

Research Article

Cleaning by beaching: introducing a new alternative for hull biofouling management in Argentina

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Abstract

Recreational vessels favor the secondary spread of exotic marine species hosted on hull biofouling communities through coastal trips. Hull biofouling is also a problem for vessel owners because it reduces the efficiency and maneuverability of the vessel. This study documents a pioneer case of alternative hull biofouling management in a context where local regulations prohibit in-water cleaning operations and where there are no shore-based facilities. We designed and put into practice a method to manually clean a 35 meter long catamaran, by beaching it in a macrotidal beach of Patagonia, Argentina. During the cleaning, all hull biofouling was removed and collected to prevent organisms from falling on the beach. A total of 12.5 m³ of biofouling was deposited in landfill following regulations for fishing discard material. In addition, qualitative and quantitative fouling samples were obtained from different hull locations of the vessel, including niche areas. A total of 53 distinct taxa were identified, including 18 exotic species for Argentina, 7 of which had not been previously reported for the study area. Cleaning by beaching can be used as a convenient biosecurity method to remove hull biofouling from small and medium size vessels when other methods or facilities on the coast are not available. Our results also provide further evidence for the potential risk of recreational vessels as vectors for the secondary spread of marine exotic species.

Key words: biological invasions, exotic species, vector management, recreational vessels

Introduction

Shipping is the main pathway for global commerce since nearly 90% of all commodities are transported by sea, involving thousands of ships connecting oceans and coastal systems (Hewitt and Campbell 2010; Kaluza et al. 2010). Within this context, ships have played a key role in the accidental spread of exotic marine species (Ruiz and Carlton 2003; Carlton 2010), transporting species either in the ballast water tanks or attached to

hulls and other submerged surfaces (biofouling). Additionally, hull biofouling exerts an economic penalty on ships because it increases friction between hull and water, generating an increase in fuel consumption, a loss in speed, and consequently unwanted delays and fines (e.g. Townsin 2003; Schultz et al. 2011). Biofouling is not homogeneously distributed across the hull (Dobretsov et al. 2014). There are niche areas such as hull protrusions, cavities, and appendages, like propellers and rudders with complex surfaces that may be subject to heavier erosion due to strong turbulence (Coutts and Taylor 2004; Dobretsov et al. 2014). Thus, antifouling coatings are susceptible to failure around these (usually called “hotspots”) areas, making them more vulnerable to colonization by a high diversity of organisms over relatively short periods of time (Moser et al. 2017). Factors related to the voyage, such as speed, time spent in ports, or periods between hull cleaning procedures, determine the success of hull biofouling organisms colonizing a vessel (Floerl et al. 2005a; Hewitt et al. 2011; Ashton et al. 2014; Ferrario et al. 2016; Martínez-Laiz et al. 2019). For example, vessels sailing slower and having a longer residence time in port, such as recreational vessels, are more susceptible to accumulate hull biofouling than those sailing faster and having a shorter residence time (Roberts and Tsamenyi 2008; Coutts et al. 2010; Hewitt and Campbell 2010). Through their usually coastal trips these recreational vessels connect marinas or bays, favoring the secondary spread of exotic species at the local and regional scale (Wasson et al. 2001; Davidson et al. 2010; Zabin et al. 2014; Peters et al. 2017). Vessels with high abundances of hull biofouling represent the greatest biosecurity threats for all ports and surrounding natural environments along their routes.

Despite the continuous development of antifouling technology, hull biofouling still occurs and cleaning is regularly required. Current methods to remove or kill hull biofouling organisms include encapsulation systems (with or without the addition of a chemical solution like chlorine or acetic acid (Roche et al. 2014)), steam (Jute and Dunphy 2017) or freshwater (Joyce et al. 2019) applications, in-water cleaning procedures by using a rotary brush system (Davidson et al. 2008a; Tribou and Swain 2015), and cleaning during dry docking. In-water cleaning with manual and simple tools is a widely used method for recreational vessels, while for large vessels dry-docking is preferred, although in-water cleaning using a variety of devices remains as the only alternative between dry-docking schedules (Hopkins and Forrest 2008; Armstrong 2013). However, several countries have put restrictions to in-water cleaning because the defouled material is rarely captured and viable organisms may settle or be dispersed by currents (Hopkins and Forrest 2008; Woods et al. 2012). In addition, it may increase the amount of biocide released into the environment (Tribou and Swain 2010), which can accumulate in high concentrations in sites with poor water circulation (Pagoropoulos et al. 2017). Although removing

vessels from water for hull cleaning is considered the most biosecure method to treat them, dry-docks and haul-out facilities for cleaning on land are uncommon since they imply substantial costs for ship owners (Morrisey et al. 2016) or simply these facilities are not available in certain locations.

Recently, hull biofouling started to receive far more attention than ever before, and there are proposals to be adopted as regulatory tools or guidelines, to minimize the transfer of biofouling organism on commercial and recreational vessels (IMO 2012). The international community advances towards a GloFouling project similar to the materialized Convention for ballast water management (IMO 2017), although it is expected to progress slowly, partially due to research limitations associated with logistic and economic difficulties in the sampling of hull biofouling. Although Southern South America has been forecasted as one of the top-four worldwide regions with the highest expected annual number of invasions by 2050 due to the increase in shipping traffic (Sardain et al. 2019), in Argentina few studies have examined hull biofouling communities, and recent findings generated concern among marine bioinvasion researchers. For example, in 2013, many exotic organisms were found living inside the sea-chests of different bulk carriers (Almada et al. 2018). Same year, numerous individuals of an exotic bivalve (*Semimytilus algosus* (Gould, 1850)) were discarded in the Nuevo Gulf (42°S) when an in-water hull cleaning procedure was performed to a squid fishing ship (Bigatti et al. 2014). Between 2016 and 2017, samplings conducted on different underwater locations of a national frigate showed a high density of the exotic barnacle *Amphibalanus amphitrite* (Darwin, 1854) (Cianis et al. 2018). Also, two new exotic isopods (*Dynamene edwardsi* (Lucas, 1849) and *Paracerceis sculpta* (Holmes, 1904)) were reported on an Argentinean scientific oceanographic vessel (Rumbold et al. 2018). Based on these events and findings, the national maritime authority, Prefectura Naval Argentina, prohibited the in-water hull cleaning in the Nuevo Gulf, an area with high native biodiversity associated with valuable ecological services.

In the summer of 2015, our research team was contacted and informed that the passenger catamaran *Regina Australe*, moored in the Nuevo Gulf, was heavily fouled and required an urgent hull cleaning. The lack of a dry dock in the region and the local prohibition for in-water hull cleaning reinforced the need for an innovative and inexpensive cleaning alternative. With very limited time due to legal, financial and logistical reasons, different stakeholders, including the catamaran owners, marine bioinvasion scientists, the provincial environmental government office (Ministerio de Ambiente y Control del Desarrollo Sustentable, MAyCDS), and the national maritime authority (Prefectura Naval Argentina, PNA), coordinated efforts to design and perform the best biosecure hull cleaning procedure possible. Taking advantage of the extreme tidal regime (4 m) and the topography of

the beach with a flat slope, all parties involved agreed on beaching the catamaran to clean it as fast as possible while surveying the hull biofouling communities in order to identify any potentially invasive species that may represent an environmental threat to the region. This work documents a pioneer case of alternative hull biofouling management through the beaching of a medium size vessel in a macrotidal beach of Patagonia, Argentina. First, we designed and put into practice a method for cleaning the ship hull on the beach which included the estimation of the volume of the hull biofouling removed, as well as the species richness and density of marine exotic species in different areas on the ship. The method also specified a way to discard the biofouling removed on land. Finally, we discussed the pros and cons of this alternative method compared to other cleaning procedures.

Materials and methods

Study Case

The *Regina Australe* is a tourist passenger catamaran with an overall length of 35 m and a beam of 10 m, with two hulls adding a total of 450 m² of hull wetted surface. It had been navigating within the Nuevo Gulf (Patagonia, Argentina) since 2012, when it was brought to Puerto Madryn harbor from Mar del Plata port (38°S) after being dry-docked and painted with antifouling coat. The ship is an emblematic tourist attraction that makes up to three trips per day for coastal sightseeing, sailing from the pier with a fixed route for about three hours at an average speed of 5 knots. However, it spends most of the year moored to the port, and the frequency of trips per month varies according to the touristic season.

In January 2015, the accumulated fouling caused a drastic reduction in the maximum speed, increasing fuel consumption and decreasing the maneuverability of the vessel (ship owner *pers. comm.*). Due to current local legislation, in-water cleaning is not allowed and, to reduce the amount of biofouling, on April 4th, 2015, the vessel sailed from Puerto Madryn to Puerto Pirámides, the closest town with an extremely flat slope across the beach (Figure 1). The staff of the company executed the intentional beaching of the ship, taking advantage of the high tide and the low terrain slope. The ship was maneuvered until it gently stranded while the tide was lowering (Figure 2). Six hours later, the *Regina Australe* was completely out of the water. The beaching of the vessel, its sampling and the subsequent hull cleaning were legally supervised by PNA and MAyCDS office.

Hull sampling

Qualitative and quantitative fouling samples were obtained from different parts of the vessel immediately after the hull was out of the water. In the qualitative fouling sampling, visual scanning was conducted by two bioinvasion

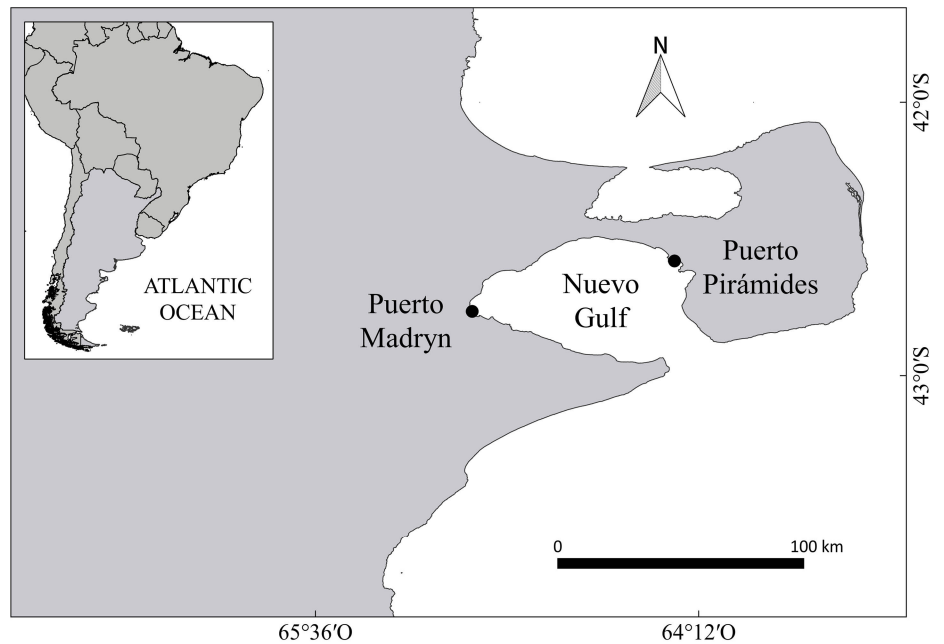


Figure 1. Localities where the catamaran *Regina Australe* remains moored during most of the year (Puerto Madryn) and where it was beached for cleaning in 2015 (Puerto Pirámides).

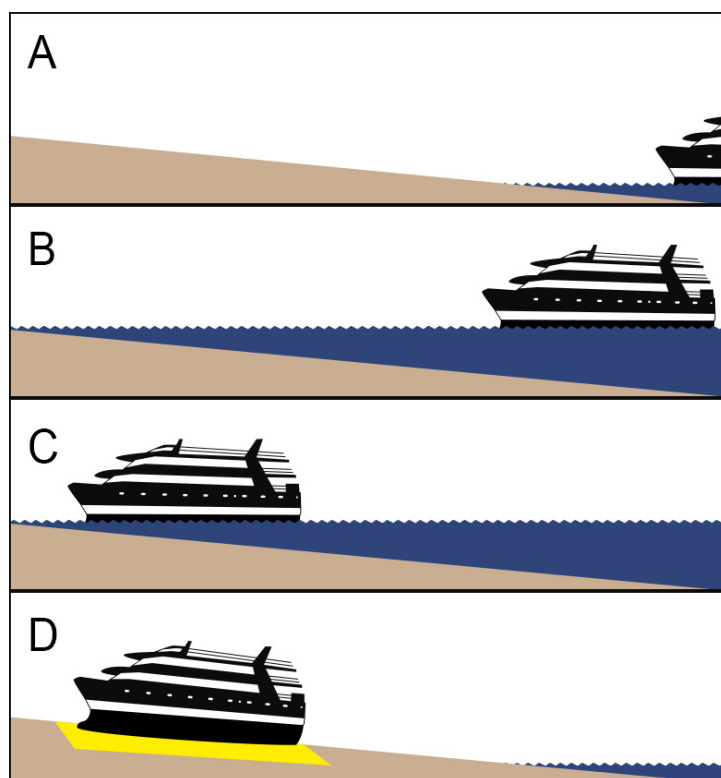


Figure 2. Schematic diagram showing the beaching procedure. (A) sailed to the beaching point, (B) waited for the high tide, (C) sailed towards the beach and (D) stranded while the tide was lowering. A nylon tarp is deployed underneath.

researchers in order to find isolated, delicate, or unusual organisms across the ship. All organisms found during the qualitative sampling were photographed, bagged, labelled and preserved. To determine species richness and density, quantitative samples were also collected by scraping 16 locations across the ship (Figure 3). General areas of the hull, such as the

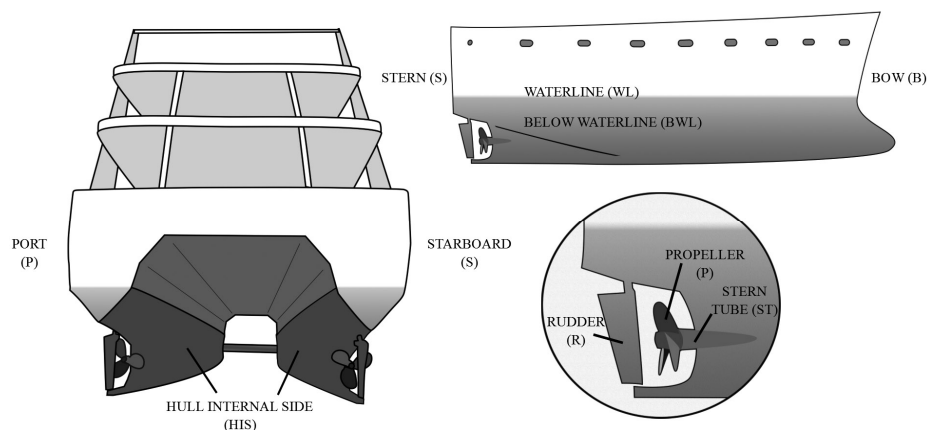


Figure 3. Schematic diagram of *Regina Australe* showing the hull locations sampled.

bow and stern sections, and both port and starboard sides, were sampled with quadrats of 20×20 cm on waterline and 2 m below it. Both internal sides of the hull of the catamaran were also sampled (Figure 3). Niche areas such as rudders, propellers and stern tubes were sampled with (10×10 cm) quadrats due to the higher surface complexity and their smaller size compared to hull locations (Figure 3). These samples were individually bagged, labeled and rapidly transported to the laboratory in coolers with ice for processing. Once in the laboratory, all macroorganisms (size > 0.5 mm) collected were fixed in formalin (4%) and then preserved in ethanol (70%), except the algae which were kept in formalin to preserve structures. Solitary organisms were counted to estimate their density and colonial organisms and algae were recorded as presence/absence in each sample. All organisms were identified to the lowest taxonomic level possible. Then, voucher samples were sent to taxonomists (see Acknowledgements) in order to ensure correct identifications. In addition, species were classified as native, cryptogenic or exotic following Chapman and Carlton (1991). Individuals of each species were deposited at the Invertebrate collection of the IBIOMAR-CONICET (CNP-INV).

Ordination techniques were carried out to analyze the relationship between assemblages of organisms found and hull locations using PRIMER 6 package (Clarke and Gorley 2006). A principal component analysis (PCA) was conducted on a covariance matrix of logarithm transformed density data. A nonmetric multidimensional scaling (MDS) was conducted on a presence/absence matrix of the whole biofouling community, using Bray Curtis similarity as resemblance measure.

Hull cleaning

Once the sampling ended, the staff of the company conducted the manual cleaning by scrapping off the organisms strongly attached on the hull surface and niche areas using shovels and paint scrapers. This aggressive method was the most convenient to detach organisms as barnacles and mussels. All the biofouling removed was accumulated on a $120 \mu\text{m}$ nylon tarp

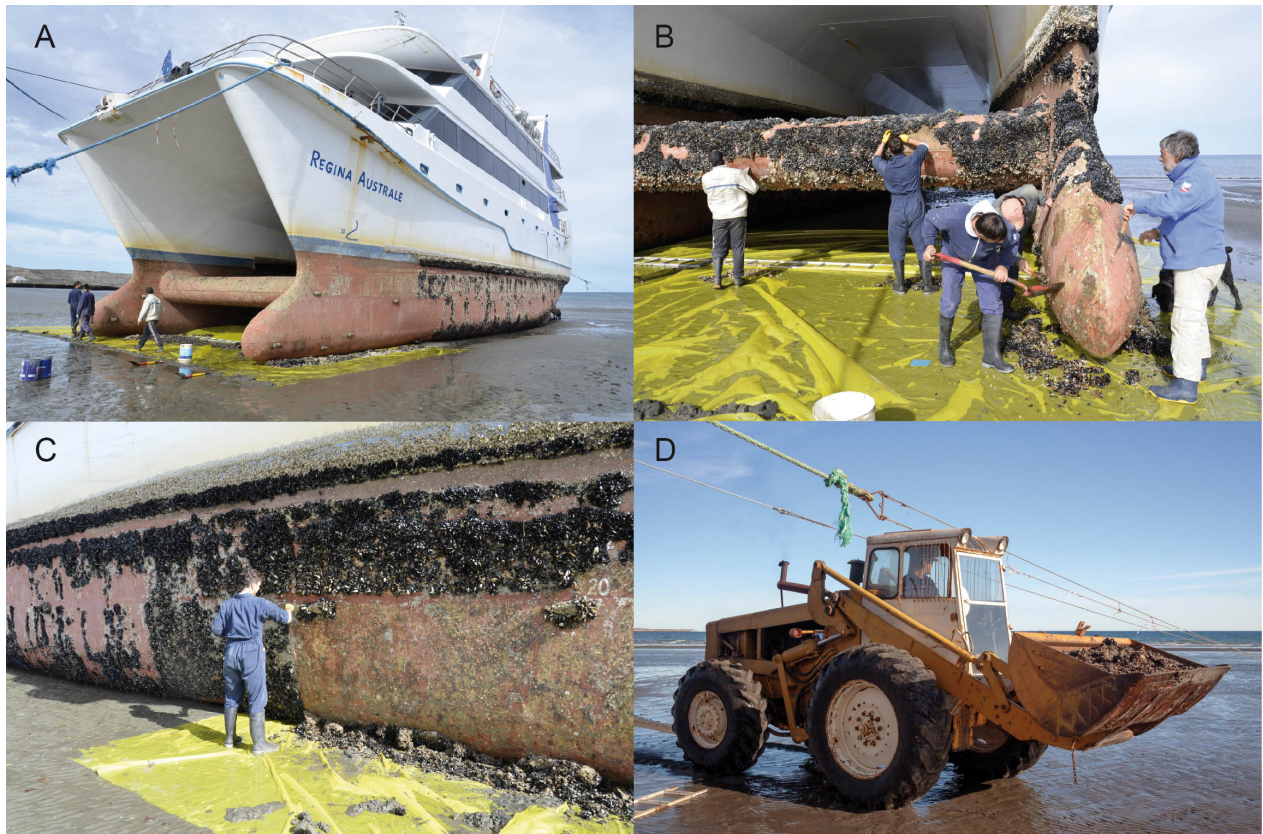


Figure 4. Hull cleaning of the catamaran (A) *Regina Australe* with nylon tarp deployed underneath, (B, C) company staff conducting manual cleaning, (D) bulldozer transporting part of the hull biofouling removed during the cleaning. Photo by A. Bortolus (A–C) and N. Battini (D)

previously deployed under the vessel to prevent organisms from falling on the beach (Figure 4A–C). Then, all the material was transported with a bulldozer (Figure 4D) and placed inside 5 m³ containers specifically deployed for that purpose. Finally, all the material in the containers was disposed in landfill as the administration of the local town usually does with fishing discard material. The applied cleaning method did not include the repainting of the hull, because this procedure is forbidden by local authorities. One year after the cleaning by beaching (2016), the ship was heavily fouled again and sailed to Mar del Plata city for dry-docking. General maintenance and hull cleaning were performed in dry-dock and the biofouling removed was again quantified using 5 m³ containers although species composition was not analyzed. Finally, the hull was painted there with a new antifouling coat and sailed back to Puerto Madryn.

Results

Hull sampling

A total of 53 distinct taxa were identified, 33% (n = 18) of them were exotic species for Argentina, 9% (n = 5) cryptogenic species, and the remaining percentage corresponded to native species and other higher taxonomic levels (Table 1). Crustaceans, algae and bryozoans were the dominant taxa (Figure 5) and the highest taxonomic richness was found in the internal side

Table 1. List of exotic (*), cryptogenic (°) and native (without marks) species recorded on the catamaran *Regina Australe*. For the complete list of taxa, location on the vessel and density see Table S1 in the supplementary material.

<p>Phylum Rhodophyta</p> <p>*<i>Anotrichium furcellatum</i> <i>Callithamnion montagnei</i> <i>Ceramium virgatum</i> *<i>Melanothamnus harveyi</i> *<i>Leptosiphonia brodiei</i> *<i>Lomentaria clavellosa</i> <i>Polysiphonia hassleri</i></p> <p>Phylum Chlorophyta</p> <p><i>Ulva rigida</i></p> <p>Phylum Ochrophyta</p> <p><i>Sphacelaria cirrosa</i> *<i>Undaria pinnatifida</i></p> <p>Phylum Chordata</p> <p>*<i>Asciidiella aspersa</i> °<i>Asterocarpa humilis</i> *<i>Botryllus schlosseri</i> *<i>Diplosoma listerianum</i></p> <p>Phylum Bryozoa</p> <p>*<i>Bugula neritina</i> *<i>Bugulina flabellata</i> <i>Celleporella hyalina sensu lato</i> *<i>Cryptosula pallasiana</i></p>	<p>Phylum Arthropoda</p> <p><i>Austromegabalanus psittacus</i> *<i>Balanus glandula</i> °<i>Caprella dilatata</i> °<i>Caprella equilibra</i> <i>Halicarcinus planatus</i> *<i>Jassa marmorata</i> *<i>Monocorophium acherusicum</i> *<i>Monocorophium insidiosum</i> *<i>Sphaeroma serratum</i> °<i>Tanais dulongii</i></p> <p>Phylum Cnidaria</p> <p>*<i>Ectopleura crocea</i> °<i>Sertularella mediterranea</i></p> <p>Phylum Mollusca</p> <p><i>Aulacomya atra</i> <i>Hiatella arctica</i> *<i>Pleurobranchaea maculata</i> <i>Siphonaria lessonii</i></p>
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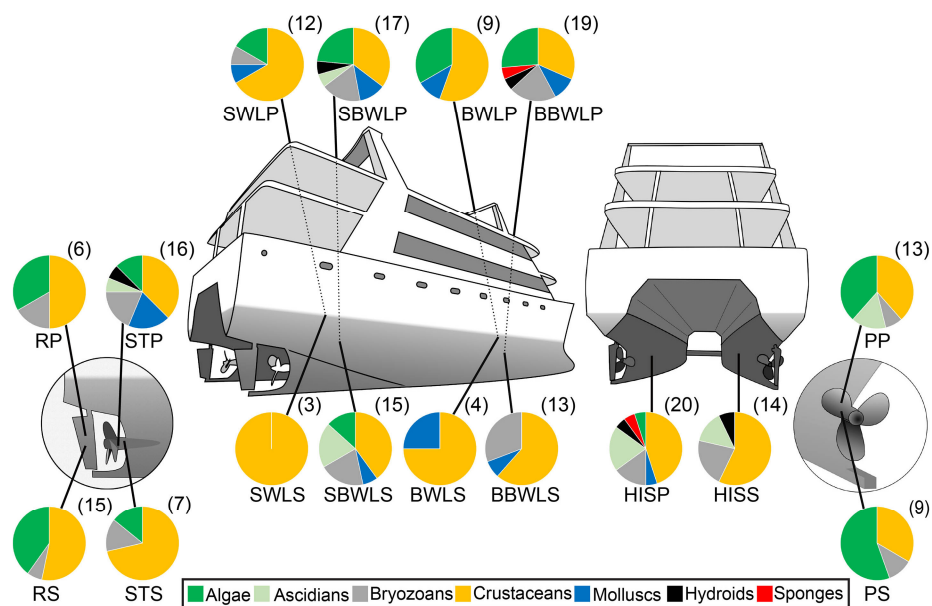


Figure 5. Proportions of taxonomic groups recorded per hull location of the ship. Abbreviations below the pie charts correspond to each of the following hull locations: BWLP (bow side at waterline port), BBWLP (bow side below waterline port), BWLS (bow side at waterline starboard), BBWLS (bow side below waterline starboard), SWLP (stern side at waterline port), SBWLP (stern side below waterline port), SWLS (stern side at waterline starboard), SBWLS (stern side below waterline starboard), HISP (hull internal side port), HISS (hull internal side starboard), RP (rudder port), RS (rudder starboard), PP (propeller port), PS (propeller starboard), STP (stern tube port), and STS (stern tube starboard). Numbers of taxa per hull location are shown in brackets.

of the hull at port (Figure 5). Principal components 1 and 2 accounted for 43.5% of the total variance (Figure 6). The hull areas at waterline differed from the other hull areas and were grouped with samples collected in niche

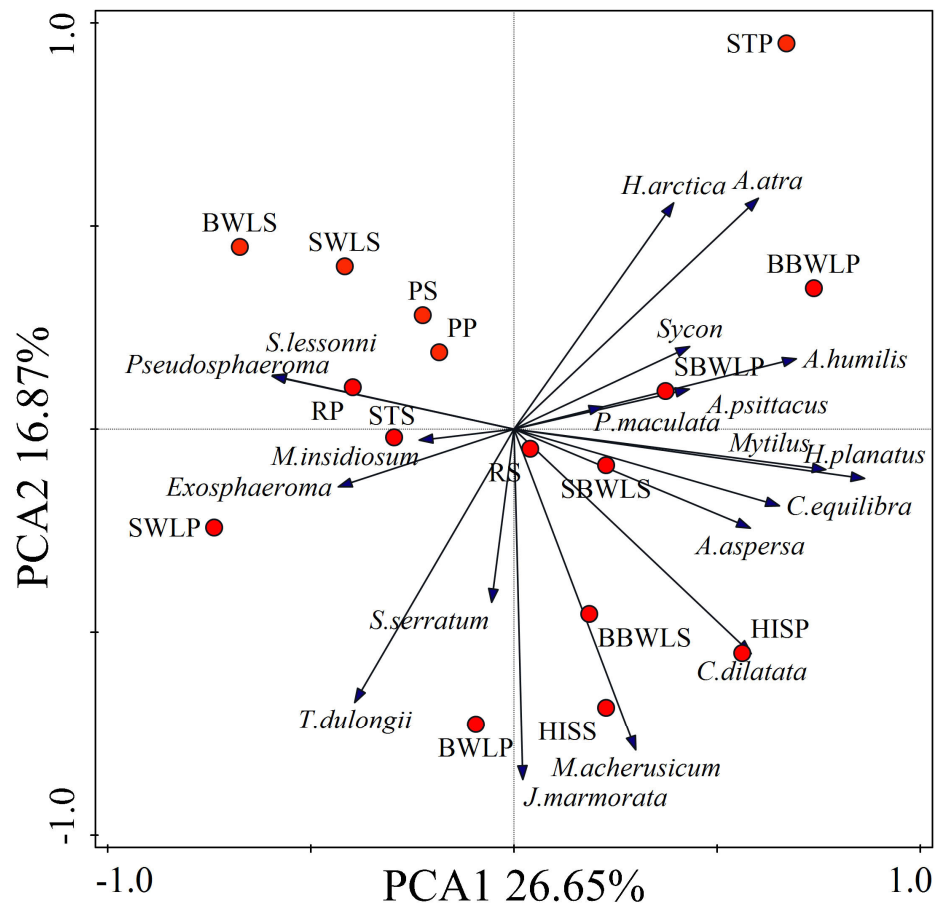


Figure 6. Principal components analysis biplot showing the ordination of hull locations and the densities of species. Principal components 1 and 2 accounted for 43.5% of the total variance. Abbreviations of species: *H. arctica* (*Hiatella arctica*), *A. atra* (*Aulacomya atra*), *Sycon* (*Sycon* sp.), *A. humilis* (*Asterocarpa humilis*), *A. psittacus* (*Astromegabalanus psittacus*), *P. maculata* (*Pleurobranchaea maculata*), *Mytilus* (*Mytilus* sp.), *H. planatus* (*Halicarcinus planatus*), *C. equilibra* (*Caprella equilibra*), *A. aspersa* (*Ascidiella aspersa*), *C. dilatata* (*Caprella dilatata*), *M. acherusicum* (*Monocorophium acherusicum*), *J. marmorata* (*Jassa marmorata*), *T. dulongii* (*Tanais dulongii*), *S. serratum* (*Sphaeroma serratum*), *Exosphaeroma* (*Exosphaeroma* sp.), *M. insidiosum* (*Monocorophium insidiosum*), *Pseudosphaeroma* (*Pseudosphaeroma* sp.), and *S. lessonii* (*Siphonaria lessonii*). Abbreviations of the hull locations are the same as Figure 4.

areas (Figure 6). The hull areas below the waterline, showed the highest abundance of *Mytilus* spp., solitary ascidians (the cryptogenic *Asterocarpa humilis* (Heller, 1878) and the exotic *Ascidiella aspersa* (Müller, 1776)), the native crab *Halicarcinus planatus* (Fabricius, 1775) and the cryptogenic amphipod *Caprella equilibra* Say, 1818 (Figure 6). The assemblages found in the internal sides of the hull, were characterized by a higher abundance of amphipods (the cryptogenic *Caprella dilatata* Krøyer, 1843 and the exotics *Monocorophium acherusicum* (Costa, 1853) and *Jassa marmorata* Holmes, 1905) compared to the other assemblages (Figure 5). The isopods *Exosphaeroma* spp., *Pseudosphaeroma* spp. and the exotic *Sphaeroma serratum* Fabricius, 1787, the exotic amphipod *Monocorophium insidiosum* (Crawford, 1937) and the native limpet *Siphonaria lessonii* Blainville, 1827 were present only in this group, and the cryptogenic tanaid *Tanais dulongii* (Audouin, 1826) showed its highest densities there (Supplementary material Table S1). The assemblage composition of biofouling



Figure 7. MDS plot of assemblage organization based on presence/absence of the whole community of organisms in hull biofouling samples. General hull locations are shown as red squares and niche areas as blue squares. WL and BWL refer to general hull locations on the waterline or below it, respectively. HIS refers to the internal sides of the hull. NA refers to niche areas. Stress: 0.13.

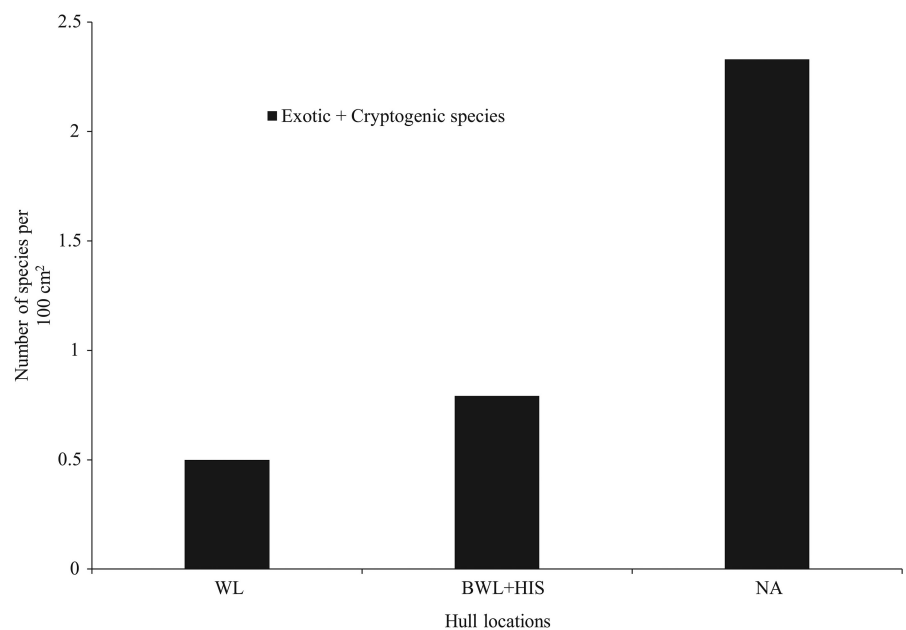


Figure 8. Number of exotic and cryptogenic species per 100 cm² found on hull locations at the waterline (WL), hull locations below the waterline and the internal sides of the hull (BWL+HIS) and, on niche areas (NA).

was different among hull locations, showing a clear separation between the hull areas located below the waterline and those located at waterline and niche areas (Figure 7).

All hull locations presented several exotic and cryptogenic species. Hull locations at the waterline presented the lower density of exotic and cryptogenic species (0.5 species per 100 cm², Figure 8). In contrast, the

niche areas showed almost five times more exotic and cryptogenic species (2.33 species per 100 cm², Figure 8). The most widespread species was the exotic amphipod *J. marmorata*, which was present on 14 of the 16 biofouling samples collected, followed by the exotic barnacle *Balanus glandula* Darwin, 1854 that covered large areas of the hull, occurring in 13 of the 16 samples (Table S1). *Jassa marmorata* was also the most abundant species collected (7868 ind per m² ± 2948 S.E.) followed by the blue mussel *Mytilus* spp. (2259 ind per m² ± 745 S.E.) and the tanaid *T. dulongii* (1037 ind per m² ± 648 S.E.) (Table S1). Polychaetes and the exotic hydroid *Ectopleura crocea* (Agassiz, 1862) were observed in the qualitative sampling.

Seven exotic species were new reports for the Nuevo Gulf, including the amphipod *M. insidiosum*, the isopod *S. serratum*, the colonial ascidians *Diplosoma listerianum* (Milne Edwards, 1841), and *Botryllus schlosseri* (Pallas, 1766), and the bryozoans *Bugula neritina* (Linnaeus, 1758), *Bugulina flabellata* (Thompson in Gray, 1848) and *Cryptosula pallasiana* (Moll, 1803). In addition, two cryptogenic amphipods were also recorded for the first time in Nuevo Gulf, *C. dilatata* and *C. equilibra*. Moreover, the cryptogenic hydroid *Sertularella mediterranea* Hartlaub, 1901 was found for the first time since its first record in 1916.

Hull cleaning

The total cleaning took 16 hours. The total volume of biofouling material accumulated on the catamaran during three years was 12.5 m³. A year later, the total volume of biofouling removed during dry-docking was 17.5 m³.

Discussion

Our study shows a pioneer case of biosecurity management of marine hull biofouling in Argentina. It also shows that the cleaning of the vessel by beaching is a convenient alternative whenever local regulations prohibit in-water cleaning operations and where there is no infrastructure or basic facilities for an appropriate dry-cleaning operation. In addition, among the organisms removed from the vessel during the cleaning process we detected 18 exotic and 5 cryptogenic species for the Argentinian coast, of which 7 exotic and 2 cryptogenic were not previously reported for the study area. These results also provide further evidence for the potential risk for recreational vessels to concentrate and spread exotic species within a region.

Hull sampling

The abundance of solitary organisms and the composition of the whole biofouling community differed among hull locations. We observed that locations below the waterline were similar to the hull internal side locations but both were different to the locations at the waterline and niche areas. Assemblage composition, distribution and abundance of hull

biofouling organisms are known to be influenced by vessel speed and by hydrodynamics forces on the hull (Davidson et al. 2009; Coutts et al. 2010). High speed merchant vessels (≥ 15 knots) tend to have flat surfaces free of biofouling but concentrate organisms in niche areas (Coutts and Taylor 2004; Coutts et al. 2010). In contrast, vessels with slower navigation speed (≤ 5 knots) usually support a greater amount of biofouling across all areas (Davidson et al. 2008b), specially vessels that are constantly kept in the water (Clarke Murray et al. 2013). Besides, there are other factors affecting the colonization by biofouling species, like the exposure to sunlight (Floerl 2005), which varies from completely exposed surfaces near the waterline, with higher degree of desiccation, to shaded and always submerged surfaces as niche areas. Both areas of the vessel here studied, i.e. general hull location at waterline and niche areas, may present stress conditions that would explain the similarities found between their biofouling assemblages. Along the waterline, the hull may host stress tolerant organisms, such as the exotic acorn barnacle *Balanus glandula* and the native limpet *Siphonaria lessonii* observed in our samples, contrary to submerged surfaces characterized by subtidal organisms such as ascidians. Although the niche areas are always submerged, these may be exposed to high levels of turbulence while the propeller (or propellers) is in motion. This turbulence also represents stressful conditions for which not all organisms are equally tolerant (Coutts and Taylor 2004; Fofonoff et al. 2003) even at low navigation speed (Clarke Murray et al. 2012). In particular, exotic species must be able to endure these challenging conditions during the transport to new habitats while attached to the vessel (Floerl et al. 2004; Coutts and Dodgshun 2007; Clarke Murray et al. 2012).

Although all hull locations of the catamaran presented several exotic and cryptogenic species, niche areas showed the highest richness. These results are consistent with surveys carried out in both merchant (Chan et al. 2015; Coutts and Dodgshun 2007) and recreational vessels (Clarke Murray et al. 2011; Ashton et al. 2014) which highlighted that niche areas are “hot spots” of exotic species. The efficiency of antifouling coatings is likely to decrease as the paint ages around those areas (Floerl et al. 2005b), but even in vessels with regular application of antifouling, the difficult access to the appendages, results in poor treatment or not treated at all (Moser et al. 2017). Consequently, these areas are susceptible to be colonized by exotic species (Davidson et al. 2009). Furthermore, among general hull areas, the internal sides accumulated more exotic and cryptogenic species than the external areas of the hull. This kind of vessel, with double hull might provide a sheltered area that enables various exotic species to colonize and survive. The design features of a ship are based on the specific requirements for its best performance, including size and type of niche areas which varies enormously among vessels (Moser et al. 2017). The great variety of types and design of vessels expands the suitable microenvironments for exotic species

to find refuge and highlights the importance of elaborating appropriate monitoring programs according to the characteristics of each vessel.

One third of all the species found during our sampling were exotics, including several range expansions for exotic and cryptogenic species already present in the Southwestern Atlantic but previously unreported within the Nuevo Gulf. Some of these are well known hull biofouling species such as the invasive ascidians *Botryllus schlosseri* and *Diplosoma listerianum*, and the erect bryozoan *Bugula neritina* (e.g. Lambert and Lambert 2003; Locke et al. 2009; Clarke Murray et al. 2011), which since their detection in this study in 2015 to date were frequently observed in the port area (Giachetti et al. *in prep.*). Marchini et al. (2015) recommended not to consider new reports the species found on their vectors, unless they are also found living outside that vector. However, since the *Regina Australe* navigates exclusively inside the study area, we know that all fouling species found on the hull must come from local ports within this same area. Although the presence of these exotic species may be due to new introduction events, regional spread is the most likely hypothesis to explain their occurrence in waters of Nuevo Gulf. A large majority of the species found in this work were first reported in port areas on the northern coast of Argentina, where major commercial ports are located. These large ports are interconnected with smaller ports distributed along the coast of Patagonia mainly by fishing and recreational vessels (Bobinac et al. 2018; Castro et al. 2018). Although the direct link between an already established species and its vector is often unclear (Ruiz et al. 2000; Minchin 2007), the key role of recreational and fishing vessels in the regional spread of several exotic species is well documented (Goldstien et al. 2010; Davidson et al. 2012; Kelly et al. 2013; Zabin et al. 2014; Peters et al. 2017). For example, the Asian kelp *Undaria pinnatifida* (Harvey) Suringar was progressively reported along the west coast of North America associated with marinas and boats travelling among marinas inside and outside San Francisco Bay (Silva et al. 2002; Zabin et al. 2009). While some recreational vessels like the *Regina Australe* may not be important for dispersing exotic species over long distances, they can promote the transfer of exotic species at a local scale, connecting port areas with adjacent natural environments. Even movement of vessels over relatively short distances, may create opportunities that increase the risk for new invasions (Wasson et al. 2001).

Despite the fact that sampling efforts in the port areas of the region have been increasing progressively over time (Rico and López Gappa 2006; Albano and Obenat 2009; Rico et al. 2010; Schwindt et al. 2014), a relatively large number of exotic and cryptogenic species detected on the catamaran had not been previously reported within the study area. It is known that differences between artificial structures and the sampling method used may have implications for monitoring and detection of exotic species in fouling communities (Campbell et al. 2007). The survey of recreational

vessels to detect potential invaders should be a tool to consider in the region. In this study, we identified many exotic species as peracarids, bryozoans and colonial ascidians, impossible to detect using only visual sampling because they either are small organisms or require dissection for a correct taxonomic identification. Furthermore, we detect an additional exotic species using a complementary qualitative sampling. The collection of scrape samples and subsequent analysis in the laboratory has been proved the most effective method to detect exotic species in recreational vessels (Peters et al. 2017). Thus, monitoring protocols that employ a combination of methods could achieve a better balance between the probability of detection and the economic costs involved.

Hull cleaning

Cleaning by beaching can be the most biosecure cleaning method to retain the solid waste during the procedure when other methods or facilities on the coast are not available. Since the ship does not crawl over the substrate during the beaching process, the possibility of detaching organisms are minimal. Cleaning on land also allowed us to quantify all the hull biofouling removed, which can be used as measure to estimate biosecurity risk of vessels (Sylvester and Floerl 2014). Generally, vessels with high abundance of hull biofouling represent the greatest threats for the introduction of invasive species (Floerl et al. 2005b), so the fate of these large amounts of biofouling removed during the cleaning should be evaluated and regulated regardless of the cleaning method. Thus, it is important for countries to facilitate and encourage the best treatment options to ensure the compliance of regulations and to maintain vessels as clean as possible. In this way, if there are no alternatives or they are too expensive, in-water cleaning restrictions can discourage small and medium size vessels owners from keeping their hulls free of biofouling (Hopkins and Forrest 2008). Leaving a fouled vessel unmanaged is not recommended because it may exacerbate the risk of introduction and spread of exotic species (Floerl et al. 2005b; Ashton et al. 2006; Hopkins and Forrest 2008) as well as being non-viable for the operation of the vessel.

When it comes to cleaning vessels, there is no perfect method, therefore managers have to evaluate the most appropriate method for each place, time and context. Only one year after beaching and cleaning of the *Regina Australe*, the hull wetted surfaces accumulated 40% more biofouling than in the previous three years. The fact that the hull could not be treated with a new antifouling coating is an issue to be considered. Hull cleaning methods help to maintain biofouling at relatively low levels during periods between dry-docking opportunities. However, some these methods, as it was described in this study, are sometimes aggressive and reduce the useful life of the antifouling coating system, consequently increasing the frequency of interventions (Floerl et al. 2005a). For this reason, the IMO

also recommended to haul recreational vessels out of the water to renew the antifouling coat every year as part of best practices to minimizing the transfer of invasive species (IMO 2012). Less abrasive methods with manual brushes, soft cloths or water jets can be suitable for removing the slime layer of hull biofouling (microfouling) preventing the establishment of extensive hull biofouling (macrofouling). The presence of macrofouling entails a more established and mature community, which constitutes a greater biosecurity risk of invasive species introductions and spread than undeveloped communities (Coutts and Taylor 2004). Therefore, preventing the extensive accumulation of hull biofouling is the most recommended action and it is an issue of interest that links the maritime industry with biosecurity researchers and managers because it simultaneously promotes the efficiency of shipping and reduces the risk of bioinvasions (Davidson et al. 2016).

The cooperation among stakeholders was essential to carry out the present work successfully. While shore-based cleaning facilities that maximize the capture of biofouling organisms are the most recommended (IMO 2012; Woods et al. 2012), this work provides an alternative management method for small and medium size vessels that can be used in regions where economical and logistical resources are scarce. In addition, although this research was performed on a single vessel and needs further replications, it greatly contributes to the search for solutions of biofouling management and to the understanding of the importance of recreational vessels as potential vectors for the secondary spread of marine exotic species.

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Supplementary material

The following supplementary material is available for this article:

Table S1. List of taxa recorded on each hull location sampled in the catamaran *Regina Australe*.

This material is available as part of online article from:

http://www.reabic.net/aquaticinvasions/2020/Supplements/AI_2020_Castro_etal_SupplementaryTable.xlsx

Table S1. List of taxa recorded on each hull location sampled in the catamaran *Regina Australe*. Density values are expressed as individuals per m². Crosses indicate the presence of colonial organisms and algae. Taxa found only during the qualitative sampling but not on the quadrats (*). Exotic species for Argentinean waters (*). BWLP: bow side at waterline port, BBWLP: bow side below waterline port, BWLS: bow side at waterline stern, BBWLS: bow side below waterline starboard, BBWLS: bow side below waterline starboard, SWLP: stern side at waterline port, SBWLP: stern side below waterline port, SWLS: stern side at waterline starboard, SBWLS: stern side below waterline starboard, HISP: hull internal side port, HISS: hull internal side starboard, RP: rudder port, RS: rudder starboard, PP: propeller port, PS: propeller starboard, STP: stern tube port, STS: stern tube starboard.

Species (or higher taxonomic level)	Hull locations										Niche areas					
	BWLP	BBWLP	SWLP	SBWLP	BWLS	BBWLS	SWLS	SBWLS	HISP	HISS	STP	STS	PP	PS	RP	RS
Crustacea: Cirripedia																
<i>Austromegabalanus psittacus</i>				100		50			50		100		200			300
<i>Balanus glandula</i> *		X	X	X	X	X	X		X		X	X	X	X	X	X
Crustacea: Amphipoda																
<i>Caprella dilatata</i>	6800	475	600	825		1875		125	950	2050	300					100
<i>Caprella equilibra</i>		125		50		175			150	1000	575	100	1400		100	200
<i>Caprella</i> spp.									675	2050						
<i>Jassa marmorata</i> *	10700	525	17700	2275	300	23825		6550	18650	40975		2200	800	300	400	700
<i>Jassa</i> spp.				1100							1000					
<i>Monocorophium acherusicum</i> *	200					75			425	1250						
<i>Monocorophium insidiosum</i> *							300			250		2000	400			
<i>Monocorophium</i> spp.		50	300						25							100
Crustacea: Isopoda																
<i>Exosphaeroma</i> spp.			7600													
<i>Pseudosphaeroma</i> spp.			600		6100											
<i>Sphaeroma serratum</i> *	100															
Crustacea: Decapoda																
<i>Halcarcinus planatus</i>		75		50		25		125	275	75	100					100
Crustacea: Tanaidacea																
<i>Tanais dulongii</i>	1400		10600			375	100	50	150	1125		600	500		1200	500
Molusca: Bivalvia																
<i>Aulacomya atra</i>		225										400				
<i>Hiatella arctica</i>												100				
<i>Mytilus</i> spp.	4200	3500		4000		3150		6300	5600		9400					
Molusca: Gastropoda																
<i>Pleurobranchaea maculata</i> *				25												
<i>Siphonaria lessonii</i>			100		500											
Bryozoa																
<i>Bugula neritina</i> *		X				X		X	X	X						
<i>Bugulina flabellata</i> *		X		X		X		X	X	X	X					
<i>Celleporella hyalina</i> sensu lato		X				X							X			
<i>Cryptosula pallasiiana</i> *		X		X		X						X				
<i>Exochella</i> sp.				X								X				
Bryozoans indet.			X					X	X	X			X	X	X	X
Chordata: Ascidiacea																
<i>Ascidella aspera</i> *	125								50	25						
<i>Asterocarpa humilis</i>	100		25					50	50	25	100		300			
<i>Botryllus schlosseri</i> *								X	X				X			
<i>Diplosoma listerianum</i> *								X	X							
Cnidaria: Hydrozoa																
<i>Ectopleura crocea</i> *																
<i>Sertularia mediterranea</i>				X												
Hydroid 1		X						X	X	X						
Porifera																
<i>Sicon</i> sp.		125														
Annelida: Polychaeta																
Eunicidae ⁺																
Polynoidea ⁺																
Terebelidae ⁺																
Polychaete 1 ⁺																
Polychaete 2 ⁺																
Algae: Rhodophyta																
<i>Anotrichium furcellatum</i> *																X
<i>Antithamnion</i> spp.																X
<i>Callithamnion montagnei</i>		X														
<i>Ceramium virgatum</i>		X		X							X	X	X	X	X	X
<i>Melanthamnion harveyi</i> *				X												
<i>Lepthosiphonia brodiei</i> *																X
<i>Lomentaria clavellosa</i> *	X	X		X				X			X		X	X		X
<i>Polysiphonia hassleri</i>		X														X
<i>Polysiphonia</i> spp.	X													X	X	
Algae: Chlorophyta																
<i>Ulva rigida</i>				X												
<i>Ulva</i> sp.	X											X	X			
<i>Ulva</i> sp. (ex <i>Enteromorpha</i>)				X								X	X			
Algae: Ochrophyta																
<i>Sphacelaria cirrosa</i>													X			X
<i>Undaria pinnatifida</i> *		X		X				X								X
Total number of taxa	53															
Total number of species	34															