached, especially for post-transit sampling (Fig. 4). The clear inference from this plot, comparing similar sampling efforts for pre- and post-transit surveys, is that our surveys underestimated the number of species present and that there were significantly more species on a per ship basis in post-transit surveys. The difference was such that pre- vs. post-transit richness measures began differentiating below a level of five samples and only 24 samples per ship were required to detect significant differences in numbers of species between surveys at 95% confidence limits. In contrast, curves for both ships do not differentiate notably from each other at each of the donor and recipient ports as samples were added. Similarly, there was a significant effect of transit on biofouling assemblage organization (Fig. 5) and a minor effect of ship (two-way ANOSIM; pre- vs. post-transit,  $r = 0.971, P < 0.001$ ; ship,  $r = 0.164, P < 0.001$ ). Importantly, the influence of ship on assemblages was more pronounced at end-point sampling rather than prior to departure in Suisun Bay.



**Figure 4** Rarefaction curves for each vessel sampled in Suisun Bay and Brownsville. The plot indicates how pre- and post-voyage curves are significantly different from each other, but assemblages did not differ significantly between ships at either Suisun Bay or Brownsville. The post-transit curves are also further from approaching asymptote indicating species richness was underestimated. Dashed and solid lines represent pre- and post-transit, respectively. The dotted partial lines (grey and black) indicate the intercept where the lowest 95% confidence limit for post-transit curves crossed with the highest 95% confidence limit for pre-transit curves.



**Figure 5** Comparison of assemblage organization using samples collected from both ships prior to and after the voyage. MDS revealed clear separation between pre-transit (white) and post-transit (grey) samples in terms of composition. The stress value for this plot was 0.1. Samples from the Florence and Point Loma are represented by triangles and circles, respectively.

Both the bryozoan, *C. chesapeakensis*, and the barnacle, *Balanus improvisus* (Darwin 1854), which were present in 98% of biological samples collected in Suisun Bay, were recorded in almost 98% of samples in Brownsville as well (see Appendix S1 in Supplementary Material). Two additional barnacles, *Balanus*

*amphitrite* (Darwin 1854) and *Lepas pacifica* (Henry 1940), were recorded in post-transit samples only, but only as a doubleton and singleton, respectively. Five species of hydroid not previously recorded during pre-transit sampling were surprisingly prevalent (see Appendix S1 in Supplementary Material). It is highly unlikely that these hydroids occurred at such prevalence and were missed in the pre-transit survey, and we re-analysed a subset of pre-transit samples to confirm this was the case – detecting no specimens of these taxa. The prevalence of one species of hydrozoan, *Turritopsis* sp., was greatly reduced between pre-transit (67% of samples) and post-transit (2%) surveys.

We detected multiple mobile crustacean species during post-transit sampling that were not present in Suisun Bay. Many of these organisms may have colonized the vessel during the transit and while in port in Brownsville, since many of the previously absent copepods and amphipods are established (both native and non-native) in the Gulf of Mexico. Because the complex, three-dimensional matrix of fouling was greatly reduced, many of the mobile organisms that inhabited the matrix in Suisun Bay were probably removed during the voyage. In terms of prevalence in samples, two amphipod species had notable reductions compared to pre-departure levels (*Melita nitida* (Smith 1873) 85% to 1% and *Gnorisphaeroma insulare* (Van Name 1940) 74% to 2%) and were not accounted for alive during preliminary sorting in Texas. The isopod *Gnorisphaeroma oregonense* (Dana 1852), however, survived the voyage and was present in 25% of samples taken across both vessels. This species is native to the Pacific coast of North America and is not known to occur in Texas waters. Overall, for species with adequate taxonomic resolution, there were seven species non-indigenous to the Texas coast found on the ships in Brownsville (five were observed alive; see Appendix S1 in Supplementary Material), and two of these are already established in Texas coastal waters. There was also a further nine taxa that are considered cryptogenic to the western Gulf of Mexico.

## DISCUSSION

### Pre-transit biofouling characteristics

Our study confirms that the extent of biofouling on RRF vessels is high, relative to commercial vessels in regular service, creating the opportunity for high-density transfer events upon movement. For the two vessels, pre-transit surveys documented nearly 100% cover of underwater surfaces by macrobiota. By contrast, biofouling on commercial vessels appears to be routinely < 10% (Coutts & Taylor, 2004; Ruiz *et al.*, 2004), although reports of high richness on commercial ships exist (Drake & Lodge, 2007).

Variation among hull locations prior to departure was minimal, despite differences in durations and vessel positioning within the fleet. Previous work on ocean-going vessels has shown how hull locations, particularly the submerged surfaces of running gears (such as rudders, propellers, and stern tubes), act as hotspots and sources of within-ship variation in biofouling composition and extent (Coutts & Taylor, 2004). We did not expect to find such differences in our comparisons, and multivariate analysis revealed that assemblages from different hull

locations were indistinguishable from each other (photo-quadrats) or barely distinguishable from each other (biological samples). In general, biotic variation among hull locations has been related to the movement of vessels, creating differences in exposure to laminar or turbulent water flow among locations (Coutts & Taylor, 2004), which was not a factor for these vessels prior to departure.

We also found little variation between the two RRF vessels in biofouling assemblages, despite ~10 years difference in residence time at Suisun Bay. In the absence of strong environmental differences across the fleet site, we would expect assemblages on ships to become less distinct over time, as experimental studies have shown (Watson & Barnes, 2004a). When in service, variation in biofouling among vessels is thought to result from differences in vessel operations (e.g. speed, port residence time, geographical route, hull husbandry; Minchin & Gollasch, 2003). Thus, while initial differences probably existed for the vessels arriving to the RRF fleet, their associated biotic assemblages have converged over time. Since our study examined ships in residence for 10 and 20 years, we cannot presently assess the rate of convergence and the extent to which biotic difference may exist among vessels due to residence time. It is also conceivable that some differences exist among vessels due to location within the fleet, and small environmental differences over the small area (~250 hectares) occupied. It is important to recognize that our analyses were intended as a pilot study to quickly assess the scope for species transfers with the RRF vessels. Thus, although we found a high degree of spatial homogeneity for the two vessels, a more extensive study is required to examine the effects of residence time and location on biota, including sampling that is appropriately stratified for each factor.

Nine (41%) of the 22 unique macroinvertebrate taxa recorded in the pre-transit fouling assemblages are considered non-native to the US West Coast (see Appendix S1 in Supplementary Material). Of these, four were of particular interest to us, as their East Pacific distributions appear to be restricted to San Francisco Bay and Delta. These species were *C. chesapeakeensis*, *Corbula amurensis* (Schrenck 1861), *Gammarus daiberi* (Bousfield 1969), and *Uromunna* sp. (Stimpson 1857). This suggests that even coastwise movement of the RRF vessels (or other vessels that have undergone long lay-ups) over relatively short distances, including adjacent bays (ports) in California, Oregon, and Washington, may create opportunities for new invasions, due to the spread (transfer) of organisms from very restricted populations.

The occurrence of the bryozoan *C. chesapeakeensis* is further noteworthy in several respects. First, the pre-transit assemblages were dominated by this organism, in terms of abundance, biomass, and physical structure. Such three-dimensional structure generally leads to increased diversity and abundance of mobile organisms in fouling communities and probably played such a role here, creating microhabitats that otherwise would not exist (Crooks, 2002). Second, *C. chesapeakeensis* has not previously been reported for the US West Coast. Described relatively recently from Chesapeake Bay (Banta *et al.*, 1995) there are limited distributional and physiological data for this species. Upon close examination of our collections from this study, and comparison with other collections, the bryozoans collected from the RRF

vessels appeared to be morphologically distinct from *C. osburni* (Soulé *et al.*, 1995), previously recorded from San Francisco Bay and elsewhere along the western US (deRivera *et al.*, 2005). Molecular analysis, comparing mitochondrial DNA from the respective collections confirms this difference, showing the *C. chesapeakensis* from Suisun Bay is similar to that from the eastern US and different from *C. osburni* from the western US (L. D. McCann, unpubl. data).

The presence of the Asian clam *C. amurensis* on the hulls of both RRF vessels was also surprising, because it is usually associated with soft sediment habitats and considered a ballast water introduction to San Francisco Bay (Cohen & Carlton, 1995; Cohen, 2005). Native to estuarine habitats in the western Pacific, with a reported range from Russia to southern China (Coan, 2002), this clam now occurs in San Francisco Bay in salinities from < 1 ppt to 32 ppt (Carlton *et al.*, 1990). Abundances of this clam are reported to be greatest in the Suisun Bay locality, where it can reach densities of 48,000 clams per m<sup>3</sup> comprising more than 95% of the benthic biomass (Cohen, 2005), and the clam has significant negative effects on phytoplankton, zooplankton, and survival of fish larvae (Alpine & Cloern, 1992; Kimmerer *et al.*, 1994; Feyrer *et al.*, 2003). The presence of juvenile clams on ships' hulls probably resulted from the colonization of extensive secondary substrate (bryozoan mats) and associated sediment accumulation. Newly settled larvae and early juveniles secrete byssus threads, allowing them to attach to particles or surfaces (Nicolini & Penry, 2000). Whether these clams can persist on the outer surfaces of ships in transit is unknown.

In addition to non-native species recorded on vessels in Suisun Bay, we detected five native species and eight cryptogenic species. The cryptogenic taxa were all uncommon and insufficiently identified to determine their origins. Only one of the five indigenous species, the amphipod *Americorophium spinicorne* (Stimpson 1857), has been recorded outside of its native range, although it is not known if non-native populations persist. It was recorded in Hawaii associated with biofouling on the hull of the USS Missouri (Brock *et al.*, 1999) and also in the Snake River, 752 km from the coast, near the barge port of Lewiston, Idaho (Lester & Clark, 2002). Thus, movement of the two ships to the Gulf of Mexico had the potential to transfer novel species, previously unrecorded in the recipient region, that are native to the US West Coast, in addition to any species that are non-native or cryptogenic in San Francisco Bay.

### Post-transit biofouling characteristics

The transit had similar and significant effects on the biofouling assemblages of both vessels. There was an expected reduction in abundance of organisms as evidenced by the photo-quadrat analysis, whereby much of the vertical three-dimensional structure of the fouling matrix was removed. However, there was also a surprising increase in species richness, detected by the biological samples analysis, which was consistent across both vessels. These two aspects of community change resulted in significant dissimilarities between start- and end-point communities (ANOSIM  $r = 0.971$ , Fig. 5).

While both vessels experienced changes in a similar direction for organism abundance and diversity, we found some differences between ships. Specifically, multivariate analysis revealed that assemblages were almost identical prior to departure but diverged significantly after transit. Although the ships were moved together by a tandem tow, there were differences in (1) the position of ships (one lead, one trailing), and (2) the timing of their separate, sequential movements, and anchorages surrounding the Panama Canal. The contribution of these factors to observed differences is not known. The most striking change was the reduction of the thick bryozoan mat observed in pre-transit surveys, losing nearly all vertical structure during the voyage. Despite this reduction in biomass, *C. chesapeakensis* was still recorded in 98% of the samples collected in Texas, such that its prevalence across samples was unchanged by the transit. Similarly, the barnacle *Balanus improvisus* remained in 98% of samples collected.

In total, nine of the 22 initial taxa were detected in post-transit surveys. The isopod *G. oregonense* was a notable West Coast native species that survived the journey in a substantial number of samples (25%). This species does not have a known non-native range, such that collections on the hulls of the RRF vessels are the first known occurrences in Texas. In addition, four other species without known occurrences in Texas were found in the post-transit surveys, but only *C. chesapeakensis* and *G. oregonense* were confirmed as alive (see Appendix S1 in Supplementary Material). The Asian bivalve *C. amurensis* was not recorded on the vessels in Brownsville, indicating the voyage greatly reduced hull-mediated transfer of this species. Overall, of the 57 species detected in Brownsville, live specimens were confirmed for 22 species, most of which were widespread across samples. Rare species were either dead or simply not encountered until more detailed sample processing was completed. These rare species were the primary reason for the increased richness encountered on both vessels in Brownsville in post-transit analyses, resulting in unsaturated species accumulation curves that differed statistically from those of the pre-transit surveys (Fig. 4).

It appears that many of the new species (absent in the pre-transit surveys) settled on the vessels during the slow transit along the coasts. While some of the new species may have been undetected in the initial pre-transit analyses, especially those that were rare, this cannot explain the overall pattern observed, since the methods and sampling effort were similar between pre- and post-transit surveys. Moreover, some species not previously recorded during pre-transit sampling were surprisingly prevalent. For example, eight of the new species occurred in > 10% of post-transit samples, including five species of hydroids (see Appendix S1 in Supplementary Material). All eight are either native or already established in the eastern Gulf of Mexico, and most are usually reported from higher salinity conditions than found in Suisun Bay, suggesting settlement during the journey or at the destination port was likely. It is also noteworthy that the hydroids found in Brownsville were generally small, consistent with recent settlement during transit or upon arrival to Brownsville.

Although the increase in species richness at the end-point of the voyage was unexpected, Carlton & Hodder (1995) also

recorded an increase in biofouling richness during the coastal voyage of the Golden Hinde II. In the latter case, the voyage included numerous lengthy calls to ports (lasting days) where it was presumed much colonization occurred. The general consensus for most ships, based on the ability of larvae to settle in moving water, is for voyages to have a negative effect on both richness and extent, with settlement occurring primarily in ports (Minchin & Gollasch, 2003). Our study, and those of other slow moving biofouling vectors (Foster & Willan, 1979; Brock *et al.*, 1999), indicates that additional settlement by new species can occur during voyages in open and coastal (non-harbour) water conditions. In our results, this is most clear-cut for the barnacle *Lepas*, which occurs in the open ocean. For most other new species, detected in Texas but not California, it is not clear whether recruitment occurred during coastwise movement or while at the ports surrounding Panama and Texas.

In our study, sea chests did not provide a novel or important source of additional biota, as reported for other ships (Coutts *et al.*, 2003). In effect, our sampling was restricted to one ship, because the sea chests on the *Florence* were blanked, being covered by a metal plate, and presumably any opening or habitat for organism entry ceased to exist. Two sea chests were examined on the *Point Loma*. One was facing vertically down, precluding sediment accumulation, and did not support any additional species to those already present in the overall fouling community. The polychaete, *N. succinea*, was the only macroorganism recorded in sediment collected from the other sea chest in Suisun Bay; this may be because no macro organisms were present or because those present were able to avoid the sampling methods, which relied on manual collection and did not include visual inspection (due to poor visibility). Thus, while we detected no unique role for sea chests in our study, the sample size was also minimal, and the results should not be considered indicative of RRF vessels more broadly. It is also worth noting that we did not evaluate any internal fouling in pipes, such as those leading from sea chests, or any aspect of potential transfers associated with the ballast tanks of RRF vessels.

### Assessing the risk of invasions

Several aspects of the history and movement of RRF vessels suggest these may be unusually potent sources of species transfer and invasion, relative to in-service commercial vessels. First, it is evident that their long (years to decades) residence times at anchorage, combined with no hull husbandry, allow the development of high-density biofouling communities. Second, when moved, the vessels are towed at low speeds, which result in lower shear forces and higher retention of the initial communities in transit. Third, upon arrival to the recipient destination, the vessels again have long residence times as they remain in-water during the dismantling process, which may increase the opportunity for species transfer to the surrounding waters, either due to reproduction or movement. Fourth, the movement of RRF vessels between a few source and destination ports is being repeated many times, increasing the frequency of transfer events of a source biota to a few focal locations.

In recent years, several cases of high-density fouling transfers have been examined (see Appendix S2 in Supplementary Material). Vessels in these studies were not typical commercial ships but ranged from barges and research vessels to floating docks and a replica 16th century sailing vessel. These examples served to underscore the potential magnitude of transfers associated with underwater surfaces of vessels: 90 tons of fouling on a trawler in New Zealand (Hay & Dodgshun, 1997); almost 26,000 kg of fouling on a barge in New Zealand (Coutts, 2002); over 140,000 amphipods on a barge in Tasmania (Lewis *et al.*, 2006); and 117 species on a retired military vessel in Puget Sound (Brock *et al.*, 1999).

Concerns about the associated invasion risk have led Godwin (2005) to suggest a management system that seeks to identify such vessels with high density biofouling for some form of quarantine or pre-entry cleaning. Using a decision tree process, the first dichotomy identifies potential high-density ships based on operational characteristics (e.g. no recent hull husbandry, long lay-up or port residence times), triggering further inspection and possible treatment prior to entry. More broadly, guidelines and management options to limit high density hull transfers are at various stages of development at state, national, and international levels (IMO, 1999; California Legislature, 2007; AQIS, in press; US Regulation 33 CFR 151.2035).

Obsolete vessels of the RRF readily fall into the category of high density biofouling vectors but also differ in the expected frequency of traffic between the same source and destination ports. This repetition or frequency is uncommon in other reports of 'unique' high-density biofouling vectors shown in Appendix S2. At the present time, there are over 200 vessels distributed among three RRF sites in the US: approximately 84 in Suisun Bay, California; 95 in Chesapeake Bay, Virginia; and 40 in Beaumont, Texas. Additional vessels continue to join these fleets and once designated obsolete, they are destined for disposal and are either used for scrap (the majority) or to create artificial reefs by sinking. As a result, without any changes to current practices, we should expect to see repeated transfers of ships with high-density biofouling communities from each of the source bays. It also appears that facilities to handle ship-breaking are currently limited, such that these ships are likely to arrive to only a few US ports, including Brownsville, and involve inter-oceanic transfers.

Our study was a quick snapshot of the fouling community on the RRF vessels in Suisun Bay, which provided a pilot survey of the scale of biofouling and effects of transit for two vessels in February 2006, and clearly does not represent a comprehensive analysis of the species pool available for transfer by these ships. Although species accumulation curves suggested pre-transit sampling provided a reasonable estimate of species richness, this was limited to macroorganisms and did not consider meiofauna, protists, and other microorganisms (including commensals, parasites, and pathogens). Even for macroorganisms, we did not evaluate the effect of season and year on pre-transit assemblages. Given the potential for high temporal variation in fouling assemblages (Watson & Barnes, 2004b), especially in estuarine sites like Suisun Bay with a reported salinity range from 0 to 18, the biofouling communities of these RRF vessels in Suisun Bay

(and the two other sites) will vary through time and cumulative species richness measures were underestimated in our study.

Thus, we surmise that the repeated (if not regular) movement of RRF vessels from Suisun Bay will result in both (1) the repeated transfer of species observed in our study and also (2) an expanded number of biofouling species arriving to Brownsville and other destination ports. Independent of the inoculation density, both the frequency and the diversity of inoculation are expected to increase invasion success (Tilman, 1997; Lonsdale, 1999; Drake & Lodge, 2005; Drake *et al.*, 2005). Furthermore, we generally expect transfers at different seasons may also serve to increase the opportunity for invasion by broadening the environmental and biological conditions available, although the effects of season on survivorship in transit and after arrival remain to be tested.

There has been little formal analysis of the risk of invasions associated with vessel movement from these reserve fleets, and others that may exist worldwide. Some guidelines exist for US federal agencies to minimize invasion risks associated with their activities, including the National Environmental Policy Act of 1970 and Presidential Executive Order 13112 (Congressional Research Service, 2002), but consideration of RRF invasion risks has been extremely limited to date. Yet, such transfers have been occurring for several years and are planned to continue, presumably until the current fleet of obsolete vessels has been removed.

It is indeed daunting to evaluate the risk of invasion for obsolete vessels from San Francisco Bay (let alone Chesapeake Bay and Beaumont) on a species-by-species level. San Francisco Bay has one of the highest reported number of invasions in the world, with over 200 non-native species documented (Cohen & Carlton, 1995). Moreover, new invasions continue to occur and the total species pool is increasing (Cohen & Carlton, 1998), as exemplified by the new discovery of *C. chesapeakeensis* in our analysis. Without extensive sampling of vessels, stratified by season, it is difficult to know what taxa will occur on the vessels, and without further detailed analysis on each species it is difficult to estimate potential for invasion at recipient ports. Adding further difficulty, some key data will simply not exist for environmental tolerance of many taxa.

As with ships' ballast water, these challenges provide a compelling case for treating the hull fouling vector at a community level. At the present time, it is not known exactly which taxa will occur associated with the hulls or ballast tanks of ships, or their probability of survivorship, and both are likely to vary substantially in space and time. However, it is clear that invasions result from such ship-mediated transfers and that invasion risk is increased by high-density, high-diversity, and repeated transfers. These associations have driven the current regulations for ballast water, which is managed to reduce organism abundance regardless of taxonomic identity. We therefore concur with previous studies (Ruiz & Carlton, 2003; Godwin, 2005) that hulls should be treated in similar fashion, to prevent all high-density transfers.

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## SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

**Appendix S1** A list of species recorded in pre- and post transit biofouling communities

**Appendix S2** Examples of stochastic high-density and magnitude biofouling vectors from the Literature

This material is available as part of the online article from:

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