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Non-indigenous species contribute equally to biofouling communities in international vs. local ports in the Biobío region, Chile.

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Running headline: Biofouling in international vs. local ports

Abstract

Growing coastal urbanization together with the intensification of maritime traffic are major processes explaining the increasing rate of biological introductions in marine environments. To investigate the link between international maritime traffic and the establishment of non-indigenous species (NIS) in coastal areas, we compared biofouling communities in 3 international and 3 nearby local ports, along 100 km of coastline in South-central Chile, using settlement panels and rapid assessment surveys. A larger number of NIS was observed in international ports, as expected in these ‘invasion hubs’. However, despite a few environmental differences between international and local ports, the two port categories did not display significant differences regarding NIS establishment and contribution to community structure, over the studied period (1.5 years). In international ports, the free space could be a limiting factor for NIS establishment. Our results also suggest that local ports should be considered in NIS surveillance programs in Chile.

Keywords: Propagule pressure, non-indigenous species, cryptogenic species, maritime traffic, predators, diversity

Introduction

Biological introductions, characterized by sudden changes of the natural distribution of species due to human-mediated transports, are among the most pervasive global changes (Simberloff et al. 2013). Human-mediated transport is indeed breaking biogeographic boundaries and contribute to the homogenization of ecosystems at global scale (Villéger et al. 2011; Capinha et al. 2015). In the marine realm, most of the attempts to eradicate established NIS have failed (Ojaveer et al. 2014; Ojaveer et al. 2015). This poor success in management and eradication is likely due to a combination of factors, including notably late detection, high dispersal abilities of many NIS and a large diversity of spreading vectors (Bax et al. 2003).

Regarding the need for early detection, surveillance should target with a high priority the points of entry of NIS, in particular ports and aquaculture facilities in the marine realm. Shipping and leisure boating (ballast water and sediment, hull biofouling) as well as aquaculture are indeed the most critical contributors to invasion in coastal ecosystems at a global scale (Drake and Lodge 2007; Molnar et al. 2008; Clarke Murray et al. 2011; Sylvester et al. 2011; Nunes et al. 2014). Current international shipping hauls almost 90 % of globally traded goods and shipping intensity is consistently increasing (Kaluza et al. 2010; Tournadre 2014). Tthe longstanding recognition of the importance of ballast water and associated sediments in NIS (including harmful organisms and pathogens) transportation has led to the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention) which entered into force on September 2017. In contrast,, no international regulation regarding hull fouling organisms – such as Craft Risk Management Standard for Biofouling in New Zealand (http://www.mpi.govt.nz/) or Biofouling Management Plan in California (http://www.slc.ca.gov/) – has been set up yet. However, in 2011, the IMO adopted Guidelines for the Control and Management of Ships' Biofouling (MEPC 2011, 2013) and is currently preparing a new project (GloFouling) to tackle this major issue. Submerged ship hulls can indeed be colonized by diverse sessile and encrusting organisms that may dislodge or reproduce in the destination port-of-call during the ship stopover. Besides, many of these sessile species are habitat-builders for a myriad of invertebrates (Sellheim et al. 2010; Thomsen et al. 2014; Leclerc and Viard 2018). As such, hull biofouling is a major pathway of introduction and spread of NIS, through both direct and facilitative processes (Bax et al. 2003; Drake and Lodge 2007; Sylvester et al. 2011; Moser et al. 2016; Pinochet et al. 2017).

Following their primary introduction, the rapid spread of marine NIS is favored by coastal hardening (Mineur et al. 2012; Airoldi et al. 2015). The increasing rate of coastal artificial structures, coined 'ocean sprawl' (sensu Duarte et al. 2012), has emerged as protection against environmental perturbations (rising sea level, erosion, storms and flooding) and to support the development of human activities (shipping, urbanization, aquaculture, energy extraction and recreation) (Firth et al. 2016). Beyond habitat-degradation and fragmentation, coastal artificial structures provide novel habitats for colonization by various species – among which NIS prevail as compared to neighboring natural reefs – and alter connectivity and local species pools (Mineur et al. 2012; Dafforn et al. 2015; Bishop et al. 2017). Interestingly ocean sprawls with shipping and boating are jointly enhancing colonization and propagule pressures (sensu Lockwood et al. 2009): besides, being points of entry, ports and marinas are "invasion hubs" (Airoldi et al. 2015). NIS establishment, spread and impacts are however context dependent, relying on a complex combination of invader traits, resource availability, abiotic conditions, and community structure of the invaded habitat (Fridley et al. 2007; Thomsen et al. 2014). The infrastructures and logistics associated to the maritime activities alter environmental factors such as temperature, salinity, hydrodynamics, sediment resuspension, contaminants (nutrients, organic matter and metals) and incident light – all susceptible to affect fouling communities and potentially select tolerant NIS, from species-specific settlement to biotic interactions (Bulleri and Chapman 2010; Dafforn et al. 2015; Johnson et al. 2017; Lawes et al.

2017). While environmental conditions artificially created may be unique at the local scale, they tend to be similar across distant locations, hence removing the potential mismatch between port-of-calls and contributing to the biotic homogenization within and among oceans (Streftaris et al. 2005; Seebens et al. 2013; Seebens et al. 2016).

In SE Pacific and more specifically along the Chilean coasts, particular attention has been paid to the numerous NIS introduced for aquaculture purposes. However, the scarcity of dedicated surveys for the presence of NIS in the marine environment is noteworthy (Castilla et al. 2005; Castilla and Neill 2009; Pinochet et al. 2017; Villaseñor-Parada et al. 2017). Past and recent research have suggested that that very few species have successfully established along SE Pacific coasts as compared to other regions in the world (Castilla and Neill 2009; Villaseñor-Parada et al. 2017).. Among possible causes, unique oceanographic properties characterized by dissolved-oxygen deficit in coastal waters together with cold north-flowing current north of 42°S and low temperature and salinity south of 42°S, had been proposed as a mechanism enabling substantial “abiotic resistance” to regional NIS establishment and spread (Castilla and Neill 2009). Recent modelling approaches regarding international trades also suggested that this environmental mismatch between source regions and SE Pacific coastline should reduce invasion risks (Keller et al. 2011; Seebens et al. 2013). Paradoxically, those barriers are elusive and may change over time (e.g. seasonality and stochasticity of upwellings): they could be overwhelmed locally by expanding international traffic, which is increasing colonization and propagule pressures (Melbourne et al. 2007; Lockwood et al. 2009; Simberloff 2009; Sylvester et al. 2011; Miller and Ruiz 2014) and by “ocean sprawl” which is increasing the NIS establishment and spread probabilities (Streftaris et al. 2005; Mineur et al. 2012; Seebens et al. 2013; Airoldi et al. 2015).

Over the last decade, the number and extent of artificial structures have dramatically expanded along the Chilean coastline in order to protect inhabited shores from storms and tsunamis and to promote economic development, through shipping trades, mining and aquaculture (Aguilera et al. 2014; Aguilera 2017). Between 18° and 41°S (3000 km of linear coastline), these infrastructures account approximately to 200 km, among which 70% are concentrated in northern (18-22°S and 23°S), central (ca. 33°S) and southern (36°S and 41°S) regions (Aguilera 2017). In parallel, Chilean ports receive international ships from diverse regions (mainly Asia, North America, Europe and Australasia). Shipping pathways have been assumed to be responsible for the introduction of 30-38 % of the NIS established in Chile (Castilla and Neill 2009). Nonetheless, the legal regulation of marine NIS surveillance has just started to be implemented in Chile Chilean authorities have enacted a Legal Procedure DIRECTEMAR A-51/002, October, 14th, 2002, based on the Ballast Water Convention, which is still under revision for being concordant with the IMO statements (Lloyd's Register 2014). However, there are currently no regulation, policies or national strategic plan to target biofouling in relation with international, regional or local shipping. In addition, research regarding NIS in Chile is generally focused on the ecology of some already-described invaders and there has been – to our knowledge – no attempt to implement dedicated NIS surveys neither in artificial nor in natural environments (Villaseñor-Parada et al. 2017). In this context there are serious knowledge gaps and uncertainties regarding the extent of NIS contribution to the fouling communities established on hard substrates (natural and artificial). Such data are urgently needed for accurate reports of NIS presence, distribution and establishment of baselines in order to implement dedicated surveillance programs of the biofouling communities in Chilean ports and nearby artificial structures. The present study aimed to investigate the diversity and structure of hard bottom communities, and specifically identify the non-indigenous components of those communities in South-Central Chile. We targeted both international and local ports, the former being primary entry points for introduction mediated by international maritime traffic while the latter being potential destination of NIS primarily introduced. To this end, a series of standardized settlement panels were deployed twice (summer and winter) in 3 international and 3 local ports, before being randomly collected after 1, 3 and 7 months. In parallel, rapid assessment visual surveys were conducted to compare the established communities in the neighboring substrates of the panels. Finally, various abiotic parameters (metal and organic matter concentration, light, and temperature) were measured to check for potential confounding effects when comparing the biofouling communities in international vs. local ports. We hypothesised that NIS richness and abundance measured on 1 month-old panels – fouled by early settlers thus being proxies of the colonization and propagule pressures – would be higher in international than in local ports. After 3 and 7 months of developments, patterns in diversity and assemblage structure were expected to differ, with potential greater contributions of NIS and cryptogenic species in international as compared to local ports.

Methods

Sampling design

The study was performed along approximately 100 km of coastline in the Bíobío region (Chile) between August 2016 and October 2017. Within the region, a total of six fully marine sites (ports) were sorted according to their shipping traffic (Fig. 1): three international ports (cargo and tanker moorings) located in Coronel (37.0304°S, 73.1540°W), San Vicente (36.7591°S, 73.1551°W), and Lirquén (36.7094°S, 72.9829W) and three local ports (fishing barges and craft moorings) located in Llico (37.1541°S, 73.5690°W), Chome (36.7735°S, 73.2137°W) and Coliumo (36.5377°S, 72.9571°W) were selected. In 2016, about 10 to 107 and 64 - 162 fishing crafts (3.5-18 m length) potentially berthed – some of which permanently – in local and international ports, respectively (http://webmail.sernapesca.cl/sernapesca/guest/web/cons_

Rpaem.asp#compuestas). During the same year, between 250 and 427 foreign commercial ships (20 - 217 national ships) berthed in the selected international ports, mainly originating from Asia, South America, North America, Europe, and Australasia (according to import-export data, *web.directemar.cl/estadisticas/puertos/default.htm*).

Biodiversity assays combined the deployment of settlement panels and rapid assessment surveys using scuba diving. In August 2016 (winter trial) and March 2017 (summer trial), a series of settlement panels (black polypropylene, 15 × 15 cm) were deployed vertically upon two experimental units (90 × 100 cm) made of a plastic fence (mesh 2.5 × 2.5 cm, Fig. 1), at two plots separated by 20-50 m within each site (local and international ports) at ca. – 4 m. Depending on the site conformation and substratum availability (e.g. jetties or mooring buoys), the experimental units were either attached to concrete/steel pilings (Coronel, San Vicente, Lirquén, Coliumo), large rocks (Chome) or to floating longline (Llico). A total of 24 panels (12 per plot) were deployed per site on each occasion. After 1, 3 and 7 months, 8 panels (4 at random per plot) were retrieved using polypropylene rubble bags (mesh < 0.5 mm) to minimize mobile fauna loss, and then stored (for up to 4 hours) within a tank filled with sea water until processing in the laboratory. Panels retrieved after 1 month of development are occupied by early-settlers (less than one-month old) and thus were hypothesized to point out mainly differences in recruitments: colonization and propagule pressures (sensu Lockwood et al. 2009; see also Sylvester et al. 2011) were estimated by richness and abundances, respectively. Panels left for a few weeks are indeed effective to detect new arrivals (Bishop, Wood, Lévêque, et al. 2015; Bishop, Wood, Yunnie, et al. 2015) and to comprehend propagule pressure *s.l.* dynamics (Sorte and Stachowicz 2011; Bouchemousse et al. 2017). Panels retrieved after 3 and 7 months were analysed to examine differences in community assembly, resulting from species interactions (Stachowicz et al. 2002; Lord and Whitlatch 2015).

In order to complement the settlement panel datasets, rapid assessment surveys were conducted by the same diver (JCL) during each trial (in November 2016 and June 2017) within established subtidal communities of hard substrates surrounding the experimental units in all locations. During 30 minutes, all species encountered were given a score of semi-abundance according to the SACFOR scale (Superabundant, Abundant, Common, Frequent, Occasional, Rare), which takes into account the individual size and growth form (Hiscock 1996). These surveys were conducted between ca. – 1.5 m and – 5 m and generally covered the horizontal distance between the experimental units (20-50m) depending on the site conformation (e.g. distance between pilings). Most sessile taxa as well as mobile taxa > 10 mm were visually identified at the lowest taxonomic level possible in the field. To ensure accurate identification, prior to this study, a series of preliminary fieldworks were performed in each study site. During these dives, specimens were photographed, collected, identified under a dissecting microscope and preserved in 95% EtOH to form a local reference collection. This preliminary work was used to prepare detailed field-worksheets and train the observer eye. In addition, during the study, a few specimens were photographed and collected to confirm field identification back in the laboratory.

Data collection

Environmental parameters

Environmental conditions of each site were assessed from a series of parameters: incident light, temperature, sediment pH, sediment organic matter content and concentration of different metals. Temperature (°C) and illuminance (Lum ft^{-2}) were measured *in situ* at 10 min intervals between March and June 2017 using data loggers (onset HOBO® data-loggers Pendant Temp-Light, Onset Computer Corporation) deployed within each site. Owing to the biofouling undergone by the loggers, light data gathered more than 4 weeks after installation were not considered. Sediment parameters were determined from superficial sediment (first cm) samples (n = 3-4) collected below experimental units in June 2017 and stored at – 20°C until analyses. In the laboratory, sediment samples were lyophilized and pulverized. The pH was measured in 1:2.5 sediment to water ratio using an electrode. The organic matter content (%OM) was determined after calcination at ca. 550ºC. Metal contents were determined using total Ray X reflexion fluorescence analysis (Towett et al. 2013, details provided in Appendix S1).

Diversity and community structure assays

In the laboratory, panels were removed from their bags, cleared from cable tiles and left with all remaining bag contents in seawater tanks until sessile fauna returned to their natural, untense state. Sessile taxa (mostly fauna, see Results) were identified under a dissecting microscope and their abundances were assessed using cover. To avoid edge effects in their distribution, a 15 mm perimeter was excluded from analysis, giving a 120 × 120 mm working area. Species cover was estimated under 100 random intersection points out of 169 created between evenly spaced lengths of string of 150 × 150 mm quadrat. Any species identified out of these intersection points was given a cover of 0.5 %. Species layering was taken into account, therefore the total cover frequently exceeded 100%. Following sessile taxa identification, panels were washed through a 500 µm mesh sieve to separate mobile fauna, ultimately stored in EtOH 95%. All specimens were identified at the lowest taxonomic level possible (generally species) by the same observer (JCL) and occasionally verified by external experts (see acknowledgments). Throughout the survey, voucher specimens were collected, dissected when appropriate and preserved in 95% EtOH in order to fill in the local reference collection (for further morphological and/or molecular examination). When appropriate, some specimens were maintained in tanks with bubbling air stone and filled with seawater at ambient temperature until they developed diagnostic size and/or characteristics. Molecular barcoding (using COI) was also employed whenever necessary and possible. While this approach did not always enable identification at the species level (e.g. because of the absence of reliable molecular reference data), it allowed to compare juveniles and adults, the latter being identified (by JCL) upon morphological characteristics and assign juveniles to identified adults. The identified specimens were categorized as ‘native’, ‘non-indigenous’ (NIS), ‘cryptogenic’ or ‘unassigned’ according to the literature (e.g. Moyano 1983; Galea 2007; Turon et al. 2016) and public databases (EASIN, WORMS/WRIMS, Pagad et al. 2016). The cryptogenic species, from unknown/uncertain origin (sensu Carlton 1996), found in this study displayed a cosmopolitan distribution and were potentially non-indigenous to the study area. Cryptogenic and NIS, both candidates for further introduction and spread, were thus considered in analyses (Dafforn et al. 2009; Leclerc and Viard 2018). When appropriate, taxa were also sorted according to their main function within the food web (carnivores, suspension-deposit-feeders, herbivores).

Statistical analyses

Patterns in environmental conditions were explored using a principal component analysis (PCA), based on normalized data. Data related to sediment conditions (pH, %OM, metal concentrations) were replicated (n = 3-4 per sites) and were all included as active variables in the PCA. All samples were given the same values for light and temperature (average and range over deployment period), therefore these data were included as supplementary (i.e. illustrative or inactive) variables (Lê et al. 2008). Environmental patterns were examined with a two-way design using a permutational multivariate analysis of variance (PERMANOVA, Anderson 2001), with 4999 permutations. Factors were ‘maritime traffic’ (hereafter ‘traffic’, fixed, 2 levels: international and local) and ‘site’ (random, nested within traffic). This analysis was based on a Euclidean distance matrix generated from normalized data of the active variables of the PCA.

Patterns in species richness, abundance and community structure of sessile taxa were examined with a four-way design using PERMANOVAs with 4999 permutations. Factors were 'traffic' (fixed, 2 levels: international and local ports), 'starting deployment period' ('starting date', random, 2 levels: August 2016 and March 2017), 'age of the settlement panel at the time of collection' ('age', fixed, 3 levels: 1, 3 and 7 months) and 'site' (random, nested within traffic). A few experimental units were lost over the course of the experiment, therefore the corresponding term (plot) was not included in analyses. Nonetheless, there were between 4 and 8 replicate panels available for each combination of Site (Traffic) × Starting date × Age. Univariate analyses were based on Euclidian distance matrices whereas multivariate analyses were based on Bray-Curtis similarity matrices generated from either raw or transformed data. In order to down-weight the importance of most abundant species (and homogenize multivariate dispersion), multivariate data were always square root-transformed. The homogeneity in univariate or multivariate dispersion was checked among the levels of the lowest interaction term Site (Traffic) × Starting date × Age using PERMDISP (Anderson et al. 2008). No transformation allowed homoscedasticity to be achieved in univariate data, except in one case (total richness). Given the balance of the design and the large number of samples, univariate PERMANOVAs (analogous to ANOVAs) were considered robust enough to cope with this issue and were run on untransformed data (Underwood 1997). As for multivariate data, samples were also ordinated using principal coordinate (PCO) analyses to support PERMANOVA results (Anderson et al. 2008). When appropriate, PERMANOVAs were followed by pairwise comparisons and *P*-values were estimated using Monte Carlo procedure. Finally, semi-quantitative data resulting from rapid assessment surveys on surrounding communities were analyzed graphically, by considering the number of reports (i.e. taxa) within each category of abundances at both sampling periods. These analyses were presented separately for non-indigenous and cryptogenic species as well as native space occupiers and potential predators.

Statistical analyses were performed either on all variables combined (including unassigned taxa as well as abiotic data in the case of community structure: bare surface, grazing scars, dead biota), native taxa, non-indigenous or cryptogenic species (Thomsen et al. 2016). By analysing sub-component of communities separately, Bray-Curtis similarity could not always be computed because of the presence of empty samples (e.g. panels not colonized by NIS): corresponding pairs of samples were thus removed from analyses (see degree of freedom in PERMANOVA results). Environmental data were analysed using R environment (Lê et al. 2008; R Development Core Team 2012) while community analyses were performed using PRIMER 7 (Clarke and Warwick 2001).

Results

Environmental parameters

According to the principal component analysis (PCA), all study sites were quite well-discriminated by their abiotic conditions (Fig. 2, Appendix S1). While the PERMANOVA confirmed these differences (Site (Traffic): $F_{4,13} = 5.01$, $P < 0.001$), it failed to detect a multivariate effect of the traffic category (i.e. international vs. local ports, $F_{1,13} = 2.75$, $P = 0.099$). In spite of a large within-site variability, the first PCA axis (50.1% of the variation) however suggested differences between ports according to their category. These differences are predominantly explained by higher concentrations of a series of metals (except Hg), but lower light (inactive variable) in international as compared to local ports.. There is however some overlap among sites: most metal concentrations and light levels were similar in Coliumo (local), Lirquén and San Vicente (international ports). This is exemplified on the second axis of the

PCA (26% of variation) which discriminates Coronel samples (57% of contribution) and the local port located in Llico (5%) from the four other sites. This discrimination is mainly due – by decreasing order of importance – to higher %OM, pH and concentrations of copper, lead and zinc. Coronel also experienced greater mean values and variation in temperature.

Diversity and community structure

During the study (combining panels examination and rapid assessment survey), a total of 119 taxa were identified, including 10 algae, 41 sessile and 37 mobile animals. Complete lists and species authorities are provided in Appendix S2. Among these taxa, 10 were classified as non-indigenous and 15 as cryptogenic. Importantly, these taxa included novel records for the region: *Bougainvillia muscus*, *Ectopleura larynx*, *E. crocea*, *Hydractinia* sp. and *Exochella* nov. sp. and *Asterocarpa humilis* (for the latter, details are provided in Pinochet et al. 2017). Within established hard bottom communities examined through rapid assessment surveys at both periods, NIS and cryptogenic species contributed to 13% and 26% of the sessile taxa richness, respectively. The highest proportions of NIS (23%) and cryptogenic species (25%) were found in Lirquén (Appendices S3, S5). Nonetheless, NIS were often rare or occasional (Fig. 3) and some of them were only found in specific habitats (e.g. crevices, mooring lines and abandoned ropes). In comparison, several native species were classified as frequent, common or abundant in both categories of ports (Fig. 3). Less NIS (i.e. cumulative numbers over sites) were reported in local (3 and 7 in November 2016 and March 2017, respectively) than in international ports (13 and 18) (Appendices S3, S5). Six NIS were found only in international ports, with three of them *Bougainvillia muscus* (rare to frequent), *Ectopleura crocea* (rare to abundant), *Ciona robusta* (absent to rare) observed in at least two sites. For cryptogenic species, the number of reports was greater, but with less difference than for NIS, between local (12-15) and international ports (21-20), with two of them only recorded in international ports (e.g. *Clytia linearis*, varying from rare to abundant depending on site and sampling date, Appendix S3).

On experimental panels, over the whole experiment, a total number of 6 and 9 NIS settled on the panels in local and international ports, respectively (details in Appendix S4). In local ports, half of the NIS were observed in one site only, and none in the three study sites. In international ports, 5 were also found in one site only but 3 NIS were observed in the three study sites. The number of cryptogenic species was more balanced between the two categories with 12 and 14 taxa reported in local and international ports, respectively. Like for NIS, the number of occurrence was however higher across sites in international ports with 6 cryptogenic species observed in the three international ports and only one in the three local ports.

Significant three-way interactions (Site × Starting date × Age) were observed with regards to either richness, sessile taxa cover or community structure, whether considering all, non-indigenous, cryptogenic or native species (Table 1-3). However, despite larger number of NIS observed in international ports and with higher occurrence, no effect of the traffic category (international vs. local ports) could be detected, regardless of the deployment period or its duration (Table 1-3, Fig. 4-6).

After one month, an average of 4 taxa settled on the panels, with an average coverage of 34% of the panel area (22% in September 2016, 45% in October 2017). Only a few non-indigenous specimens were observed at this stage (2 in local and 6 in international ports on total, both sampling periods combined), with up to 1 taxa for a maximum cover of 2% in Lirquén (Fig. 4-5, Appendices S6-7). No clear pattern could be detected for cryptogenic species (5 and 9 taxa in local and international ports, respectively) richness and cover, although they represented half of the richness and up to 87% of the cover in Llico (Fig. 4-5, Appendices S6-7). The PCO illustrates the three-way interaction (Site × Starting date× Age, Table 3): traffic category did not explain settlement patterns, but important site-to-site differences were observed within each category and depending on the period (Fig. 6). NIS poorly influenced these patterns, which are more likely due to some species-specific recruitments. For instance, the native barnacle *Austromegabalanus psicattus* showed greater settlement in Llico during the second trial (March 2017) as compared to all other sites (Fig. 6).

After 3 months, site-to-site differences – though depending on the period – increased (Fig. 4-6, Appendices 6-8). The average richness per panel varied between 3 (Chome) and 9 (Lirquén) after the first deployment and between 3 (Chome) and 10 (San Vicente) after the second one (Appendix S6). Total cover of sessile taxa varied between 40% (Chome) and 180% (San Vicente) after the first deployment and between 20% (Lirquén) and 100% (in Llico, Coliumo and Coronel) after the second (Appendix S7). At this stage of development (both periods combined), a total of 4 and 5 NIS was observed on panels in local and international ports, respectively (5 and 14 cryptogenic species). All non-indigenous, cryptogenic and native species apparently contributed to differences among sites (Appendices S6-7, Table 1-2). The global multivariate structure allowed to discriminate all sites and to illustrate within site-similarity between panels deployed in winter and summer (the first two axes of the PCO explained a total of 44%, Fig. 6). Importantly, it also illustrates the absence of clear discrimination according to the traffic categories (Table 3): for instance, grazing scars (apparently due to urchins, JCL pers. obs.) – correlated with bare surface – contributed to community structure in both Chome (local) and Lirquén (international port), at least in summer, while barnacle *A. psicattus* was an important space occupier in both Llico (local) and San Vicente (international port). Similarly, by analysing separately non-indigenous, cryptogenic and native assemblage structures, it is clear that several species contributed to spatial patterns, regardless of traffic categories (Fig. 6). For instance, while *B. muscus* contributed to NIS community structure in all international ports, *Exochella* n. sp. was abundant in both Coliumo (local port, where the species has been observed for the first time) and San Vicente (international). After 7 months, panels were generally fouled by more species and in greater abundances (involving either NIS, cryptogenic, native or a combination of all, depending on site or sampling occasion, Appendix S5), but the observed patterns were generally consistent with those observed after 3 months (Fig. 4-6, Appendices S6-8). At this stage, a total of 5 and 8 NIS and a total of 8 and 9 cryptogenic species were observed on panels from local and international ports, respectively.

Discussion

International ports are often coined as ‘introduction hotspots’ or’ invasion hubs’ because maritime traffic occurring in these areas is expected to enhance the colonization and propagule pressures. The largest number of NIS observed in international ports in our study supports this scenario. However, a roughly similar number of NIS was also observed in local port. Thus, conversely to our working hypotheses, no significant effect of the traffic category (international vs. local ports) could be detected on the biotic component, at any stage of development of the biofouling community. NIS early settlement was low in all sites, including in international ports, as measured on experimental panels collected after 1 month at any trial period. While contrasting biofouling communities developed 3 and 7 months after panel deployment, NIS played either a minor role or an equal contribution across both categories of ports. Altogether, important site-to-site differences were observed regarding abiotic conditions, richness, sessile taxa cover and community structure, but without discriminating ports according to their traffic categories.

Overall, unique communities developed in each study site irrespective of their traffic category and in spite of some variations in environmental parameters (metal concentration, light) between them. For instance, higher metal concentrations were reported in the international ports, as shown in other studies(Dafforn et al. 2012). Resuspension of sediment-bound contaminants, such as metals, may influence biofouling communities by affecting either settlement cues or the biofilm. In the more contaminated site of this study (Coronel), the average concentrations of copper, lead and zinc were of 41, 34 and 73 mg kg^{-1}, respectively. While such values are in the range of some impacted estuaries (e.g. in New South wales) (Dafforn et al. 2012), they are below the reference range of possible adverse biological effects (Macdonald et al. 1996) and about 10 times lower than highly contaminated sediments (from Port Kembla, New South Wales) which have recently been used to demonstrate impacts on bacterial biofilm interactions and macro-fouling communities (Lawes et al. 2017). Under the conditions herein measured, only experimental approaches considering possible interactions with a series of other stressors (Johnston and Keough 2002; Lawes et al. 2017), which may display more complex dynamics (such as the dissolved-oxygen in the study area, Ahumada B. et al. 1983; Valle-Levinson et al. 2003) would allow to disentangle among oceanographic and anthropogenic factors which influence the studied communities.

NIS represented less than 1% of the surface colonized on settlement panels collected after 1 month at both occasions, suggesting that all study sites undergo a similar and low propagule pressure by NIS. In addition, most of these NIS apparently settled on panels with relative abundances representative of those also identified within the surrounding established communities or in older panel assemblages (e.g. *Bougainvillia muscus* in Lirquén, *Ectopleura crocea* in Coronel, *Bugulina flabellata* in Lirquén and Llico, *Exochella* nov. sp. in Coliumo and San Vicente; Fig. 6, Appendix S2) – a pattern quite consistent with the one observed for native species (e.g. *Austromegabalanus psicattus* in Llico, *Semimytilus algosus* in Coronel, *Balanus laevis* in Coliumo and San Vicente). It is therefore possible that a significant part of the observed propagule pressure mainly results from local (site-specific) populations, and thus involve more complex histories of species introductions. In the present study, most of the novel records were hydroids which, as compared to well-studied groups in Chile (e.g. Bryozoans, Viviani 1969; Moyano 1983), have received less attention until recent campaigns in Patagonian fjords (Galea 2007; Galea et al. 2007). These campaigns resulted in novel records of various non-native (*Bougainvillia muscoides*, *B. pyramidata*) and cryptogenic hydroids (e.g. *Clytia linearis*, *Amphisbetia operculata*), some of them widely distributed (Galea 2007; Galea et al. 2007). Interestingly, among the novel taxa herein recorded, *Ectopleura crocea* has been observed in all studied international ports. This common biofouling species is considered non-native in many regions worldwide, including in the NE Pacific along which – decades ago – its hydrants have been demonstrated to survive long distances and disperse from the hulls of sailing vessels (Carlton and Hodder 1995). First time report does not mean that the introduction is recent (e.g. Provan et al. 2005), especially in under-studied taxonomic groups. Like other non-native species reported for the first time, including in this study, this species has probably been introduced along the SE Pacific, and more specifically in Chile, a long time ago, spread and established widely, likely beyond the geographic span of this study. In order to test whether the recruitment patterns herein observed result from local populations or external sources, thorough surveys of ship tanks and hulls, as well as small craft hulls, arriving in the studied ports would have been needed. For the time being and according to worldwide literature on the topic, it seems however unlikely that arriving international ships do not transport NIS (Bax et al. 2003; Drake and Lodge 2007; Gollasch et al. 2007). A recent study of international ship hulls in Talcahuano port, located in our study area, revealed the presence of the non-indigenous tunicate *Asterocarpa humilis* – putativelly native from New Zealand – within their sea chest (Pinochet et al. 2017). Although no effect of the traffic category could be have been detected on recruitments over the studied period, the larger number of NIS reported in international ports suggests that international shipping have influenced their local establishment at some point. Nonetheless, the environment of the receiving ports may mismatch with the abiotic requirements of many introduced species for reproduction and propagule survival (Castilla and Neill 2009; Seebens et al. 2013). In addition, regardless of their survival, some propagules may simply not be retained: although generally constructed within bays, none of the study ports were enclosed by breakwaters or seawalls, therefore it is possible that the propagule pressure was locally limited in favour of outward dispersal (Floerl and Inglis 2003; Bishop et al. 2017). Dispersal and survival modelling based on local oceanographic features and species life history traits would help to test whether these hypotheses could explain the generally low propagule pressure and the current distribution of NIS among all sampling sites.

After 3 and 7 months, NIS contribution to community structure was highly variable – in terms of identity and abundances – among sites and there was no indication that international maritime traffic contributed to this pattern. While NIS richness and cover respectively increased in 4 and 3 sites between 1 and 3 months, they only increased in 1 and 3 sites between 3 and 7 months, irrespective of their associated traffic (Appendix S9). In comparison, native richness and cover respectively increased in 4 and 5 sites between 1 and 3 months and both in 4 sites between 3 and 7 months. The apparent poor contribution of NIS to community development patterns is potentially in agreement with Castilla and Neill (2009) who suggested that the uniqueness of the Chilean coastline in terms of oceanographic conditions could contribute to limit the regional establishment and spread of NIS, less ‘adapted’ than most native species under specific conditions (e.g. Castilla et al. 2004; Caro et al. 2011). In order to be validated, such a hypothesis would however require thorough test of the propagule survival and growth of the NIS herein found (as well as native species), under controlled conditions. With regards to the temporal changes in richness and cover undergone by native species (generally increasing, Appendix S9) on experiment panels, the influence of these species in limiting NIS establishment (i.e. biotic resistance hypothesis) deserves further experimental scrutiny (Stachowicz et al. 2002; Clark and Johnston 2009; Lord and Whitlatch 2015). In all the studied international ports, pilings were generally completely fouled (JCL, pers. obs.) and various native sessile taxa were classified as common, abundant or even super-abundant (Figure 3), i.e. with species-specific covers varying between 10 to more than 80% depending on the growth forms (crust or massive)-. In parallel, only a few NIS fell in these categories, most of them being rare or occasional (Fig. 3). The native assemblages may have contributed to limit NIS establishment in the studied sites by limiting at least one of the most limiting resources of biofouling communities: free space (Elton 1958; Stachowicz et al. 2002). Should the influence of native space occupiers in limiting NIS establishment be further investigated in the study area, the complexity of possible interactions (e.g. facilitation, predation) between native and non-indigenous taxa could not be overlooked (Bulleri et al. 2008; Rogers et al. 2016; Leclerc and Viard 2018). Various NIS (e.g. *Ectopleura crocea*, *Bugulina flabelatta*, *Asterocarpa humilis*, *Diplosoma listerianum*) and cryptogenic species (e.g. *Alcyonidioides mytili*, *Amathia* cf. *gracilis*, *Plumularia* cf. *setacea*) were, for instance, herein frequently observed in abundance when developing upon massive native habitat builders, such as *Pyura chilensis* and *Austromegabalanus piscattus*. In the present survey, several native predators (e.g. fish, urchins, crabs, Caprellid amphipods) were also observed and generally classified as frequent or common depending on study sites, in either local or international ports (Fig. 3, Appendix S5). Nonetheless, multivariate analyses suggested that grazing (apparently due to sea urchins) influences the community with contrasting dynamics in at least two sites – an international (Lirquén) and a local port (Chome) – by increasing the free space (Fig. 6). As such, the specific functions of native predators in controlling some NIS populations may deserve further investigation (Dumont et al. 2011; Rogers et al. 2016), although careful attention should be paid regarding direct and indirect effects (Connell 2001).

In a more regional context, the influence of local shipping and ocean sprawl in favoring NIS establishment and spread should also be considered (Wasson et al. 2001; Mineur et al.

2012; Aguilera et al. 2014; Johnson et al. 2017). For instance, unlike all the studied international ports, Coliumo jetty did open to regional traffic (fishing boats) only one year before the beginning of our survey and its construction dated back a couple of years following 2010 tsunami – common catastrophic events along the Chilean coast (Aguilera 2017). At this site, only seldom and poorly abundant native taxa were initially observed on pilings (Appendix S5), still covered by antifouling paint. Later on (e.g. in June 2017), several NIS were recorded (e.g. *Exochella* nov. sp., *Bugula neritina*, *Bugulina flabelatta*, *Asterocarpa humilis*, *Diplosoma listerianum*) and some of them (e.g. *Exochella* nov. sp.) showed important increase in cover (and apparent overgrowth of native taxa) on experimental panels between sampling dates (Appendix S9). These results strengthen the hypothesis that free space may be an important limiting factor for NIS establishment (Stachowicz et al. 2002). In this context, it is noteworthy that NIS cover varied on average between 14 and 29% after 7 months, which are values greater than those observed within the surrounding communities (Fig. 3). Unless they get colonized by abundant native taxa, the creation of novel artificial structures may thus contribute to the local establishment of NIS, and potentially act as stepping stones for subsequent spread (Wasson et al. 2001; Mineur et al. 2012; Airoldi et al. 2015), potentially facilitated by regional transport (Clarke Murray et al. 2011; Ferrario et al. 2017) – herein mainly due to fishing barge and crafts: in the present survey, *Exochella* nov. sp. was detected for the first time underneath the local jetty of Coliumo in March 2017 and later recruited and contributed to community structure on experimental panels in both Coliumo and San Vicente (international port). If Chilean authorities and research groups were to implement NIS surveillance programs, the present study performed locally, along with others conducted abroad (e.g. Clarke Murray et al. 2011; Bishop, Wood, Yunnie, et al. 2015; Johnson et al. 2017), strongly supports that the diversity of putative vectors (including hull biofouling of local crafts) and stepping stones (including local mooring facilities) should be taken into account.

In conclusion, there is no clear evidence that international ports, in which intense international maritime traffic is occurring, play the most important role for NIS establishment in the study region. Without challenging the fact that international ports are invasion hubs, our surveys also pointed out the importance of local ports for NIS establishment and potential subsequent spread, including in nearby international ports from where they can be afterwards transported over longer distance. Overall, NIS recruitment appeared low during the study period and NIS contribution to community structure depended on study sites, regardless of traffic categories. Site-specific processes are most likely to explain these patterns, among which the influence of environmental conditions, regional transport, the availability of free space to be colonized and NIS interactions with the recipient communities should deserve further attention.

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Table legends

Table 1. Results of PERMANOVA tests for differences in sessile taxa richness among levels of the main factors (traffic, starting date, age and site) and their interactions. Tests are presented for all, non-indigenous, cryptogenic and native species, separately. Post-hoc pairwise tests are summarized in Appendix S9.

Richness	Total	NIS	Cryptogenic	Native
Transformation	square-root	none	none	none
PERMDISP	$F_{1,35} = 1.199$, $P = 0.464$	$F_{1,35} = 5.478$, $P < 0.001$	$F_{1,35} = 2.429$, $P = 0.002$	$F_{1,35} = 5.227$, $P < 0.001$

Source	df	MS	Pseudo-*F*	*P*	MS	Pseudo-*F*	*P*	MS	Pseudo-*F*	*P*	MS	Pseudo-*F*	*P*
Traffic = T	1	12.2	3.14	0.160	14.9	1.64	0.328	76.1	3.85	0.111	131.9	3.23	0.170
Starting date = S	1	0.0	0.00	0.994	8.2	1.87	0.240	2.1	0.23	0.650	1.0	0.23	0.642
Age = A	2	19.4	13.43	**0.001**	20.0	6.49	**0.010**	6.9	1.19	0.414	122.2	8.17	**0.003**
Site(T) = Si	4	3.3	3.04	0.162	11.7	2.60	0.206	20.2	2.19	0.228	35.9	8.26	**0.036**
T × S	1	1.0	0.99	0.359	0.3	0.07	0.791	2.4	0.26	0.629	7.0	1.65	0.262
T × A	2	1.6	1.12	0.425	0.1	0.52	0.799	8.4	2.07	0.175	6.0	0.61	0.737
S × A	2	0.3	0.68	0.518	1.7	1.58	0.254	2.7	1.25	0.358	1.5	0.46	0.658
Si(T) × S	4	1.1	13.43	**<0.001**	4.5	15.40	**<0.001**	9.2	15.90	**<0.001**	4.3	6.79	**<0.001**
Si(T) × A	8	1.2	2.61	0.099	1.6	1.42	0.310	5.0	2.25	0.140	14.2	4.34	**0.028**
T × S × A	2	0.6	1.43	0.296	0.7	0.66	0.534	0.2	0.08	0.926	1.2	0.36	0.711
Si(T) × S × A	8	0.5	5.74	**<0.001**	1.1	3.78	**0.001**	2.2	3.85	**<0.001**	3.3	5.12	**<0.001**
Res.	232	0.1			0.3			0.6			0.6		
Total	267												

Table 2. Results of PERMANOVA tests for differences in sessile taxa cover among levels of the main factors (traffic, starting date, age and site) and their interactions. Tests are presented for all, non-indigenous, cryptogenic and native species, separately. Post-hoc pairwise tests are summarized in Appendix S9.

Cover (%)	Total	NIS	Cryptogenic	Native
Transformation	none	none	none	none
PERMDISP	$F_{1,35} = 2.933, P < 0.001$	$F_{1,35} = 11.050, P < 0.001$	$F_{1,35} = 8.518, P < 0.001$	$F_{1,35} = 6.804, P < 0.001$

Source	df	MS	Pseudo-*F*	*P*	MS	Pseudo-*F*	*P*	MS	Pseudo-*F*	*P*	MS	Pseudo-*F*	*P*
Traffic = T	1	30415.0	0.43	0.763	3848.3	1.70	0.327	22184.0	1.95	0.277	24686.0	6.09	0.057
Starting date = S	1	5568.8	1.02	0.360	2148.7	3.38	0.146	7011.5	0.67	0.454	5124.7	0.49	0.513
Age = A	2	135330.0	8.13	**0.004**	9494.6	5.15	**0.022**	2790.4	0.53	0.797	19033.0	4.18	**0.034**
Site(T) = Si	4	66246.0	11.90	**0.023**	2524.8	3.91	0.094	16589.0	1.54	0.336	3880.6	0.36	0.839
T × S	1	18956.0	3.47	0.148	162.0	0.25	0.640	546.8	0.05	0.834	1968.3	0.19	0.658
T × A	2	3733.2	0.74	0.662	920.9	1.12	0.447	3071.6	0.78	0.635	5732.6	1.55	0.280
S × A	2	11498.0	3.58	0.087	1122.3	1.44	0.287	3047.5	2.02	0.197	1715.9	0.58	0.581
Si(T) × S	4	5569.2	12.64	**<0.001**	645.2	3.29	**0.014**	10749.0	42.26	**<0.001**	10663.0	119.35	**<0.001**
Si(T) × A	8	5670.3	1.73	0.232	893.2	1.13	0.438	5129.3	3.34	0.053	3631.8	1.20	0.405
T × S × A	2	3892.8	1.21	0.343	645.9	0.83	0.467	833.8	0.55	0.596	2079.5	0.70	0.522
Si(T) × S × A	8	3278.6	7.44	**<0.001**	791.5	4.03	**<0.001**	1537.4	6.04	**<0.001**	3032.9	33.95	**<0.001**
Res.	232	440.8			196.4			254.4			89.3		
Total	267												

Table 3. Results of PERMANOVA tests for differences in community structure among levels of the main factors (traffic, starting date, age and site) and their interactions. Tests are presented for all, non-indigenous, cryptogenic and native species separately. Post-hoc pairwise tests are summarized in Appendix S9.

Community	Total				NIS				Cryptogenic				Native			
Transformation	square-root				square-root				square-root				square-root			
PERMDISP	$F_{1,35} = 5.628$, $P < 0.001$				$F_{1,26} = 3.049$, $P = 0.017$				$F_{1,35} = 8.216$, $P < 0.001$				$F_{1,27} = 7.580$, $P < 0.001$			
Source	df	MS	Pseudo-*F*	*P*	df	MS	Pseudo-*F*	*P*	df	MS	Pseudo-*F*	*P*	df	MS	Pseudo-*F*	*P*
Traffic = T	1	82673.0	1.31	0.365	1	10978.0	1.05	0.440	1	119060.0	2.53	0.150	1	20885.0	1.47	0.146
Starting date = S	1	15105.0	1.54	0.238	1	2576.5	1.44	0.340	1	12569.0	1.46	0.262	1	7040.3	1.95	0.212
Age = A	2	57578.0	3.67	**0.008**	2	6360.8	1.38	0.293	2	13968.0	1.21	0.288	2	21733.0	1.76	0.127
Site(T) = Si	4	64219.0	6.44	**<0.001**	4	24980.0	7.83	**0.001**	4	52250.0	5.29	**<0.001**	4	34322.0	3.64	**<0.001**
T × S	1	7766.1	0.79	0.549	1	3304.5	1.64	0.280	1	5998.5	0.70	0.616	1	7770.6	1.99	0.201
T × A	2	7594.0	0.95	0.552	2	2316.0	0.97	0.565	2	8961.3	1.08	0.403	2	15629.0	1.21	0.356
S × A	2	6876.6	1.12	0.380	2	3014.7	0.81	0.605	2	6108.0	0.88	0.563	2	7671.4	1.34	0.310
Si(T) × S	4	9972.9	19.27	**<0.001**	4	3492.9	3.49	**0.001**	4	9885.4	8.01	**<0.001**	4	9612.5	8.58	**<0.001**
Si(T) × A	8	10725.0	1.71	**0.017**	6	6553.4[c]	1.38	0.376	8	12917.0	1.62	**0.030**	5	10475[c]	1.77	0.118
T × S × A	2	3919.7	0.64	0.769	1	2217.8[c]	0.51	0.627	2	3587.6	0.52	0.905	1	9209.2[c]	1.66	0.204
Si(T) × S × A	8	6289.0	12.15	**<0.001**	2	4595.8[c]	4.60	**<0.001**	8	7957.3	6.45	**<0.001**	4	5951.4[c]	5.31	**<0.001**
Res.	232	517.5			121	1000.0			202	1233.6			165	1119.8		
Total	267				147				237				192			

[c] : the corresponding term has one or more empty cells

Figures

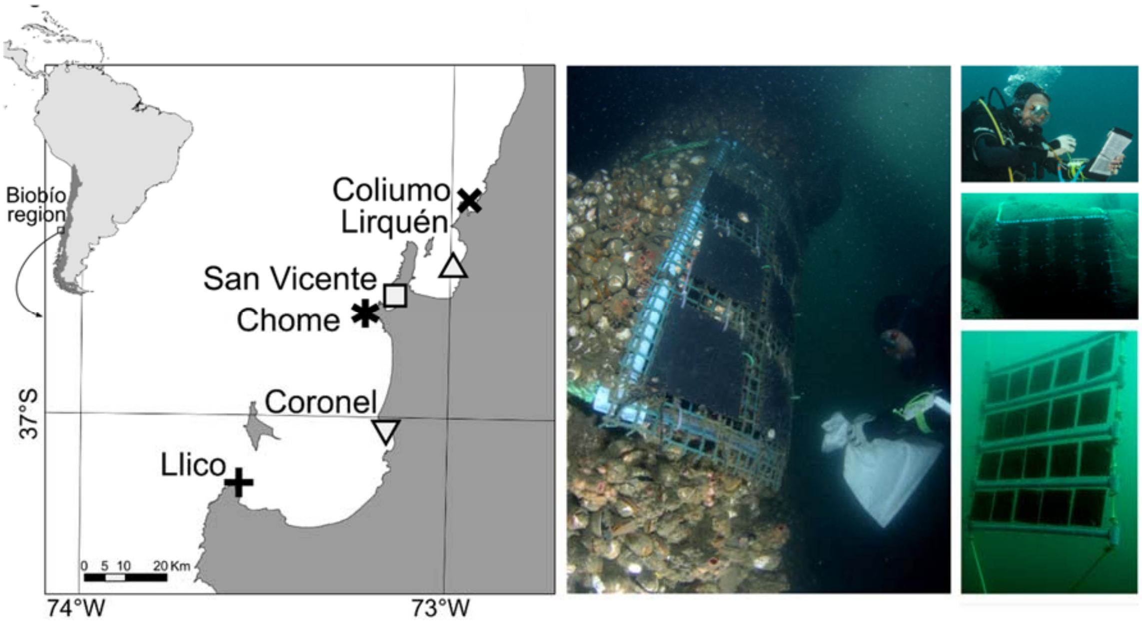

Figure 1. Location of the study sites along Bíobío region: International (white symbols) and local ports (black symbols).

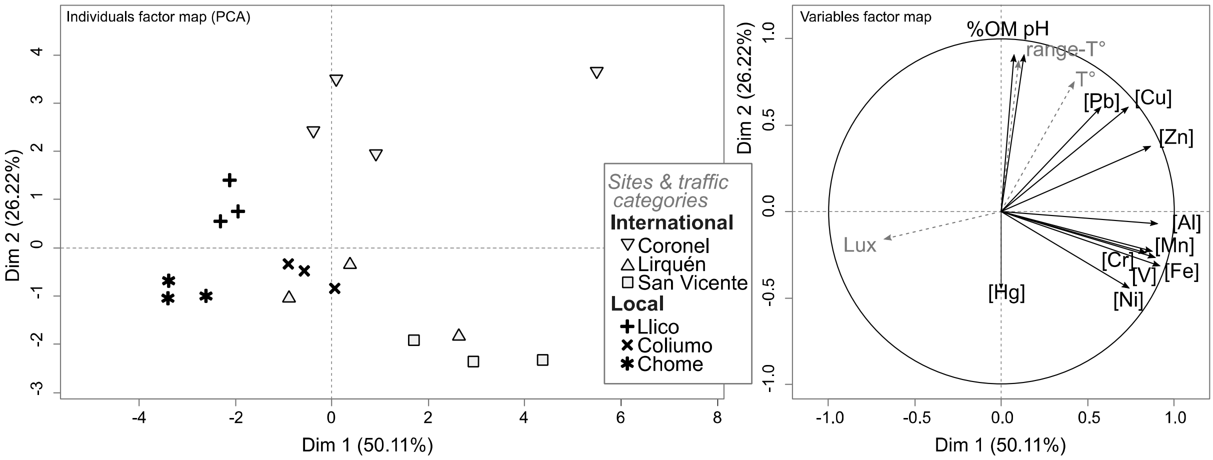

Figure 2. Principal Component Analyses (PCA) describing environmental conditions in international and local ports (individual factor map, left panel). Active (black arrows) and inactive (dashed grey arrows) variables are indicated on the vector plot (right panel).

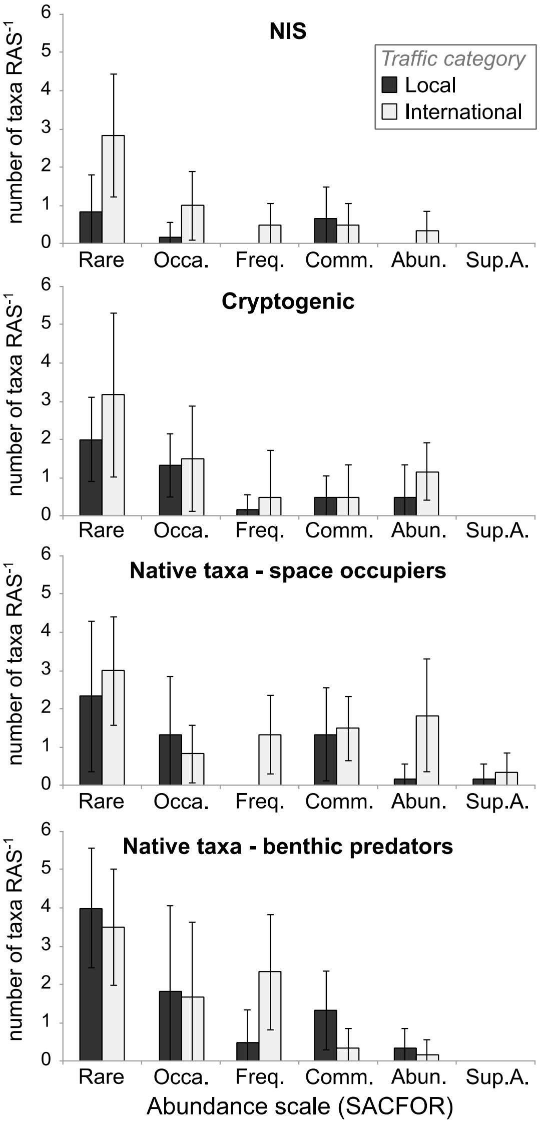

Figure 3. Number of non-indigenous, cryptogenic and native species (space occupiers and benthic predators) encountered within communities surrounding panels for the two categories of ports, depending on their traffic (international and local ports). Data are presented as the average number of reports (species or taxa) per site and sampling occasion (± S.D.) within each category of abundance (SACFOR). Detailed data are presented in Appendix S5.

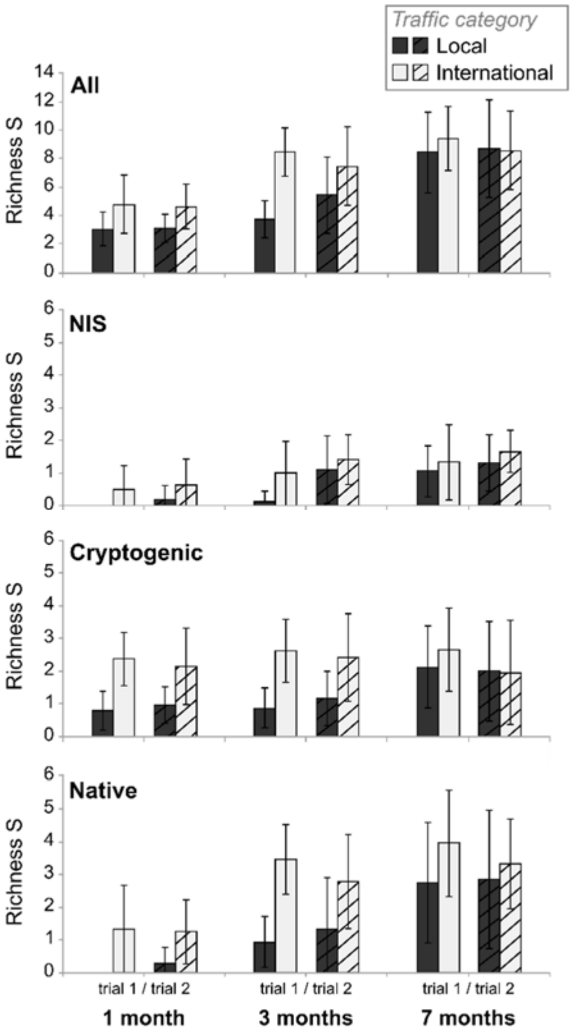

Figure 4. Sessile taxa (total, NIS, cryptogenic and native) richness on the experimental panels collected after 1, 3 and 7 months within each traffic category. Data are averaged (± S.D.) for all panels deployed in winter (monochrome) and summer (dashed). Detailed data are presented in Appendix S6.

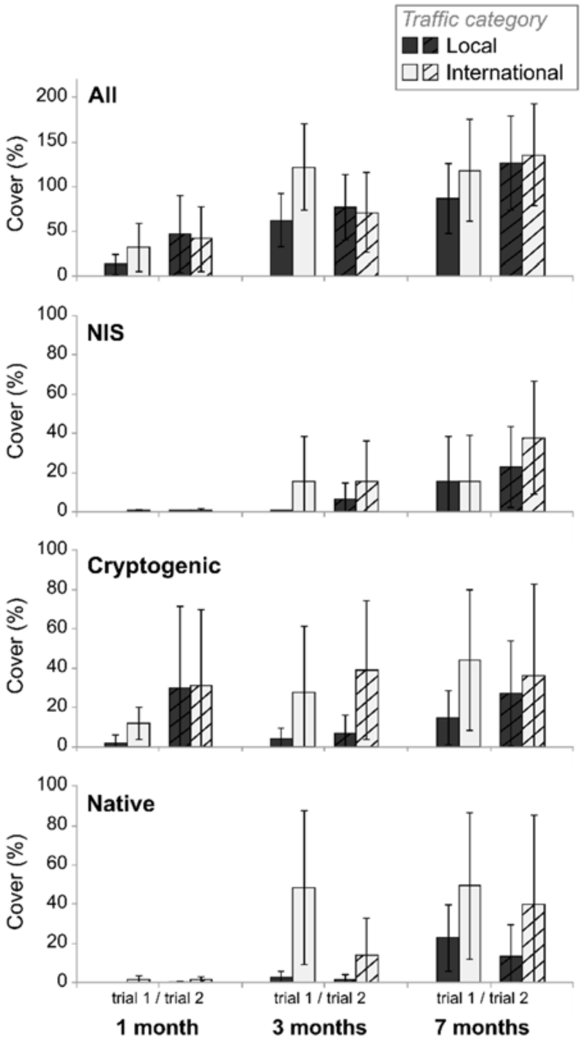

Figure 5. Sessile taxa (total, NIS, cryptogenic and native) cover (%) on the experimental panels collected after 1, 3 and 7 months within each traffic category. Data are averaged (± S.D.) for all panels deployed in winter (monochrome) and summer (dashed).. Detailed data are presented in Appendix S7.

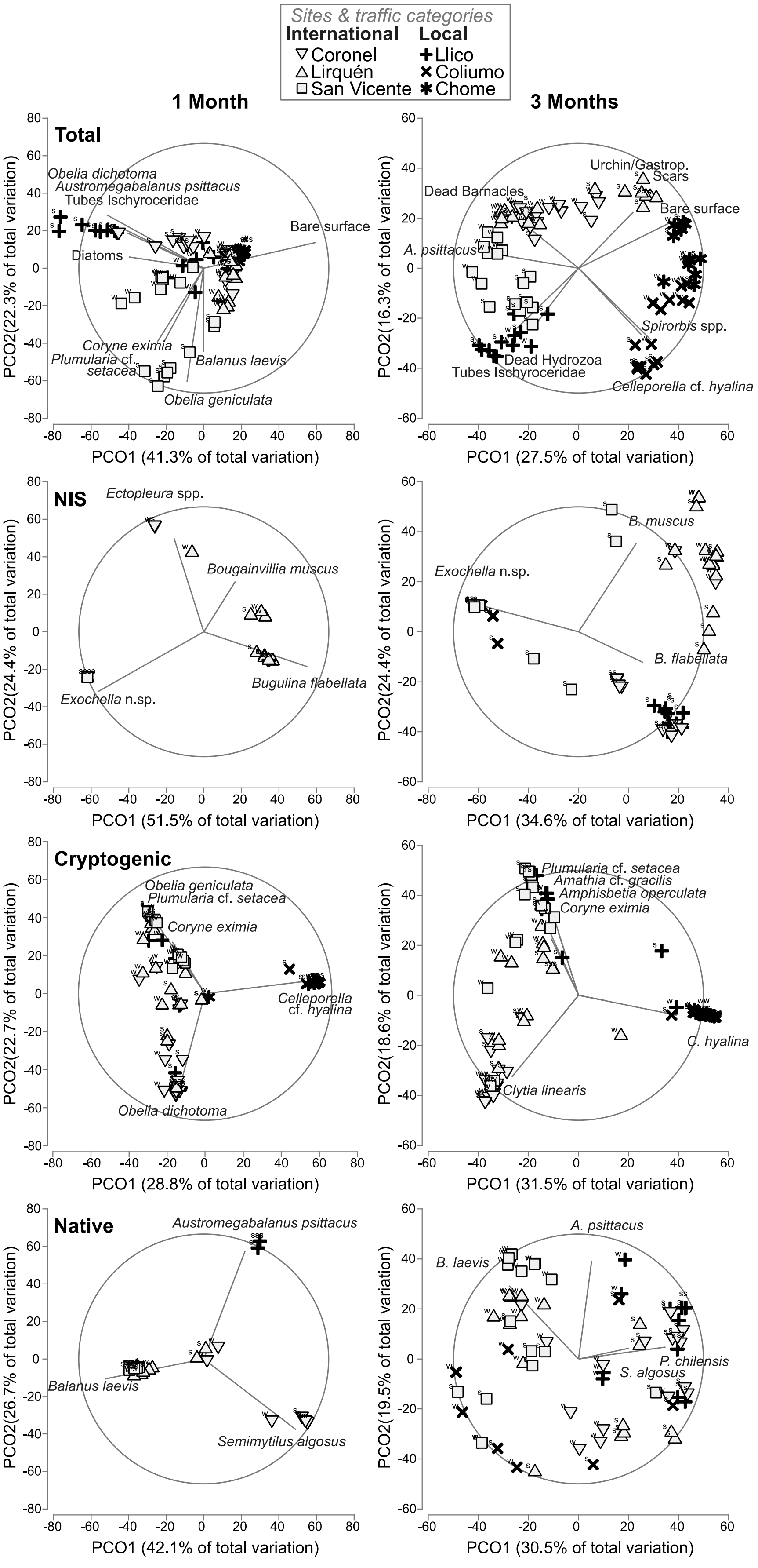

Figure 6. Principal coordinate analyses describing the assemblage structure of all, non-native,

cryptogenic and native species on the panels collected after 1 and 7 months in all study sites for each trial period (^{w}: winter and ^{s}: summer). Vector plots of variables correlated with the PCO axes are indicated within the same panels, with r > 0.6 for all species and r > 0.4 for NIS, cryptogenic and native species. Detailed data (including 3 months-panels) are presented in Appendix S8.

Supplementary information

Appendix S1. Environmental parameters (average ± S.D.) measured in all sites and protocol related to metal concentration measurements

Appendix S2. Complete list of taxa observed during the survey (including rapid assessment surveys and identification on settlement plates)

Appendix S3. List and relative abundance of species identified during rapid assessment surveys.

Appendix S4. List and relative abundance of sessile taxa identified on settlement plates throughout the experiment.

Appendix S5. Detailed number of observations within each site per sampling occasion during the rapid assessment surveys.

Appendix S6. Detailed richness of sessile taxa identified on panels within each site per sampling occasion.

Appendix S7. Detailed abundance of sessile taxa identified on panels within each site per sampling occasion.

Appendix S8. Detailed multivariate structure of sessile taxa identified on panels within each site per sampling occasion.

Appendix S9. Summary of pairwise tests following univariate and multivariate PERMANOVAs.

Supplementary material accompanying:

Non-indigenous species contribute equally to biofouling communities in international vs. local ports in the Biobío region, Chile

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Biofouling

Table S1. Environmental parameters measured in all sites. Data are expressed as average ± S.D, calculated either from 3-4 replicates (pH, %OM and metal concentrations, June 2017) or from 10 min interval measurements during a defined period (March-April 2017 for light, March-June 2017 for temperature).

Traffic	Local			International		
Site	Llico	Chomé	Coliumo	Coronel	San Vicente	Lirquén
pH	8.3 ± 0.1	7.1 ± 0.1	7.4 ± 0.1	8.6 ± 0.1	7.1 ± 0	7.5 ± 0.1
%OM	14.5 ± 9.9	1.5 ± 0.3	3.1 ± 0.7	28 ± 10.1	1.7 ± 0.2	3.2 ± 0.7
Zn (mg kg^{-1})	14.8 ± 2	8.0 ± 1.7	34.0 ± 4.3	73.3 ± 27.6	46.1 ± 10.3	53.2 ± 17.6
Cu (mg kg^{-1})	2.7 ± 0.7	2.4 ± 0.6	5.5 ± 1.8	40.9 ± 19.9	16 ± 4.2	14.4 ± 6.9
Pb (mg kg^{-1})	15.7 ± 2.4	9.3 ± 1.8	17.8 ± 1	34.4 ± 19.7	18.2 ± 4.1	18.7 ± 15.2
V (mg kg^{-1})	15.3 ± 1.6	4.5 ± 1.6	24.3 ± 13.5	37.9 ± 22.3	87.1 ± 13	51.5 ± 16.6
Cr (mg kg^{-1})	11.6 ± 1.3	7.8 ± 5.9	22.3 ± 3.2	34.2 ± 15	54.9 ± 4.4	47.6 ± 32
Mn (mg kg^{-1})	97.8 ± 6.3	21.0 ± 13	267.8 ± 60.1	199 ± 105.5	400.2 ± 116.5	130.4 ± 46.1
Ni (mg kg^{-1})	4.2 ± 1.6	2.8 ± 1.6	6.3 ± 2.4	13.0 ± 8.3	60.8 ± 15.9	13.5 ± 2.8
Hg (mg kg^{-1})	1.9 ± 1.3	15 ± 7.8	2.1 ± 0.3	0.6 ± 0.3	8.5 ± 3.7	41 ± 25.2
Al (mg kg^{-1})	3455 ± 885.9	1669.5 ± 818.5	6646.7 ± 774.3	7428.8 ± 7037	9822.5 ± 5071.2	8463.3 ± 3913.4
Fe (mg kg^{-1})	8185 ± 545.3	4831.5 ± 5642.6	20430 ± 4132.8	20642.5 ± 7946.3	33420 ± 3704.2	22613.3 ± 16321.2
T (°C)	14.3 ± 1	13.8 ± 0.7	14.1 ± 0.9	14.6 ± 0.7	14.1 ± 0.6	14.3 ± 0.8
Light (Lum ft^{-2})	62.9 ± 120.9	62.1 ± 116.5	7.8 ± 29.4	< 0.1	17.4 ± 49.6	4.7 ± 17.4

Method related to sediment conditions: In the laboratory, sediment samples were lyophilized and pulverized. From each samples, 3 replicates were taken as quality control. The pH was measured in 1:2.5 sediment to water ratio using an electrode. The organic matter content (%OM) was determined after calcination at ca. 550ºC. Metal contents (of Al, Cr, Cu, Fe, Hg, Mn, Ni, Pb, V and Zn) were determined using total Ray X reflexion fluorescence (TXRF) analysis (Towett et al. 2013). Initially, the samples were digested by aqua regia solutions (ISO 11466.2002) (1:3 HNO_3/HCl) in a digester brand SCP SCIENCE, model DigiPrep. Samples were then siliconized: ten µL silicone solution in isopropanol were dropped onto the surface of the quartz carriers and dried upon a hot plate (110 °C). Then, ten microliters of each digestion sample containing 1000 µg L^{-1} Ga as internal standard were dropped onto the siliconized carriers. Finally, samples were drought at 80°C before TWRF analyses. The benchtop TXRF system (S4 TStar, Bruker® AXS Microanalysis GmbH, Berlin, Germany) is equipped with a 50 W X-ray tube with molybdenum (Mo) anode and a multilayer monochromator (17.5 keV). The characteristic radiation emitted by elements present in the sample is detected by a silicon drift detector with 60 mm^2 area and an energy resolution < 145 eV, working at 50 kV and 750 µA in air. Measurement live time is 500 s. Qualitative analysis and spectra deconvolution were performed using the spectrometer software (Tstar 1.0, Bruker). Elements are identified automatically referring to the software database. Deconvolution is done with the “super bias optimized fit” function of the SPECTRA software, which calculates it on basis of measured reference spectra present in the software database. The background correction is carried out in a maximum value of 1000 iterations.

Table S2. Complete list of taxa observed during the survey (including rapid assessment surveys and identification on settlement plates)

Chlorophyta
Ulva sp. Linnaeus, 1753

Ochrophyta
Ectocarpales Bessey, 1907
Macrocystis pyrifera (Linnaeus) C.Agardh, 1820 (Linnaeus) C.Agardh, 1820
Bacillariophyceae Haeckel, 1878

Rhodophyta
Antithamnionella ternifolia (J.D.Hooker & Harvey) Lyle, 1922 (J.D.Hooker & Harvey) Lyle, 1922
Crustose Corallinales
Gigartinales Schmitz, 1892
Mazzaella laminarioides (Bory de Saint-Vincent) Fredericq, 1993 (Bory de Saint-Vincent) Fredericq, 1993
Non-clacareous crust
Rhodymeniales Schmitz in Engler, 1892

Ciliophora
Ciliophora indet.

Brachiopoda
Discinisca cf. *tenuis* (Sowerby, 1847)

Porifera
Clathria sp. Schmidt, 1862
Haliclona sp. Grant, 1841
Leucosolenia sp. Bowerbank, 1864

Cnidaria
Amphisbetia operculata (Linnaeus, 1758) (Linnaeus, 1758)
Bougainvillia sp. (*B. muscus* (Allman, 1863)) Lesson, 1830
Clytia linearis (Thorneley, 1900)
Coryne eximia Allman, 1859
Ectopleura crocea (Agassiz, 1862)
Ectopleura larynx (Ellis & Solander, 1786)
Eudendrium sp. Ehrenberg, 1834
Halecium sp. Oken, 1815
Halecium sp2. Oken, 1815
Hydractinia pacifica Hartlaub, 1905
Hydractinia sp. Van Beneden, 1844
Obelia geniculata (Linnaeus, 1758)
Obelia dichotoma (Linnaeus, 1758)
Orthopyxis sp. (*O. mollis*) L. Agassiz, 1862
Phiaella cf. *quadrata*
Plumularia setacea (Linnaeus, 1758)
Sertularellidae Maronna et al., 2016
Cereus herpetodes (McMurrich, 1904)
Anthothoe chilensis (Lesson, 1830)
Phymactis papillosa (Lesson, 1830)
Oulactis concinnata (Drayton in Dana, 1846)
Acontiaria sp. 2 (Haussermann, 2004, PhD thesis)

Bryozoa
Aetea sp. Lamouroux, 1812
Alcyonidiodies mytilii (Dalyell, 1848)
Amathia cf. *gracilis* (Leidy, 1855)
Amathia sp. (*A. imbricata*) (Adams, 1798)
Bugula neritina (Linnaeus, 1758)
Bugulina flabellata (Thompson in Gray, 1848)
Cauloramphus spiniferum (Johnston, 1832)
Celleporella cf. *hyalina* (Linnaeus, 1767)
Electra monostachys (Busk, 1854)
Exochella nov. sp. Jullien, 1888 (status checked by D. Gordon)
Lagenicella variablilis Moyano, 1991
Schyzoporella maulina Moyano, 1983
Scruparia ambigua (d'Orbigny, 1841)
Membranipora isabelleana (d'Orbigny, 1842)
Tricellaria acuelata (d'Orbigny, 1842)
Tubulipora sp. Lamarck, 1816
Turbicellepora sp. Ryland, 1963

Annelida
Terebellidae Johnston, 1846
Spionidae Grube, 1850
Sabellariidae Johnston, 1865

Spirorbis sp. Daudin, 1800

Mollusca

Choromytilus chorus (Molina, 1782)
Aulacomya atra (Molina, 1782)
Semimytilus algosus (Gould, 1850)
Mytilus cf. *edulis* Linnaeus, 1758
Hiatella sp. Bosc, 1801
Petricolinae d'Orbigny, 1840
Bivalvia indet. Linnaeus, 1758
Chiton cumingsii Frembly, 1827
Acanthina monodon (Pallas, 1774)
Tegula spp. Lesson, 1832
Fissurella spp. Bruguière, 1789
Concholepas concholepas (Bruguière, 1789)
Crepipatella fecunda (Lamarck, 1822)
Thecacera darwini Pruvot-Fol, 1950
Phidiana lottini (Lesson, 1831)
Robsonella fontaniana (d'Orbigny, 1834 in 1834-1847)
Xanthochorus cassidiformis (Blainville, 1832)

Crustacea

Austromegabalanus psittacus (Molina, 1788)
Balanus laevis Bruguière, 1789
Notobalanus flosculus (Darwin, 1854)
Verruca laevigata (Sowerby, 1827)
Caprellidae Leach, 1814
Corophiidae Leach, 1814
Ischyroceridae Stebbing, 1899
Rhynchocinetes typus H. Milne Edwards, 1837
Petrolisthes laevigatus (Guérin, 1835)
Homalaspis plana (H. Milne Edwards, 1834)
Metacarcinus edwardsii (Bell, 1835)
Romaleon setosum (Molina, 1782)
Cancer plebejus Poeppig, 1836
Cancer porteri Rathbun, 1930
Taliepus dentalus (H. Milne Edwards, 1834)
Pilumnoides perlatus (Poeppig, 1836)
Neotrypaea uncinata (H. Milne Edwards, 1837)
Caridea Dana, 1852
Alpheidae Rafinesque, 1815

Echinodermata

Heterocucumis godeffroyi (Semper, 1867)
Ophiactis sp. Lütken, 1856
Patiria chilensis (Lutken, 1859)
Asteriidae Gray, 1840
Arbacia dufresnii (Blainville, 1825)
Stichaster striatus Müller & Troschel, 1840
Tetrapygus niger (Molina, 1782)
Meyenaster gelatinosus (Meyen, 1834)

Chordata

Aplidium peruvianum Sanamyan & Schories, 2004
Asterocarpa humilis (Heller, 1878)
Ciona robusta Hoshino & Tokioka, 1967
Corella eumyota Traustedt, 1882
Didemnum studeri Hartmeyer, 1911
Diplosoma listerianum (Milne Edwards, 1841)
Paramolgula sp. Traustedt, 1885
Pyura chilensis Molina, 1782
Bovichtus chilensis Regan, 1913
Genypterus chilensis (Guichenot, 1848)
Hypsoblennius sordidus (Bennett, 1828)
Sicyases sanguineus Müller & Troschel, 1843
Helcogrammoides cunninghami (Smitt, 1898)
Leptonotus blainvilleanus (Eydoux & Gervais, 1837)
Blenniidae Rafinesque, 1810
Prolatilus jugularis (Valenciennes, 1833)
Otaria flavescens Shaw, 1800

Table S3. List and relative abundance of species identified during rapid assessment surveys performed around experimental units within each site at two dates (November 2016 & June 2017). Relative abundances were estimated using the SACFOR scale: ******: Superabundant, *****: Abundant, ****: Common, ***: Frequent, **: Occasional, *: Rare. Status and main trophic group (TG) are indicated: carnivores (C), suspension feeders (SF), herbivores (G), primary producers (PP)

			Traffic	Local						International					
			Site	Llico		Chome		Coliumo		Coronel		San Vicente		Lirquén	
Taxon	Status	TG	Date	Nov16	Apr17	Nov16	Apr17	Nov16	Apr17	Nov16	Apr17	Nov16	Apr17	Nov16	Apr17
Chlorophyta															
Ulva sp.	Unassigned	PP		**	****	*		**	*				*		
Ochrophyta															
Ectocarpales	Unassigned	PP													
Macrocystis pyrifera	Native	PP			**	*	*								
Rhodophyta															
Antithamnionella ternifolia	NIS	PP										****	****		
Crustose Corallinales	Unassigned	PP				******	******	****	*****						
Gigartinales	Unassigned	PP		**				**	*			***	**		
Mazaella laminiorides	Native	PP			**										
Non-clacareous crust	Unassigned	PP				***	***								
Rhodymeniales	Unassigned	PP			*	**						***	*		
Porifera															
Clathria sp.	Unassigned	SF										*		*	*
Haliclona sp.	Unassigned	SF													
Leucosolenia sp.	Unassigned	SF										*	*	*	*
Cnidaria															
Amphisbetia operculata	Cryptogenic	SF		*								****	*****	*	
Bougainvillia sp. (*B. muscus*)	NIS	SF								***	***	*	*	**	**
Clytia linearis	Cryptogenic	SF								*****	**	*****	**	*	*
Coryne eximia	Cryptogenic	SF		***							*		***	*	
Ectopleura crocea	NIS	SF								*****	****	*	*	*****	**
Ectopleura larynx	NIS	SF								*	*				
Eudendrium sp.	Unassigned	SF										*			
Halecium sp.	Unassigned	SF										*	*		
Hydractinia pacifica	Native	SF							*		*	*	*		
Hydractinia sp.	NIS	SF										**	**		
Obelia spp.	Cryptogenic	SF		*	**				*	*****	****	*	***	*	*
Orthopyxis sp. (O. *mollis*)	Native	SF											*		
Phiaella cf. *quadrata*	Cryptogenic	SF													*
Plumularia setacea	Cryptogenic	SF		*****	*****							****	*****		
Sertularellidae	Unassigned	SF			*										
Cereus herpetodes	Native	C		****	**							*			

Anthothoe chilensis	Native	C											*****	****
Phymactis papillosa	Native	C						*					*	*
Oulactis concinnata	Native	C							****	*****				
Acontiaria sp. 2	Native	C							*****	*****				
Bryozoa														
Aetea sp.	Unassigned	SF										*		
Alcyonidiodies mytilii	Cryptogenic	SF	**	*				**	**	**	*	***		*
Amathia cf. *gracilis*	Cryptogenic	SF		*							*	**	**	*
Bugula neritina	NIS	SF						*						*
Bugulina flabellata	NIS	SF	*	****				**	*	*		*	**	***
Cauloramphus spiniferum	Cryptogenic	SF				*								
Celleporella cf. *hyalina*	Cryptogenic	SF			*	*	**	**					*	
Electra monostachys	Cryptogenic	SF							*	**				
Exochella n. sp.	NIS	SF						****				*		
Lagenicella variablilis	Native	SF	*											
Schyzoporella maulina	Native	SF						*						
Scruparia ambigua	Native	SF										*		
Membranipora isabelleana	Native	SF		*										
Tricellaria acuelata	Native	SF		*										
Annelida														
Sabellariidae	Unassigned	SF												*
Spirorbis sp	Cryptogenic	SF			**	**	****	****						
Mollusca														
Choromytilus chorus	Native	SF	****	**				*	*****	*****			****	**
Aulacomya atra	Native	SF	**	*					***	***	*		******	*****
Semimytilus algosus	Native	SF	*	****					*****	*****	*			
Mytilus cf. *edulis*	Cryptogenic	SF	****	*****				**	**	*			*****	*****
Hiatella sp.	Unassigned	SF	*						*					
Chiton cumingsii	Native	H				*		*						
Acanthina monodon	Native	C						*						
Tegula spp.	Native	H			****	****	****	****						*
Fissurella spp.	Native	H			*	***					*	**		
Concholepas concholepas	Native	C										*		
Crepipatella fecunda	Native	H	**	***			*	****	*****	*****	****	*****	****	*****
Thecacera darwini	Native	C	*	**			*	**			*	*	***	***
Phidiana lottini	Native	C	**	*					*	*		*	***	
Robsonella fontaniana	Native	C	*										***	
Xanthochorus cassidiformis	Native	C												*
Crustacea														

Austromegabalanus psittacus	Native	SF	*****	****			****	****	*	**	******	*****	*	*
Balanus laevis	Native	SF					**	****	***	*	***	***	****	**
Notobalanus flosculus	Native	SF								*	***	**		
Verruca laevigata	Native	SF								*				
Ischyroceridae (Tubes)	Unassigned	SF	*****	******										
Caprellidae	Unassigned	C	******	******										
Rhynchocinetes typus	Native	C			****	****	***		*		*			
Petrolisthes laevigatus	Native	SF			*						*	*		**
Homalaspis plana	Native	C							*					
Metacarcinus edwardsii	Native	C					*		*					
Romaleon setosum	Native	C	*	**			****	**	***	***	***	**	**	***
Cancer plebejus	Native	C		**			****	****			***	***	***	*
Cancer porteri	Native	C												
Taliepus dentalus	Native	C	*	*			*	**			*	**		**
Pilumnoides perlatus	Native	C		*										
Neotrypaea uncinata	Native	C											**	
Caridea	Native	C											*	
Alpheidae	Native	C								*	*	**		
Echinodermata														
Heterocucumis godeffroyi	Native	SF											***	*****
Ophiactis sp.	Unassigned	SF	*						******	******			**	
Patiria chilensis	Native	C			*	*	****	**					***	**
Asteriidae	Unassigned	C											**	**
Arbacia dufresnii	Native	C				*							****	*****
Stichaster striatus	Native	C			***	****	*	*						
Tetrapygus niger	Native	C			*****	*****							***	***
Meyenaster gelatinosus	Native	C				*								
Chordata														
Aplidium peruvianum	Native	SF	*											
Asterocarpa humilis	NIS	SF	*	*				*						*
Ciona robusta	NIS	SF							*	*	*	*		*
Corella eumyota	Cryptogenic	SF	*	*	*			*	**		*	*	*	
Didemnum studeri	Native	SF	*											
Diplosoma listerianum	NIS	SF	****	****				*						*
Paramolgula sp.	Native	SF	**	*					**	*				*
Pyura chilensis	Native	SF	******	****				**	***	****	****	****	****	****
Bovichtus chilensis	Native	C			*									
Genypterus chilensis	Native	C			***									
Hypsoblennius sordidus	Native	C	****	**	*			**			****	***	*	**

Sicyases sanguineus	Native	C			*	*	*	*						
Helcogrammoides cunninghami	Native	C				*					*	**		
Leptonotus blainvilleanus	Native	C	**											*
Blenniidae	Native	C												
Prolatilus jugularis	Native	C				*								
Otaria flavescens	Native	C	*		**		*		*	*	*			

Table S4. List and relative abundance of sessile taxa identified on settlement plates throughout the experiment. Relative abundances are expressed according to occurrences: ******: 80-100%, *****: 60-79%, ****: 40-59%, ***: 20-39%, **: 5-19%, *: 0-4%. Status are indicated.

Traffic		Local			International		
Site		Llico	Chome	Coliumo	Coronel	San V.	Lirquén
Taxon	Status						
Chlorophyta							
Ulva sp.	Unassigned		**	**		*	
Ochrophyta							
Ectocarpales	Unassigned	*				**	
Macrocystis pyrifera							
Bacillariophyceae	Unassigned	***			**	*****	***
Rhodophyta							
Antithamnionella ternifolia	Unassigned					****	
Crustose Corallinales	Unassigned		******	*****			
Gigartinales							
Mazzaella laminarioides	Native	**					
Non-clacareous crust	Unassigned	*	****	**			
Rhodymeniales	Unassigned		**	**		**	
Ciliophora							
Ciliophora indet.	Unassigned	**			*	*	
Brachiopoda							
Discinisca cf. *tenuis*	Unassigned				**		*
Porifera							
Leucosolenia sp.	Unassigned	*		*		**	***
Cnidaria							
Amphisbetia operculata	Cryptogenic					****	
Bougainvillia sp. (*B. muscus*)	NIS	*			**	*	*****
Clytia linearis	Cryptogenic	**			*****	***	*****
Coryne eximia	Cryptogenic	*			**	*****	**
Ectopleura spp. (E. crocea & E. Larynx)	NIS				****		
Eudendrium sp.	Unassigned					***	
Halecium sp.	Unassigned						*
Halecium sp2.	Unassigned					*	
Hydractinia pacifica	Native				**		
Hydractinia sp.	NIS					*	**
Obelia geniculata	Cryptogenic	**			**	***	**
Obelia dichotoma	Cryptogenic	****		*	****		**
Orthopyxis sp. (O. *mollis*)	Native					*	
Phiaella cf. *quadrata*	Cryptogenic	*				**	***
Plumularia setacea	Cryptogenic	****		*		******	
Cereus herpetodes	Native	*					
Acontiaria sp. 2	Native				****		
Bryozoa							
Alcyonidiodies mytilii	Cryptogenic	*			**	**	**
Amathia cf. *gracilis*	Cryptogenic	**		**	*	****	***
Amathia sp. (*A. imbricata*)	NIS				*		
Bugula neritina	NIS			*			*
Bugulina flabellata	NIS	****		*	***	**	*****
Cauloramphus spiniferum	Cryptogenic		**				
Celleporella cf. *hyalina*	Cryptogenic	**	****	******			*
Electra monostachys	Cryptogenic				*		
Exochella n. sp.	NIS		**	****		***	
Lagenicella variablilis	Native	**	*				
Schyzoporella maulina	Native			**			
Scruparia ambigua	Native	*				**	
Tricellaria acuelata	Native	**					
Tubulipora sp.	Unassigned	*					
Turbicellepora sp.	Unassigned		*				

Annelida							
Terebellidae/Spionidae (Tubes)	Unassigned	**		*	**	**	**
Spirorbis sp.	Unassigned		******	******			
Mollusca							
Choromytilus chorus	Native	*			**		**
Aulacomya atra	Native	*		**	***	*	**
Semimytilus algosus	Native	***			*****	*	*
Mytilus cf. *edulis*	Cryptogenic	**		**	*	*	**
Petricolinae	Unassigned				*		
Bivalvia	Unassigned	*			*		*
Crepipatella fecunda	Native	**		***	****	**	******
Crustacea							
Austromegabalanus psittacus	Native	*****		***	***	****	**
Balanus laevis	Native	*		****	***	*****	*****
Verruca laevigata	Native				*		
Ischyroceridae (Tubes)	Unassigned	******					*
Chordata							
Asterocarpa humilis	NIS	**					*
Ciona robusta	NIS				**	*	**
Corella eumyota	Cryptogenic	***			*	*	
Didemnidae	Unassigned						*
Diplosoma listerianum	NIS	****		*			
Paramolgula sp.	Native	***			**	*	***
Pyura chilensis	Native	****		**	*****	***	****
Egg masses	Unassigned	**		**	***	**	*

Supplement S5. Detailed number of observations within each site per sampling occasion during the rapid assessment surveys.

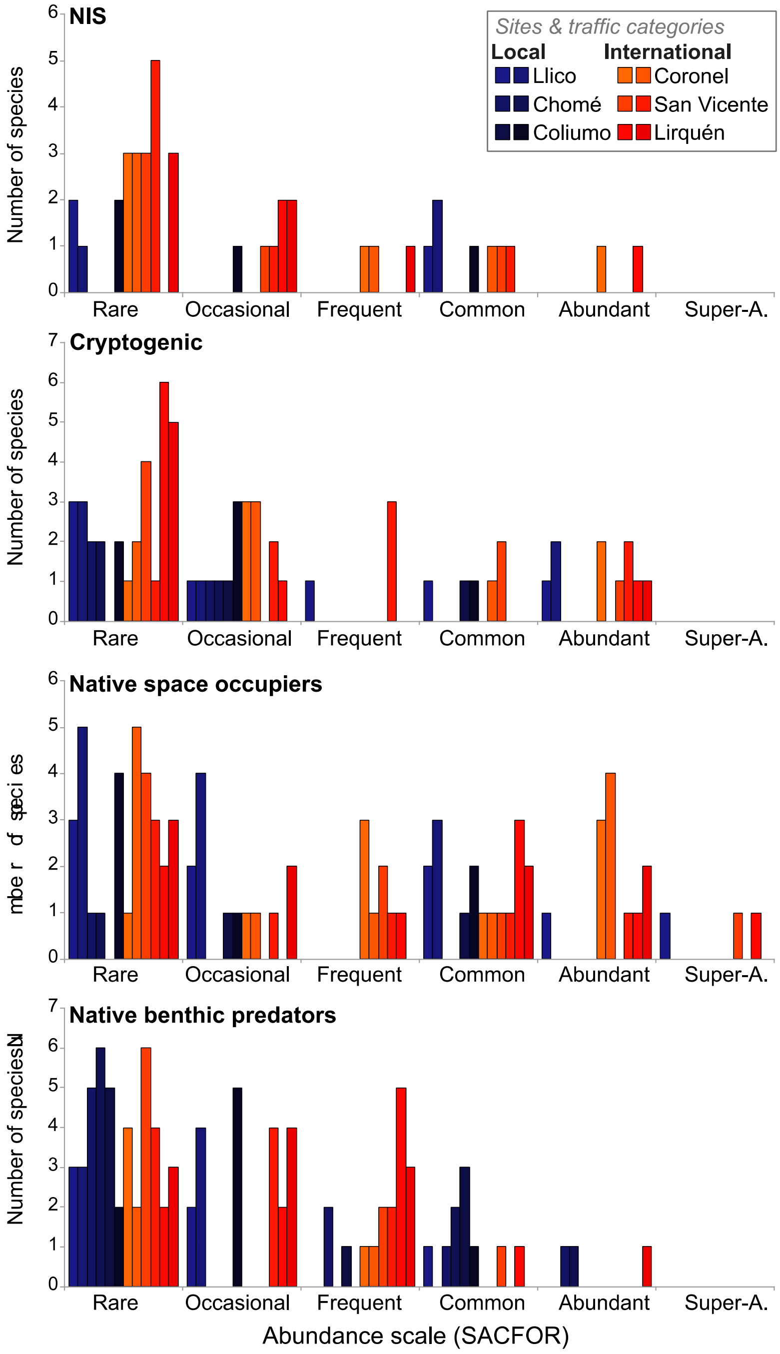

Figure S5. Number of NIS, cryptogenic species, native space occupiers and benthic predators encountered within surrounding communities at each study site (international ports in pale patterns, local piers in dark). Data are presented as the number of reports (taxa) within each category of abundance (SACFOR) during winter (monochrome) and summer (dashed) trials.

Supplement S6. Detailed richness of sessile taxa identified on panels within each site per sampling occasion.

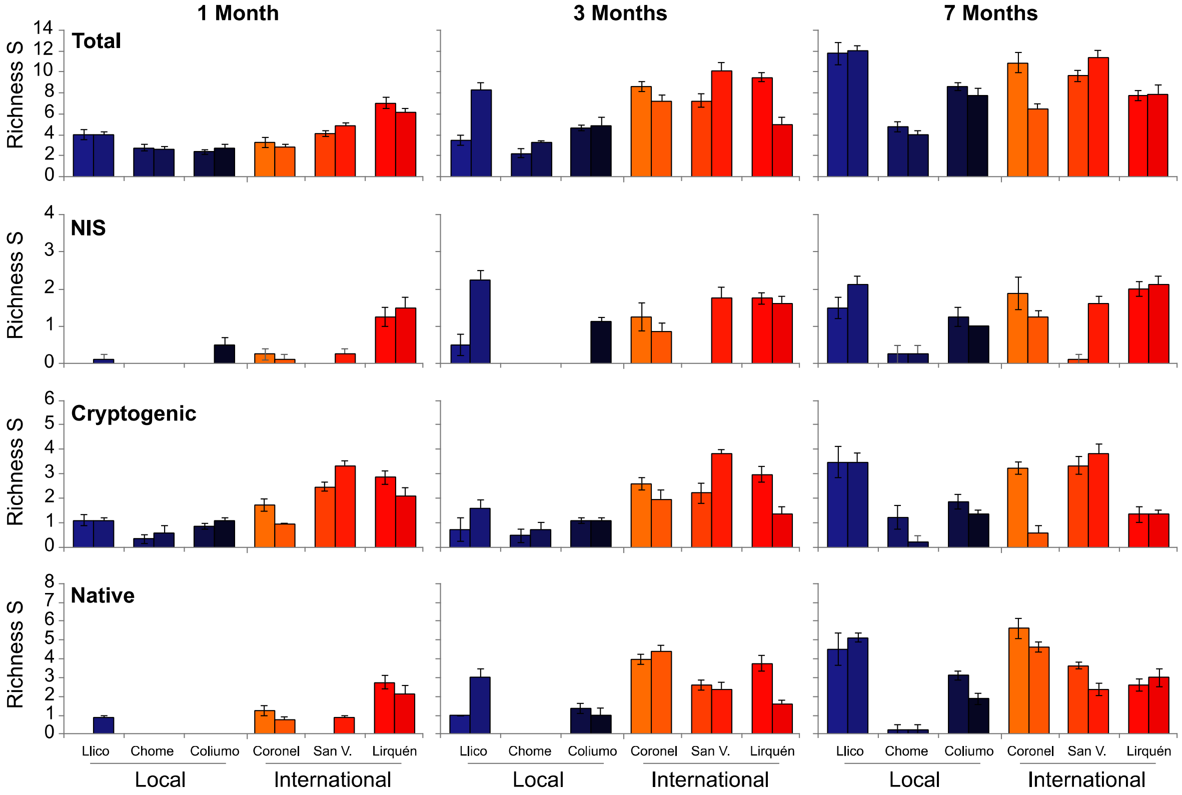

Figure S6. Sessile taxa (total, NIS, cryptogenic and native) richness on the experimental panels collected after 1, 3 and 7 months in all study sites at each sampling occasion (± S.E.). Legend is the same as in figure S5.

Supplement S7. Detailed abundance of sessile taxa identified on panels within each site per sampling occasion.

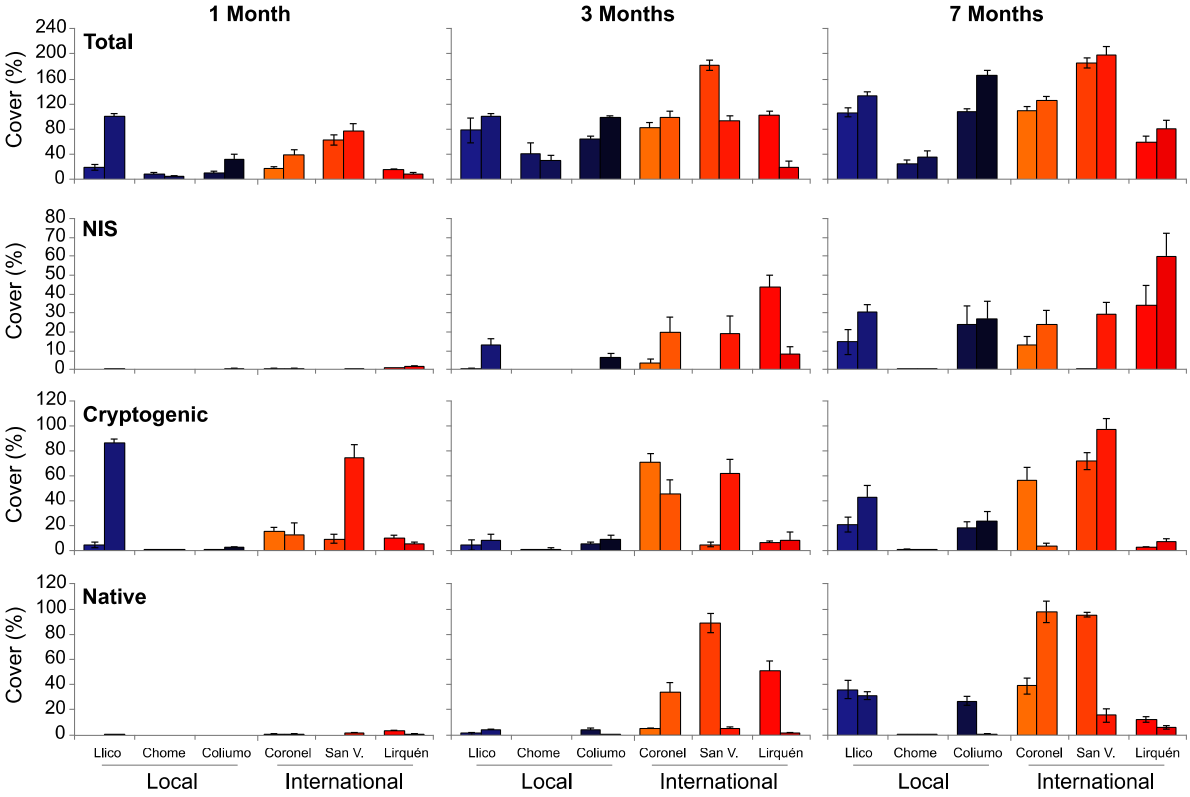

Figure S7. Sessile taxa (total, NIS, cryptogenic and native) cover (%) on the experimental panels collected after 1, 3 and 7 months in all study sites at each sampling occasion (± S.E.). Legend is the same as in figure S5.

Supplement S8. Detailed multivariate structure of sessile taxa identified on panels within each site per sampling occasion.

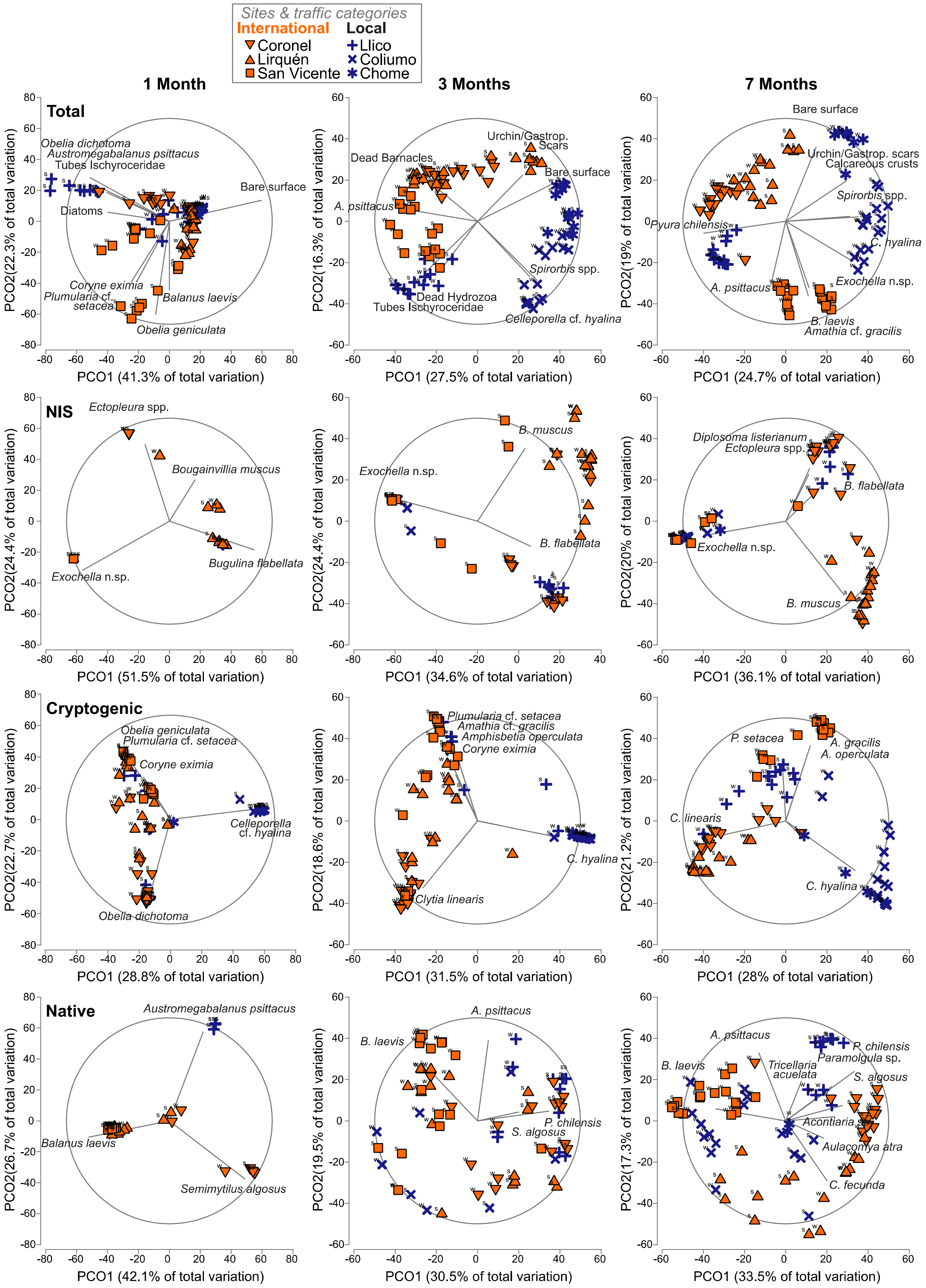

Figure S8. Principal coordinate analyses describing the assemblage structure of all, non-native, cryptogenic and native species on the panels collected after 1, 3 and 7 months in all study sites for each trial (^{w}: winter and ^{s}: summer). Vector plots of variables correlated with the PCO axes are indicated within the same panels, with r > 0.6 for all species and r > 0.4 for NIS, cryptogenic and native species.

Supplement S9. Summary of pairwise tests following univariate and multivariate PERMANOVAs.

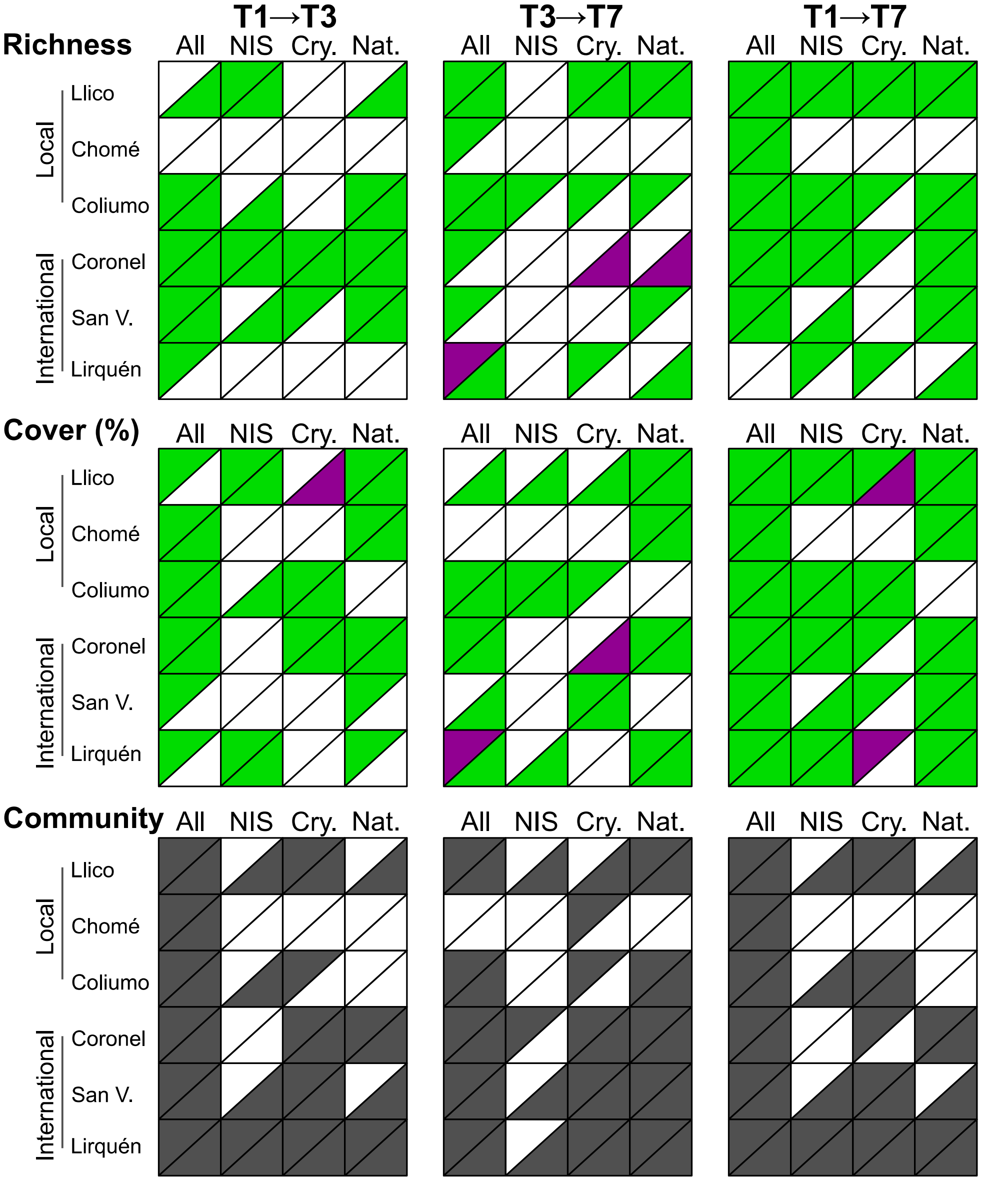

Figure S9. Summary of pairwise tests following univariate and multivariate PERMANOVAs testing for differences in sessile taxa richness, cover and community structure. Tests are presented for all, non-indigenous (NIS), cryptogenic (Cry.) and native (Nat.) species, separately. Results are illustrated as changes between levels of the factor ‘time’ (panels collected after 1, 3 or 7 months) within each site and at each sampling occasion (first and second trials are illustrated within left and right corners, respectively. Neutral (white), positive (pale green), negative (purple) and non-directional (grey) changes were considered at α = 0.05 (based on Monte Carlo procedure).