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

4
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22
23 **Running headline:** Biofouling in international vs. local ports
24
25

26 **Abstract**

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

39

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

42

43 **Introduction**

44 Biological introductions, characterized by sudden changes of the natural distribution of
45 species due to human-mediated transports, are among the most pervasive global changes
46 (Simberloff et al. 2013). Human-mediated transport is indeed breaking biogeographic
47 boundaries and contribute to the homogenization of ecosystems at global scale (Villéger et al.
48 2011; Capinha et al. 2015). In the marine realm, most of the attempts to eradicate established
49 NIS have failed (Ojaveer et al. 2014; Ojaveer et al. 2015). This poor success in management
50 and eradication is likely due to a combination of factors, including notably late detection, high
51 dispersal abilities of many NIS and a large diversity of spreading vectors (Bax et al. 2003).

52 Regarding the need for early detection, surveillance should target with a high priority
53 the points of entry of NIS, in particular ports and aquaculture facilities in the marine realm.
54 Shipping and leisure boating (ballast water and sediment, hull biofouling) as well as aquaculture
55 are indeed the most critical contributors to invasion in coastal ecosystems at a global scale
56 (Drake and Lodge 2007; Molnar et al. 2008; Clarke Murray et al. 2011; Sylvester et al. 2011;
57 Nunes et al. 2014). Current international shipping hauls almost 90 % of globally traded goods
58 and shipping intensity is consistently increasing (Kaluza et al. 2010; Tournadre 2014). Tthe
59 longstanding recognition of the importance of ballast water and associated sediments in NIS
60 (including harmful organisms and pathogens) transportation has led to the International
61 Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM
62 Convention) which entered into force on September 2017. In contrast,, no international
63 regulation regarding hull fouling organisms – such as Craft Risk Management Standard for
64 Biofouling in New Zealand (<http://www.mpi.govt.nz/>) or Biofouling Management Plan in
65 California (<http://www.slc.ca.gov/>) – has been set up yet. However, in 2011, the IMO adopted
66 Guidelines for the Control and Management of Ships' Biofouling (MEPC 2011, 2013) and is
67 currently preparing a new project (GloFouling) to tackle this major issue. Submerged ship hulls

68 can indeed be colonized by diverse sessile and encrusting organisms that may dislodge or
69 reproduce in the destination port-of-call during the ship stopover. Besides, many of these sessile
70 species are habitat-builders for a myriad of invertebrates (Sellheim et al. 2010; Thomsen et al.
71 2014; Leclerc and Viard 2018). As such, hull biofouling is a major pathway of introduction and
72 spread of NIS, through both direct and facilitative processes (Bax et al. 2003; Drake and Lodge
73 2007; Sylvester et al. 2011; Moser et al. 2016; Pinochet et al. 2017).

74 Following their primary introduction, the rapid spread of marine NIS is favored by
75 coastal hardening (Mineur et al. 2012; Airoidi et al. 2015). The increasing rate of coastal
76 artificial structures, coined ‘ocean sprawl’ (sensu Duarte et al. 2012), has emerged as protection
77 against environmental perturbations (rising sea level, erosion, storms and flooding) and to
78 support the development of human activities (shipping, urbanization, aquaculture, energy
79 extraction and recreation) (Firth et al. 2016). Beyond habitat-degradation and fragmentation,
80 coastal artificial structures provide novel habitats for colonization by various species – among
81 which NIS prevail as compared to neighboring natural reefs – and alter connectivity and local
82 species pools (Mineur et al. 2012; Dafforn et al. 2015; Bishop et al. 2017). Interestingly ocean
83 sprawls with shipping and boating are jointly enhancing colonization and propagule pressures
84 (sensu Lockwood et al. 2009): besides, being points of entry, ports and marinas are “invasion
85 hubs” (Airoidi et al. 2015). NIS establishment, spread and impacts are however context
86 dependent, relying on a complex combination of invader traits, resource availability, abiotic
87 conditions, and community structure of the invaded habitat (Fridley et al. 2007; Thomsen et al.
88 2014). The infrastructures and logistics associated to the maritime activities alter environmental
89 factors such as temperature, salinity, hydrodynamics, sediment resuspension, contaminants
90 (nutrients, organic matter and metals) and incident light – all susceptible to affect fouling
91 communities and potentially select tolerant NIS, from species-specific settlement to biotic
92 interactions (Bulleri and Chapman 2010; Dafforn et al. 2015; Johnson et al. 2017; Lawes et al.

93 2017). While environmental conditions artificially created may be unique at the local scale,
94 they tend to be similar across distant locations, hence removing the potential mismatch between
95 port-of-calls and contributing to the biotic homogenization within and among oceans (Streftaris
96 et al. 2005; Seebens et al. 2013; Seebens et al. 2016).

97 In SE Pacific and more specifically along the Chilean coasts, particular attention has
98 been paid to the numerous NIS introduced for aquaculture purposes. However, the scarcity of
99 dedicated surveys for the presence of NIS in the marine environment is noteworthy (Castilla et
100 al. 2005; Castilla and Neill 2009; Pinochet et al. 2017; Villaseñor-Parada et al. 2017). Past and
101 recent research have suggested that that very few species have successfully established along
102 SE Pacific coasts as compared to other regions in the world (Castilla and Neill 2009; Villaseñor-
103 Parada et al. 2017).. Among possible causes, unique oceanographic properties characterized by
104 dissolved-oxygen deficit in coastal waters together with cold north-flowing current north of
105 42°S and low temperature and salinity south of 42°S, had been proposed as a mechanism
106 enabling substantial “abiotic resistance” to regional NIS establishment and spread (Castilla and
107 Neill 2009). Recent modelling approaches regarding international trades also suggested that
108 this environmental mismatch between source regions and SE Pacific coastline should reduce
109 invasion risks (Keller et al. 2011; Seebens et al. 2013). Paradoxically, those barriers are elusive
110 and may change over time (e.g. seasonality and stochasticity of upwellings): they could be
111 overwhelmed locally by expanding international traffic, which is increasing colonization and
112 propagule pressures (Melbourne et al. 2007; Lockwood et al. 2009; Simberloff 2009; Sylvester
113 et al. 2011; Miller and Ruiz 2014) and by “ocean sprawl” which is increasing the NIS
114 establishment and spread probabilities (Streftaris et al. 2005; Mineur et al. 2012; Seebens et al.
115 2013; Airoldi et al. 2015).

116 Over the last decade, the number and extent of artificial structures have dramatically
117 expanded along the Chilean coastline in order to protect inhabited shores from storms and

118 tsunamis and to promote economic development, through shipping trades, mining and
119 aquaculture (Aguilera et al. 2014; Aguilera 2017). Between 18° and 41°S (3000 km of linear
120 coastline), these infrastructures account approximately to 200 km, among which 70% are
121 concentrated in northern (18-22°S and 23°S), central (ca. 33°S) and southern (36°S and 41°S)
122 regions (Aguilera 2017). In parallel, Chilean ports receive international ships from diverse
123 regions (mainly Asia, North America, Europe and Australasia). Shipping pathways have been
124 assumed to be responsible for the introduction of 30-38 % of the NIS established in Chile
125 (Castilla and Neill 2009). Nonetheless, the legal regulation of marine NIS surveillance has just
126 started to be implemented in Chile Chilean authorities have enacted a Legal Procedure
127 DIRECTEMAR A-51/002, October, 14th, 2002, based on the Ballast Water Convention, which
128 is still under revision for being concordant with the IMO statements (Lloyd's Register 2014).
129 However, there are currently no regulation, policies or national strategic plan to target
130 biofouling in relation with international, regional or local shipping. In addition, research
131 regarding NIS in Chile is generally focused on the ecology of some already-described invaders
132 and there has been – to our knowledge – no attempt to implement dedicated NIS surveys neither
133 in artificial nor in natural environments (Villaseñor-Parada et al. 2017). In this context there are
134 serious knowledge gaps and uncertainties regarding the extent of NIS contribution to the fouling
135 communities established on hard substrates (natural and artificial). Such data are urgently
136 needed for accurate reports of NIS presence, distribution and establishment of baselines in order
137 to implement dedicated surveillance programs of the biofouling communities in Chilean ports
138 and nearby artificial structures. The present study aimed to investigate the diversity and
139 structure of hard bottom communities, and specifically identify the non-indigenous components
140 of those communities in South-Central Chile. We targeted both international and local ports,
141 the former being primary entry points for introduction mediated by international maritime
142 traffic while the latter being potential destination of NIS primarily introduced. To this end, a

143 series of standardized settlement panels were deployed twice (summer and winter) in 3
144 international and 3 local ports, before being randomly collected after 1, 3 and 7 months. In
145 parallel, rapid assessment visual surveys were conducted to compare the established
146 communities in the neighboring substrates of the panels. Finally, various abiotic parameters
147 (metal and organic matter concentration, light, and temperature) were measured to check for
148 potential confounding effects when comparing the biofouling communities in international vs.
149 local ports. We hypothesised that NIS richness and abundance measured on 1 month-old panels
150 – fouled by early settlers thus being proxies of the colonization and propagule pressures – would
151 be higher in international than in local ports. After 3 and 7 months of developments, patterns in
152 diversity and assemblage structure were expected to differ, with potential greater contributions
153 of NIS and cryptogenic species in international as compared to local ports.

154

155 **Methods**

156 *Sampling design*

157 The study was performed along approximately 100 km of coastline in the BíoBío region
158 (Chile) between August 2016 and October 2017. Within the region, a total of six fully marine
159 sites (ports) were sorted according to their shipping traffic (Fig. 1): three international ports
160 (cargo and tanker moorings) located in Coronel (37.0304°S, 73.1540°W), San Vicente
161 (36.7591°S, 73.1551°W), and Lirquén (36.7094°S, 72.9829°W) and three local ports (fishing
162 barges and craft moorings) located in Llico (37.1541°S, 73.5690°W), Chome (36.7735°S,
163 73.2137°W) and Coliumo (36.5377°S, 72.9571°W) were selected. In 2016, about 10 to 107 and
164 64 - 162 fishing crafts (3.5-18 m length) potentially berthed – some of which permanently – in
165 local and international ports, respectively
166 (<http://webmail.sernapesca.cl/sernapesca/guest/web/cons>

167 *Rpaem.asp#compuestas*). During the same year, between 250 and 427 foreign commercial ships
168 (20 - 217 national ships) berthed in the selected international ports, mainly originating from
169 Asia, South America, North America, Europe, and Australasia (according to import-export
170 data, web.directemar.cl/estadisticas/puertos/default.htm).

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

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

207

208 ***Data collection***

209 *Environmental parameters*

210 Environmental conditions of each site were assessed from a series of parameters:
211 incident light, temperature, sediment pH, sediment organic matter content and concentration of
212 different metals. Temperature (°C) and illuminance (Lum ft⁻²) were measured *in situ* at 10 min
213 intervals between March and June 2017 using data loggers (onset HOBO® data-loggers
214 Pendant Temp-Light, Onset Computer Corporation) deployed within each site. Owing to the
215 biofouling undergone by the loggers, light data gathered more than 4 weeks after installation

216 were not considered. Sediment parameters were determined from superficial sediment (first cm)
217 samples (n = 3-4) collected below experimental units in June 2017 and stored at – 20°C until
218 analyses. In the laboratory, sediment samples were lyophilized and pulverized. The pH was
219 measured in 1:2.5 sediment to water ratio using an electrode. The organic matter content
220 (%OM) was determined after calcination at ca. 550°C. Metal contents were determined using
221 total Ray X reflexion fluorescence analysis (Towett et al. 2013, details provided in Appendix
222 S1).

223

224 *Diversity and community structure assays*

225 In the laboratory, panels were removed from their bags, cleared from cable tiles and left
226 with all remaining bag contents in seawater tanks until sessile fauna returned to their natural,
227 untense state. Sessile taxa (mostly fauna, see Results) were identified under a dissecting
228 microscope and their abundances were assessed using cover. To avoid edge effects in their
229 distribution, a 15 mm perimeter was excluded from analysis, giving a 120 × 120 mm working
230 area. Species cover was estimated under 100 random intersection points out of 169 created
231 between evenly spaced lengths of string of 150 × 150 mm quadrat. Any species identified out
232 of these intersection points was given a cover of 0.5 %. Species layering was taken into account,
233 therefore the total cover frequently exceeded 100%. Following sessile taxa identification,
234 panels were washed through a 500 µm mesh sieve to separate mobile fauna, ultimately stored
235 in EtOH 95%. All specimens were identified at the lowest taxonomic level possible (generally
236 species) by the same observer (JCL) and occasionally verified by external experts (see
237 acknowledgments). Throughout the survey, voucher specimens were collected, dissected when
238 appropriate and preserved in 95% EtOH in order to fill in the local reference collection (for
239 further morphological and/or molecular examination). When appropriate, some specimens were
240 maintained in tanks with bubbling air stone and filled with seawater at ambient temperature

241 until they developed diagnostic size and/or characteristics. Molecular barcoding (using COI)
242 was also employed whenever necessary and possible. While this approach did not always enable
243 identification at the species level (e.g. because of the absence of reliable molecular reference
244 data), it allowed to compare juveniles and adults, the latter being identified (by JCL) upon
245 morphological characteristics and assign juveniles to identified adults. The identified specimens
246 were categorized as ‘native’, ‘non-indigenous’ (NIS), ‘cryptogenic’ or ‘unassigned’ according
247 to the literature (e.g. Moyano 1983; Galea 2007; Turon et al. 2016) and public databases
248 (EASIN, WORMS/WRIMS, Pagad et al. 2016). The cryptogenic species, from
249 unknown/uncertain origin (sensu Carlton 1996), found in this study displayed a cosmopolitan
250 distribution and were potentially non-indigenous to the study area. Cryptogenic and NIS, both
251 candidates for further introduction and spread, were thus considered in analyses (Dafforn et al.
252 2009; Leclerc and Viard 2018). When appropriate, taxa were also sorted according to their main
253 function within the food web (carnivores, suspension-deposit-feeders, herbivores).

254

255 *Statistical analyses*

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

265 was based on a Euclidean distance matrix generated from normalized data of the active
266 variables of the PCA.

267 Patterns in species richness, abundance and community structure of sessile taxa were
268 examined with a four-way design using PERMANOVAs with 4999 permutations. Factors were
269 ‘traffic’ (fixed, 2 levels: international and local ports), ‘starting deployment period’ (‘starting
270 date’, random, 2 levels: August 2016 and March 2017), ‘age of the settlement panel at the time
271 of collection’ (‘age’, fixed, 3 levels: 1, 3 and 7 months) and ‘site’ (random, nested within
272 traffic). A few experimental units were lost over the course of the experiment, therefore the
273 corresponding term (plot) was not included in analyses. Nonetheless, there were between 4 and
274 8 replicate panels available for each combination of Site (Traffic) \times Starting date \times Age.
275 Univariate analyses were based on Euclidian distance matrices whereas multivariate analyses
276 were based on Bray-Curtis similarity matrices generated from either raw or transformed data.
277 In order to down-weight the importance of most abundant species (and homogenize multivariate
278 dispersion), multivariate data were always square root-transformed. The homogeneity in
279 univariate or multivariate dispersion was checked among the levels of the lowest interaction
280 term Site (Traffic) \times Starting date \times Age using PERMDISP (Anderson et al. 2008). No
281 transformation allowed homoscedasticity to be achieved in univariate data, except in one case
282 (total richness). Given the balance of the design and the large number of samples, univariate
283 PERMANOVAs (analogous to ANOVAs) were considered robust enough to cope with this
284 issue and were run on untransformed data (Underwood 1997). As for multivariate data, samples
285 were also ordinated using principal coordinate (PCO) analyses to support PERMANOVA
286 results (Anderson et al. 2008). When appropriate, PERMANOVAs were followed by pairwise
287 comparisons and *P*-values were estimated using Monte Carlo procedure. Finally, semi-
288 quantitative data resulting from rapid assessment surveys on surrounding communities were
289 analyzed graphically, by considering the number of reports (i.e. taxa) within each category of

290 abundances at both sampling periods. These analyses were presented separately for non-
291 indigenous and cryptogenic species as well as native space occupiers and potential predators.

292 Statistical analyses were performed either on all variables combined (including unassigned
293 taxa as well as abiotic data in the case of community structure: bare surface, grazing scars, dead
294 biota), native taxa, non-indigenous or cryptogenic species (Thomsen et al. 2016). By analysing
295 sub-component of communities separately, Bray-Curtis similarity could not always be
296 computed because of the presence of empty samples (e.g. panels not colonized by NIS):
297 corresponding pairs of samples were thus removed from analyses (see degree of freedom in
298 PERMANOVA results). Environmental data were analysed using R environment (Lê et al.
299 2008; R Development Core Team 2012) while community analyses were performed using
300 PRIMER 7 (Clarke and Warwick 2001).

301

302 **Results**

303

304 *Environmental parameters*

305 According to the principal component analysis (PCA), all study sites were quite well-
306 discriminated by their abiotic conditions (Fig. 2, Appendix S1). While the PERMANOVA
307 confirmed these differences (Site (Traffic): $F_{4,13} = 5.01$, $P < 0.001$), it failed to detect a
308 multivariate effect of the traffic category (i.e. international vs. local ports, $F_{1,13} = 2.75$, $P =$
309 0.099). In spite of a large within-site variability, the first PCA axis (50.1% of the variation)
310 however suggested differences between ports according to their category. These differences are
311 predominantly explained by higher concentrations of a series of metals (except Hg), but lower
312 light (inactive variable) in international as compared to local ports.. There is however some
313 overlap among sites: most metal concentrations and light levels were similar in Coliumo (local),
314 Lirquén and San Vicente (international ports). This is exemplified on the second axis of the

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

319

320 *Diversity and community structure*

321 During the study (combining panels examination and rapid assessment survey), a total
322 of 119 taxa were identified, including 10 algae, 41 sessile and 37 mobile animals. Complete
323 lists and species authorities are provided in Appendix S2. Among these taxa, 10 were classified
324 as non-indigenous and 15 as cryptogenic. Importantly, these taxa included novel records for the
325 region: *Bougainvillia muscus*, *Ectopleura larynx*, *E. crocea*, *Hydractinia* sp. and *Exochella* nov.
326 sp. and *Asterocarpa humilis* (for the latter, details are provided in Pinochet et al. 2017). Within
327 established hard bottom communities examined through rapid assessment surveys at both
328 periods, NIS and cryptogenic species contributed to 13% and 26% of the sessile taxa richness,
329 respectively. The highest proportions of NIS (23%) and cryptogenic species (25%) were found
330 in Lirquén (Appendices S3, S5). Nonetheless, NIS were often rare or occasional (Fig. 3) and
331 some of them were only found in specific habitats (e.g. crevices, mooring lines and abandoned
332 ropes). In comparison, several native species were classified as frequent, common or abundant
333 in both categories of ports (Fig. 3). Less NIS (i.e. cumulative numbers over sites) were reported
334 in local (3 and 7 in November 2016 and March 2017, respectively) than in international ports
335 (13 and 18) (Appendices S3, S5). Six NIS were found only in international ports, with three of
336 them *Bougainvillia muscus* (rare to frequent), *Ectopleura crocea* (rare to abundant), *Ciona*
337 *robusta* (absent to rare) observed in at least two sites. For cryptogenic species, the number of
338 reports was greater, but with less difference than for NIS, between local (12-15) and

339 international ports (21-20), with two of them only recorded in international ports (e.g. *Clytia*
340 *linearis*, varying from rare to abundant depending on site and sampling date, Appendix S3).

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

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

355 After one month, an average of 4 taxa settled on the panels, with an average coverage
356 of 34% of the panel area (22% in September 2016, 45% in October 2017). Only a few non-
357 indigenous specimens were observed at this stage (2 in local and 6 in international ports on
358 total, both sampling periods combined), with up to 1 taxa for a maximum cover of 2% in
359 Lirquén (Fig. 4-5, Appendices S6-7). No clear pattern could be detected for cryptogenic species
360 (5 and 9 taxa in local and international ports, respectively) richness and cover, although they
361 represented half of the richness and up to 87% of the cover in Llico (Fig. 4-5, Appendices S6-
362 7). The PCO illustrates the three-way interaction (Site \times Starting date \times Age, Table 3): traffic
363 category did not explain settlement patterns, but important site-to-site differences were

364 observed within each category and depending on the period (Fig. 6). NIS poorly influenced
365 these patterns, which are more likely due to some species-specific recruitments. For instance,
366 the native barnacle *Austromegabalanus psicattus* showed greater settlement in Llico during the
367 second trial (March 2017) as compared to all other sites (Fig. 6).

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

389 (international). After 7 months, panels were generally fouled by more species and in greater
390 abundances (involving either NIS, cryptogenic, native or a combination of all, depending on
391 site or sampling occasion, Appendix S5), but the observed patterns were generally consistent
392 with those observed after 3 months (Fig. 4-6, Appendices S6-8). At this stage, a total of 5 and
393 8 NIS and a total of 8 and 9 cryptogenic species were observed on panels from local and
394 international ports, respectively.

395

396 **Discussion**

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

410

411 Overall, unique communities developed in each study site irrespective of their traffic
412 category and in spite of some variations in environmental parameters (metal concentration,
413 light) between them. For instance, higher metal concentrations were reported in the

414 international ports, as shown in other studies(Dafforn et al. 2012). Resuspension of sediment-
415 bound contaminants, such as metals, may influence biofouling communities by affecting either
416 settlement cues or the biofilm. In the more contaminated site of this study (Coronel), the average
417 concentrations of copper, lead and zinc were of 41, 34 and 73 mg kg⁻¹, respectively. While such
418 values are in the range of some impacted estuaries (e.g. in New South wales) (Dafforn et al.
419 2012), they are below the reference range of possible adverse biological effects (Macdonald et
420 al. 1996) and about 10 times lower than highly contaminated sediments (from Port Kembla,
421 New South Wales) which have recently been used to demonstrate impacts on bacterial biofilm
422 interactions and macro-fouling communities (Lawes et al. 2017). Under the conditions herein
423 measured, only experimental approaches considering possible interactions with a series of other
424 stressors (Johnston and Keough 2002; Lawes et al. 2017), which may display more complex
425 dynamics (such as the dissolved-oxygen in the study area, Ahumada B. et al. 1983; Valle-
426 Levinson et al. 2003) would allow to disentangle among oceanographic and anthropogenic
427 factors which influence the studied communities.

428 NIS represented less than 1% of the surface colonized on settlement panels collected
429 after 1 month at both occasions, suggesting that all study sites undergo a similar and low
430 propagule pressure by NIS. In addition, most of these NIS apparently settled on panels with
431 relative abundances representative of those also identified within the surrounding established
432 communities or in older panel assemblages (e.g. *Bougainvillia muscus* in Lirquén, *Ectopleura*
433 *crocea* in Coronel, *Bugulina flabellata* in Lirquén and Llico, *Exochella* nov. sp. in Coliumo and
434 San Vicente; Fig. 6, Appendix S2) – a pattern quite consistent with the one observed for native
435 species (e.g. *Austromegabalanus psicattus* in Llico, *Semimytilus algosus* in Coronel, *Balanus*
436 *laevis* in Coliumo and San Vicente). It is therefore possible that a significant part of the observed
437 propagule pressure mainly results from local (site-specific) populations, and thus involve more
438 complex histories of species introductions. In the present study, most of the novel records were

439 hydroids which, as compared to well-studied groups in Chile (e.g. Bryozoans, Viviani 1969;
440 Moyano 1983), have received less attention until recent campaigns in Patagonian fjords (Galea
441 2007; Galea et al. 2007). These campaigns resulted in novel records of various non-native
442 (*Bougainvillia muscoides*, *B. pyramidata*) and cryptogenic hydroids (e.g. *Clytia linearis*,
443 *Amphisbetia operculata*), some of them widely distributed (Galea 2007; Galea et al. 2007).
444 Interestingly, among the novel taxa herein recorded, *Ectopleura crocea* has been observed in
445 all studied international ports. This common biofouling species is considered non-native in
446 many regions worldwide, including in the NE Pacific along which – decades ago – its hydrants
447 have been demonstrated to survive long distances and disperse from the hulls of sailing vessels
448 (Carlton and Hodder 1995). First time report does not mean that the introduction is recent (e.g.
449 Provan et al. 2005), especially in under-studied taxonomic groups. Like other non-native
450 species reported for the first time, including in this study, this species has probably been
451 introduced along the SE Pacific, and more specifically in Chile, a long time ago, spread and
452 established widely, likely beyond the geographic span of this study. In order to test whether
453 the recruitment patterns herein observed result from local populations or external sources,
454 thorough surveys of ship tanks and hulls, as well as small craft hulls, arriving in the studied
455 ports would have been needed. For the time being and according to worldwide literature on the
456 topic, it seems however unlikely that arriving international ships do not transport NIS (Bax et
457 al. 2003; Drake and Lodge 2007; Gollasch et al. 2007). A recent study of international ship
458 hulls in Talcahuano port, located in our study area, revealed the presence of the non-indigenous
459 tunicate *Asterocarpa humilis* – putatively native from New Zealand – within their sea chest
460 (Pinochet et al. 2017). Although no effect of the traffic category could be have been detected
461 on recruitments over the studied period, the larger number of NIS reported in international ports
462 suggests that international shipping have influenced their local establishment at some point.
463 Nonetheless, the environment of the receiving ports may mismatch with the abiotic

464 requirements of many introduced species for reproduction and propagule survival (Castilla and
465 Neill 2009; Seebens et al. 2013). In addition, regardless of their survival, some propagules may
466 simply not be retained: although generally constructed within bays, none of the study ports were
467 enclosed by breakwaters or seawalls, therefore it is possible that the propagule pressure was
468 locally limited in favour of outward dispersal (Floerl and Inglis 2003; Bishop et al. 2017).
469 Dispersal and survival modelling based on local oceanographic features and species life history
470 traits would help to test whether these hypotheses could explain the generally low propagule
471 pressure and the current distribution of NIS among all sampling sites.

472 After 3 and 7 months, NIS contribution to community structure was highly variable – in
473 terms of identity and abundances – among sites and there was no indication that international
474 maritime traffic contributed to this pattern. While NIS richness and cover respectively increased
475 in 4 and 3 sites between 1 and 3 months, they only increased in 1 and 3 sites between 3 and 7
476 months, irrespective of their associated traffic (Appendix S9). In comparison, native richness
477 and cover respectively increased in 4 and 5 sites between 1 and 3 months and both in 4 sites
478 between 3 and 7 months. The apparent poor contribution of NIS to community development
479 patterns is potentially in agreement with Castilla and Neill (2009) who suggested that the
480 uniqueness of the Chilean coastline in terms of oceanographic conditions could contribute to
481 limit the regional establishment and spread of NIS, less ‘adapted’ than most native species
482 under specific conditions (e.g. Castilla et al. 2004; Caro et al. 2011). In order to be validated,
483 such a hypothesis would however require thorough test of the propagule survival and growth
484 of the NIS herein found (as well as native species), under controlled conditions. With regards
485 to the temporal changes in richness and cover undergone by native species (generally
486 increasing, Appendix S9) on experiment panels, the influence of these species in limiting NIS
487 establishment (i.e. biotic resistance hypothesis) deserves further experimental scrutiny
488 (Stachowicz et al. 2002; Clark and Johnston 2009; Lord and Whitlatch 2015). In all the studied

489 international ports, pilings were generally completely fouled (JCL, pers. obs.) and various
490 native sessile taxa were classified as common, abundant or even super-abundant (Figure 3), i.e.
491 with species-specific covers varying between 10 to more than 80% depending on the growth
492 forms (crust or massive)-. In parallel, only a few NIS fell in these categories, most of them
493 being rare or occasional (Fig. 3). The native assemblages may have contributed to limit NIS
494 establishment in the studied sites by limiting at least one of the most limiting resources of
495 biofouling communities: free space (Elton 1958; Stachowicz et al. 2002). Should the influence
496 of native space occupiers in limiting NIS establishment be further investigated in the study area,
497 the complexity of possible interactions (e.g. facilitation, predation) between native and non-
498 indigenous taxa could not be overlooked (Bulleri et al. 2008; Rogers et al. 2016; Leclerc and
499 Viard 2018). Various NIS (e.g. *Ectopleura crocea*, *Bugulina flabelatta*, *Asterocarpa humilis*,
500 *Diplosoma listerianum*) and cryptogenic species (e.g. *Alcyonidioides mytili*, *Amathia* cf.
501 *gracilis*, *Plumularia* cf. *setacea*) were, for instance, herein frequently observed in abundance
502 when developing upon massive native habitat builders, such as *Pyura chilensis* and
503 *Austromegabalanus piscattus*. In the present survey, several native predators (e.g. fish, urchins,
504 crabs, Caprellid amphipods) were also observed and generally classified as frequent or common
505 depending on study sites, in either local or international ports (Fig. 3, Appendix S5).
506 Nonetheless, multivariate analyses suggested that grazing (apparently due to sea urchins)
507 influences the community with contrasting dynamics in at least two sites – an international
508 (Lirquén) and a local port (Chome) – by increasing the free space (Fig. 6). As such, the specific
509 functions of native predators in controlling some NIS populations may deserve further
510 investigation (Dumont et al. 2011; Rogers et al. 2016), although careful attention should be paid
511 regarding direct and indirect effects (Connell 2001).

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

514 2012; Aguilera et al. 2014; Johnson et al. 2017). For instance, unlike all the studied international
515 ports, Coliumo jetty did open to regional traffic (fishing boats) only one year before the
516 beginning of our survey and its construction dated back a couple of years following 2010
517 tsunami – common catastrophic events along the Chilean coast (Aguilera 2017). At this site,
518 only seldom and poorly abundant native taxa were initially observed on pilings (Appendix S5),
519 still covered by antifouling paint. Later on (e.g. in June 2017), several NIS were recorded (e.g.
520 *Exochella* nov. sp., *Bugula neritina*, *Bugulina flabelatta*, *Asterocarpa humilis*, *Diplosoma*
521 *listerianum*) and some of them (e.g. *Exochella* nov. sp.) showed important increase in cover
522 (and apparent overgrowth of native taxa) on experimental panels between sampling dates
523 (Appendix S9). These results strengthen the hypothesis that free space may be an important
524 limiting factor for NIS establishment (Stachowicz et al. 2002). In this context, it is noteworthy
525 that NIS cover varied on average between 14 and 29% after 7 months, which are values greater
526 than those observed within the surrounding communities (Fig. 3). Unless they get colonized by
527 abundant native taxa, the creation of novel artificial structures may thus contribute to the local
528 establishment of NIS, and potentially act as stepping stones for subsequent spread (Wasson et
529 al. 2001; Mineur et al. 2012; Airoidi et al. 2015), potentially facilitated by regional transport
530 (Clarke Murray et al. 2011; Ferrario et al. 2017) – herein mainly due to fishing barge and crafts:
531 in the present survey, *Exochella* nov. sp. was detected for the first time underneath the local
532 jetty of Coliumo in March 2017 and later recruited and contributed to community structure on
533 experimental panels in both Coliumo and San Vicente (international port). If Chilean authorities
534 and research groups were to implement NIS surveillance programs, the present study performed
535 locally, along with others conducted abroad (e.g. Clarke Murray et al. 2011; Bishop, Wood,
536 Yunnice, et al. 2015; Johnson et al. 2017), strongly supports that the diversity of putative vectors
537 (including hull biofouling of local crafts) and stepping stones (including local mooring
538 facilities) should be taken into account.

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

549

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783

784 **Table legends**

785

786 **Table 1.** Results of PERMANOVA tests for differences in sessile taxa richness among levels of the main factors (traffic, starting date, age and
 787 site) and their interactions. Tests are presented for all, non-indigenous, cryptogenic and native species, separately. Post-hoc pairwise tests are
 788 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- <i>F</i>	<i>P</i>	MS	Pseudo- <i>F</i>	<i>P</i>	MS	Pseudo- <i>F</i>	<i>P</i>	MS	Pseudo- <i>F</i>	<i>P</i>
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												

789

790 **Table 2.** Results of PERMANOVA tests for differences in sessile taxa cover among levels of the main factors (traffic, starting date, age and site)
 791 and their interactions. Tests are presented for all, non-indigenous, cryptogenic and native species, separately. Post-hoc pairwise tests are
 792 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- <i>F</i>	<i>P</i>	MS	Pseudo- <i>F</i>	<i>P</i>	MS	Pseudo- <i>F</i>	<i>P</i>	MS	Pseudo- <i>F</i>	<i>P</i>
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												

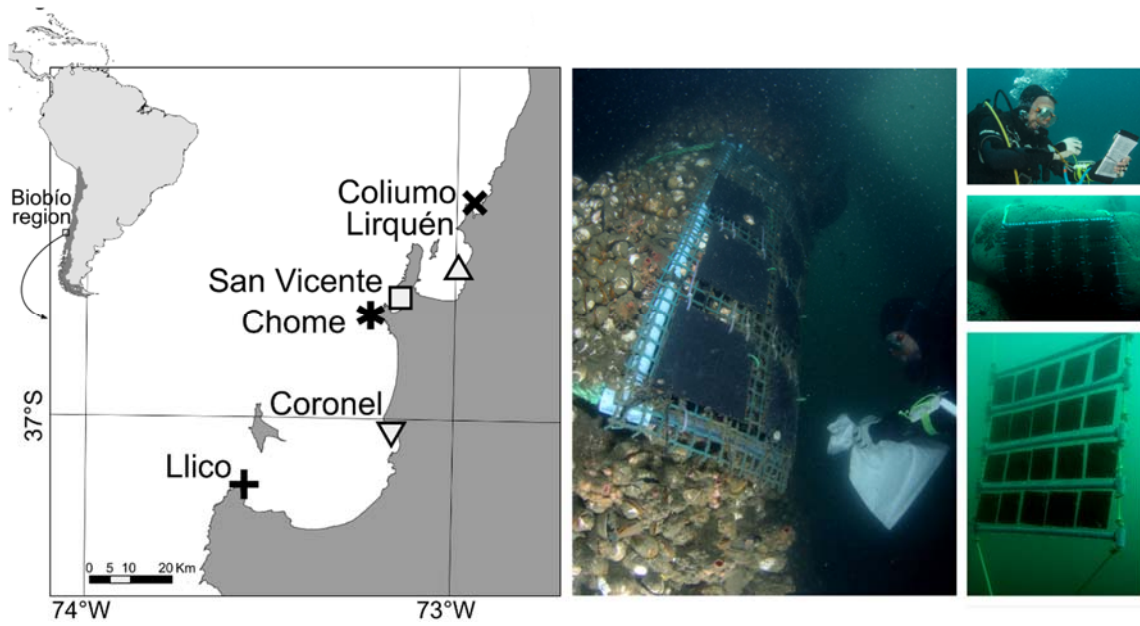
793

794 **Table 3.** Results of PERMANOVA tests for differences in community structure among levels of the main factors (traffic, starting date, age and
 795 site) and their interactions. Tests are presented for all, non-indigenous, cryptogenic and native species separately. Post-hoc pairwise tests are
 796 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- <i>F</i>	<i>P</i>	df	MS	Pseudo- <i>F</i>	<i>P</i>	df	MS	Pseudo- <i>F</i>	<i>P</i>	df	MS	Pseudo- <i>F</i>	<i>P</i>
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

797

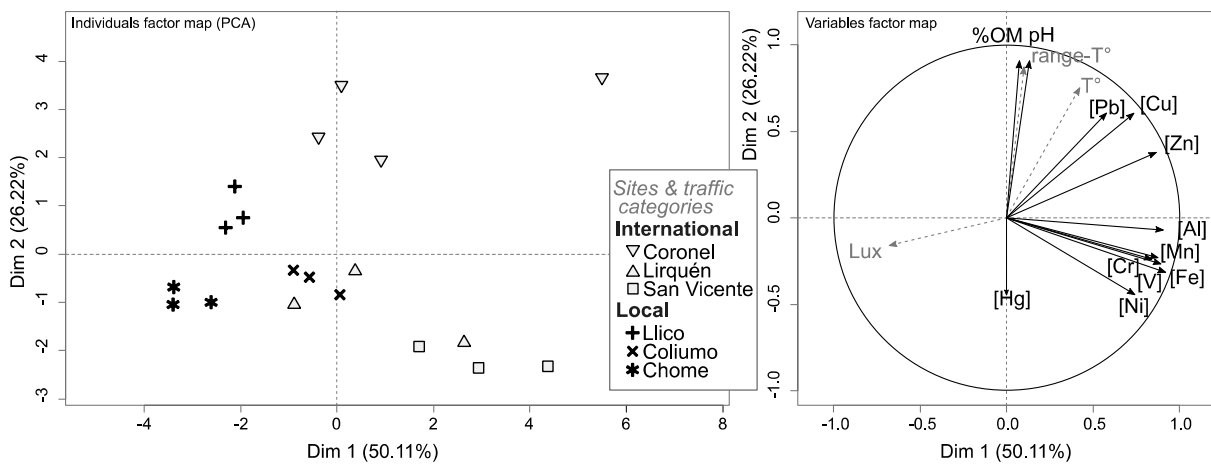


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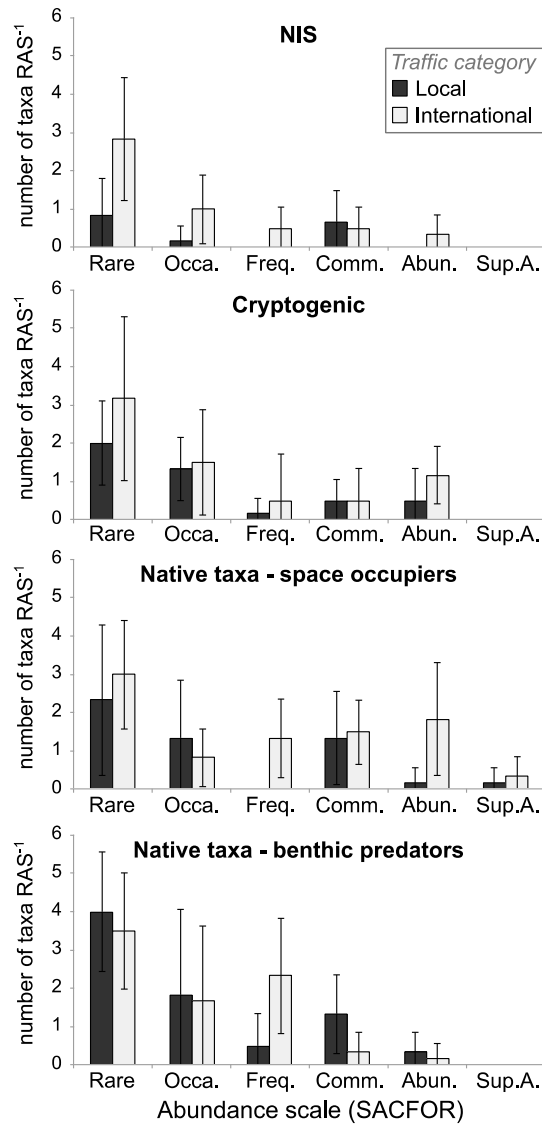
801 **Figure 1.** Location of the study sites along Bío-Bío region: International (white symbols) and
 802 local ports (black symbols).

803



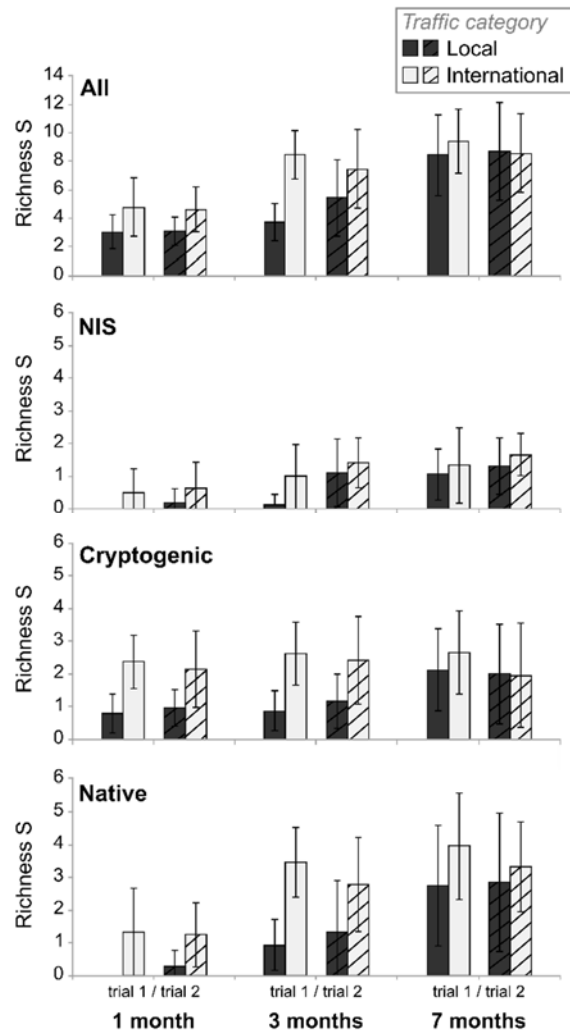
804

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



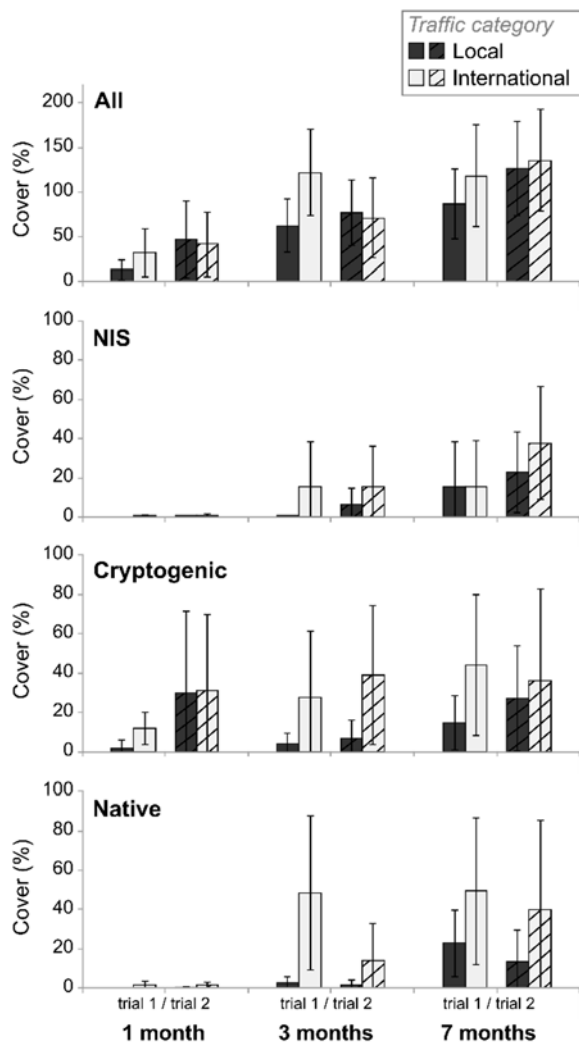
808

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



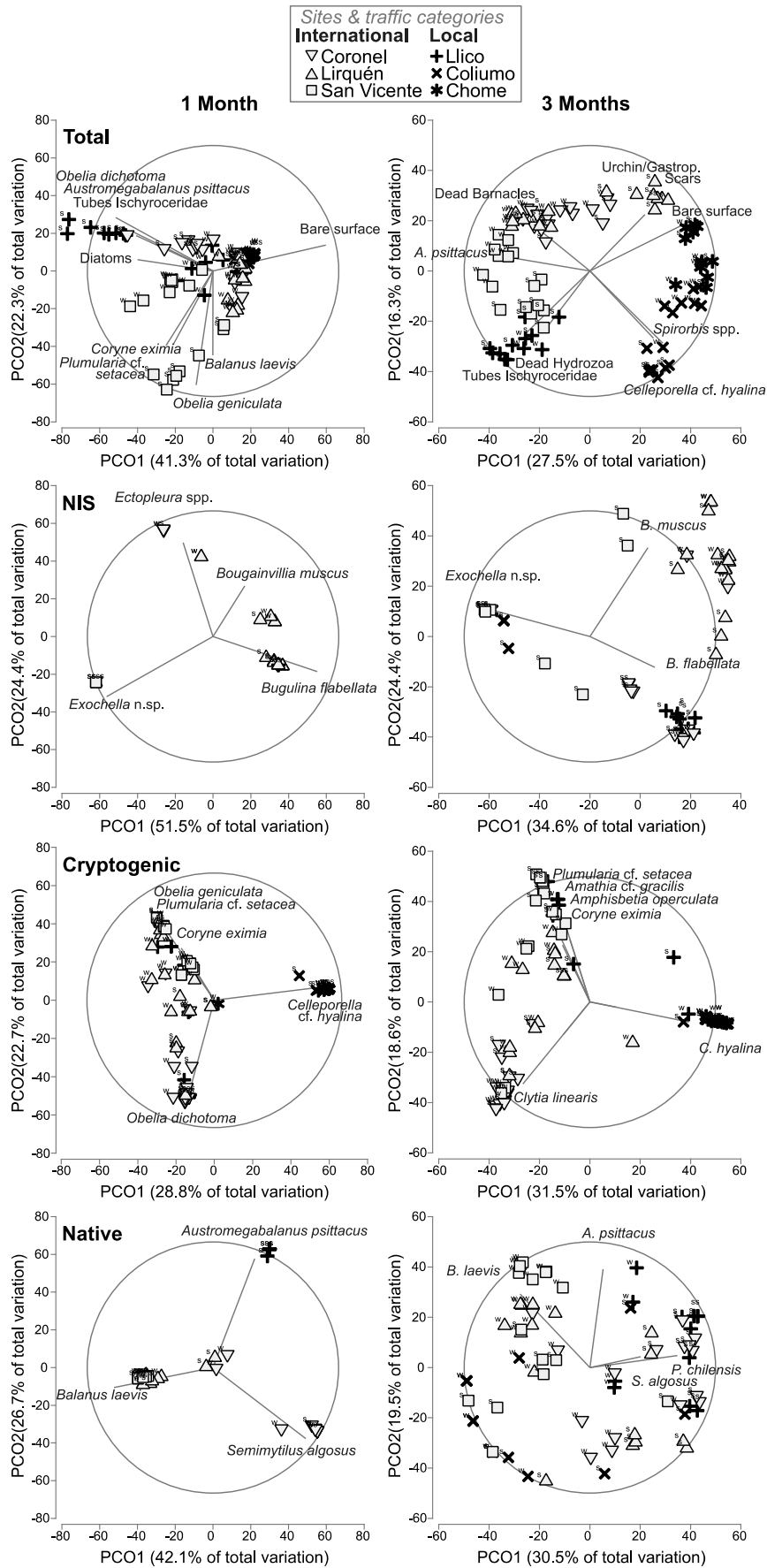
814

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



819

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



824

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

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

831

832

833 *Supplementary information*

834

835 **Appendix S1.** Environmental parameters (average \pm S.D.) measured in all sites and protocol
836 related to metal concentration measurements

837

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

840

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

843

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

846

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

849

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

852

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

855

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

858

859 **Appendix S9.** Summary of pairwise tests following univariate and multivariate

860 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 \pm 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 \pm 0.1	7.1 \pm 0.1	7.4 \pm 0.1	8.6 \pm 0.1	7.1 \pm 0	7.5 \pm 0.1
%OM	14.5 \pm 9.9	1.5 \pm 0.3	3.1 \pm 0.7	28 \pm 10.1	1.7 \pm 0.2	3.2 \pm 0.7
Zn (mg kg ⁻¹)	14.8 \pm 2	8.0 \pm 1.7	34.0 \pm 4.3	73.3 \pm 27.6	46.1 \pm 10.3	53.2 \pm 17.6
Cu (mg kg ⁻¹)	2.7 \pm 0.7	2.4 \pm 0.6	5.5 \pm 1.8	40.9 \pm 19.9	16 \pm 4.2	14.4 \pm 6.9
Pb (mg kg ⁻¹)	15.7 \pm 2.4	9.3 \pm 1.8	17.8 \pm 1	34.4 \pm 19.7	18.2 \pm 4.1	18.7 \pm 15.2
V (mg kg ⁻¹)	15.3 \pm 1.6	4.5 \pm 1.6	24.3 \pm 13.5	37.9 \pm 22.3	87.1 \pm 13	51.5 \pm 16.6
Cr (mg kg ⁻¹)	11.6 \pm 1.3	7.8 \pm 5.9	22.3 \pm 3.2	34.2 \pm 15	54.9 \pm 4.4	47.6 \pm 32
Mn (mg kg ⁻¹)	97.8 \pm 6.3	21.0 \pm 13	267.8 \pm 60.1	199 \pm 105.5	400.2 \pm 116.5	130.4 \pm 46.1
Ni (mg kg ⁻¹)	4.2 \pm 1.6	2.8 \pm 1.6	6.3 \pm 2.4	13.0 \pm 8.3	60.8 \pm 15.9	13.5 \pm 2.8
Hg (mg kg ⁻¹)	1.9 \pm 1.3	15 \pm 7.8	2.1 \pm 0.3	0.6 \pm 0.3	8.5 \pm 3.7	41 \pm 25.2
Al (mg kg ⁻¹)	3455 \pm 885.9	1669.5 \pm 818.5	6646.7 \pm 774.3	7428.8 \pm 7037	9822.5 \pm 5071.2	8463.3 \pm 3913.4
Fe (mg kg ⁻¹)	8185 \pm 545.3	4831.5 \pm 5642.6	20430 \pm 4132.8	20642.5 \pm 7946.3	33420 \pm 3704.2	22613.3 \pm 16321.2
T (°C)	14.3 \pm 1	13.8 \pm 0.7	14.1 \pm 0.9	14.6 \pm 0.7	14.1 \pm 0.6	14.3 \pm 0.8
Light (Lum ft ⁻²)	62.9 \pm 120.9	62.1 \pm 116.5	7.8 \pm 29.4	< 0.1	17.4 \pm 49.6	4.7 \pm 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. 550°C. 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 HNO₃/HCl) in a digester brand SCP SCIENCE, model DigiPrep. Samples were then siliconized: ten μ L 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 μ g 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 mm² area and an energy resolution < 145 eV, working at 50 kV and 750 μ A 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

Antithamionella 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)

Acontiarina 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 variabilis 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)

Crepidatella 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 Poëppig, 1836

Cancer porteri Rathbun, 1930

Taliepus dentalus (H. Milne Edwards, 1834)

Pilumnoides perlatus (Poëppig, 1836)

Neotrypaea uncinata (H. Milne Edwards, 1837)

Caridea Dana, 1852

Alpheidae Rafinesque, 1815

Echinodermata

Heterocucumis godeffroyi (Semper, 1867)

Ophiactis sp. Lütken, 1856

Patiria chilensis (Lütken, 1859)

Asteriidae Gray, 1840

Arbacia dufresnii (Blainville, 1825)

Stichaster striatus Müller & Troschel, 1840

Tetrapygyus 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 blainvillianus (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)

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

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

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

<i>Sicyases sanguineus</i>	Native	C		*	*	*	*			
<i>Helcogrammoides cunninghami</i>	Native	C			*			*	**	
<i>Leptonotus blainvilleanus</i>	Native	C	**							*
Blenniidae	Native	C								
<i>Prolatilus jugularis</i>	Native	C			*					
<i>Otaria flavescens</i>	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							
<i>Ulva</i> sp.	Unassigned		**	**		*	
Ochrophyta							
Ectocarpales	Unassigned	*				**	
<i>Macrocystis pyrifera</i>							
Bacillariophyceae	Unassigned	***			**	*****	***
Rhodophyta							
<i>Antithamnionella ternifolia</i>	Unassigned					****	
Crustose Corallinales	Unassigned		*****	*****			
Gigartinales							
<i>Mazzaella laminarioides</i>	Native	**					
Non-clacareous crust	Unassigned	*	****	**			
Rhodymeniales	Unassigned		**	**		**	
Ciliophora							
Ciliophora indet.	Unassigned	**			*	*	
Brachiopoda							
<i>Discinisca</i> cf. <i>tenuis</i>	Unassigned				**		*
Porifera							
<i>Leucosolenia</i> sp.	Unassigned	*		*		**	***
Cnidaria							
<i>Amphisbetia operculata</i>	Cryptogenic					****	
<i>Bougainvillia</i> sp. (<i>B. muscus</i>)	NIS	*			**	*	*****
<i>Clytia linearis</i>	Cryptogenic	**			*****	***	*****
<i>Coryne eximia</i>	Cryptogenic	*			**	*****	**
<i>Ectopleura</i> spp. (<i>E. crocea</i> & <i>E. Larynx</i>)	NIS				*****		
<i>Eudendrium</i> sp.	Unassigned					**	
<i>Halecium</i> sp.	Unassigned						*
<i>Halecium</i> sp2.	Unassigned					*	
<i>Hydractinia pacifica</i>	Native				**		
<i>Hydractinia</i> sp.	NIS					*	**
<i>Obelia geniculata</i>	Cryptogenic	**			**	**	**
<i>Obelia dichotoma</i>	Cryptogenic	****		*	*****		**
<i>Orthopyxis</i> sp. (<i>O. mollis</i>)	Native					*	
<i>Phiaella</i> cf. <i>quadrata</i>	Cryptogenic	*				**	***
<i>Plumularia setacea</i>	Cryptogenic	****		*		*****	
<i>Cereus herpetodes</i>	Native	*					
<i>Acontiarina</i> sp. 2	Native				****		
Bryozoa							
<i>Alcyonidiodies mytilii</i>	Cryptogenic	*			**	**	**
<i>Amathia</i> cf. <i>gracilis</i>	Cryptogenic	**		**	*	****	***
<i>Amathia</i> sp. (<i>A. imbricata</i>)	NIS				*		
<i>Bugula neritina</i>	NIS			*			*
<i>Bugulina flabellata</i>	NIS	****		*	***	**	*****
<i>Cauloramphus spiniferum</i>	Cryptogenic		**				
<i>Celleporella</i> cf. <i>hyalina</i>	Cryptogenic	**	****	*****			*
<i>Electra monostachys</i>	Cryptogenic				*		
<i>Exochella</i> n. sp.	NIS		**	****		**	
<i>Lagenicella variabilis</i>	Native	**	*				
<i>Schyzoporella maulina</i>	Native			**			
<i>Scruparia ambigua</i>	Native	*				**	
<i>Tricellaria acuelata</i>	Native	**					
<i>Tubulipora</i> sp.	Unassigned	*					
<i>Turbicellepora</i> sp.	Unassigned		*				

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

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

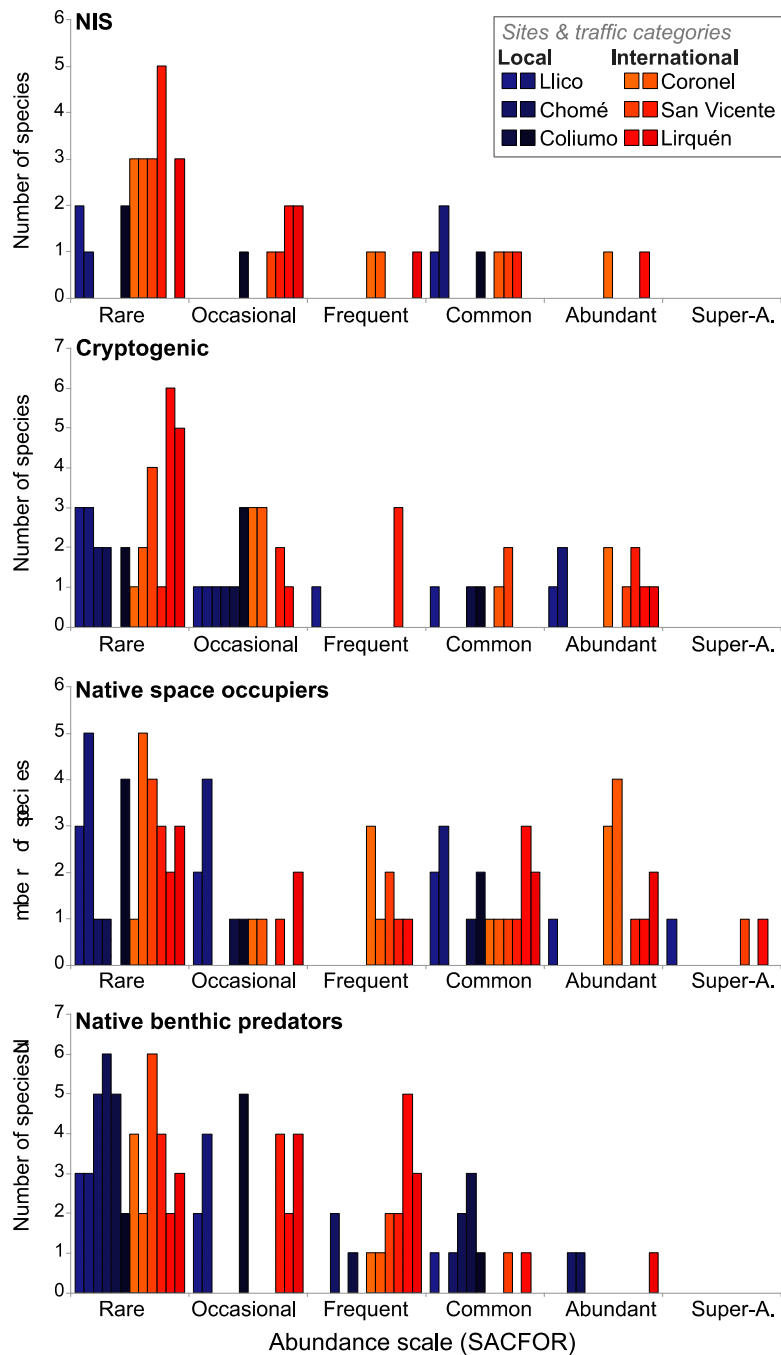


Figure S5. Number of NIS, cryptogenic species, native space occupiers and benthic predators encountered within surrounding communities at each study site (international ports in pale patterns, local piers in dark). Data are presented as the number of reports (taxa) within each category of abundance (SACFOR)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

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

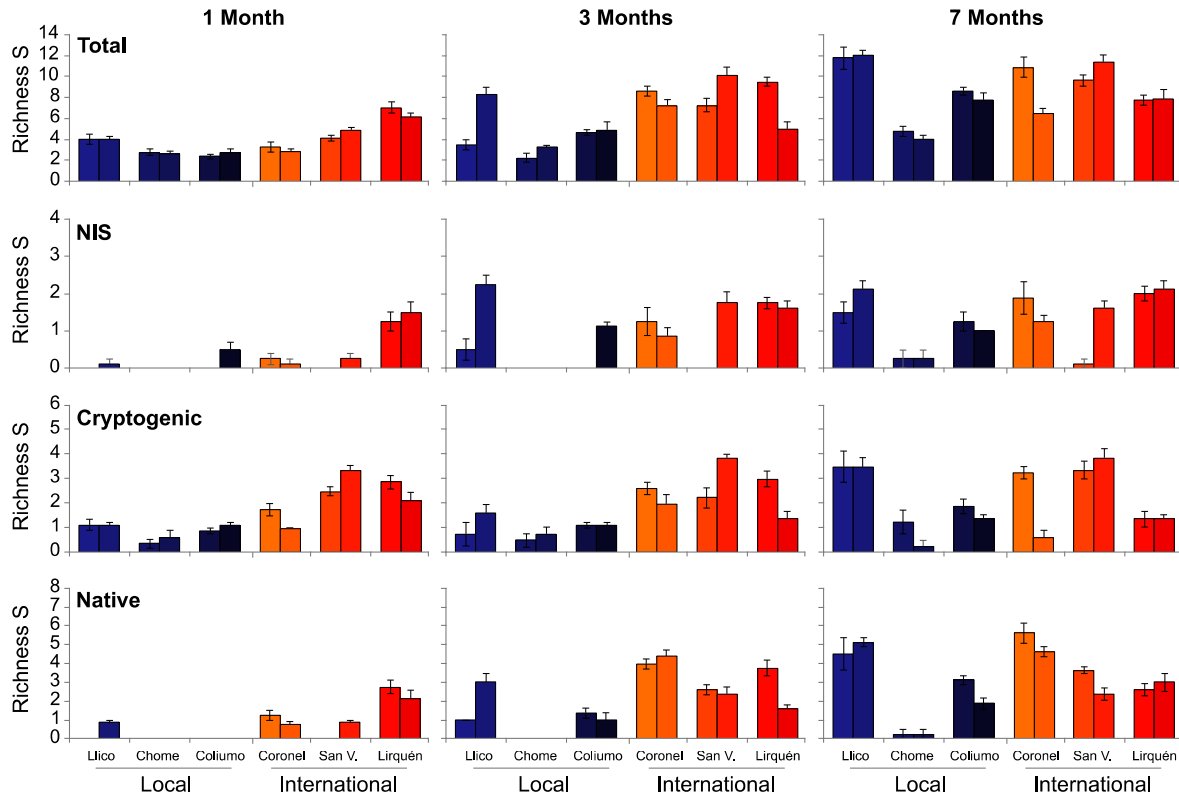


Figure S6. Sessile taxa (total, NIS, cryptogenic and native) richness on the experimental panels collected after 1, 3 and 7 months in all study sites at each sampling occasion (\pm S.E.). Legend is the same as in figure S5.

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

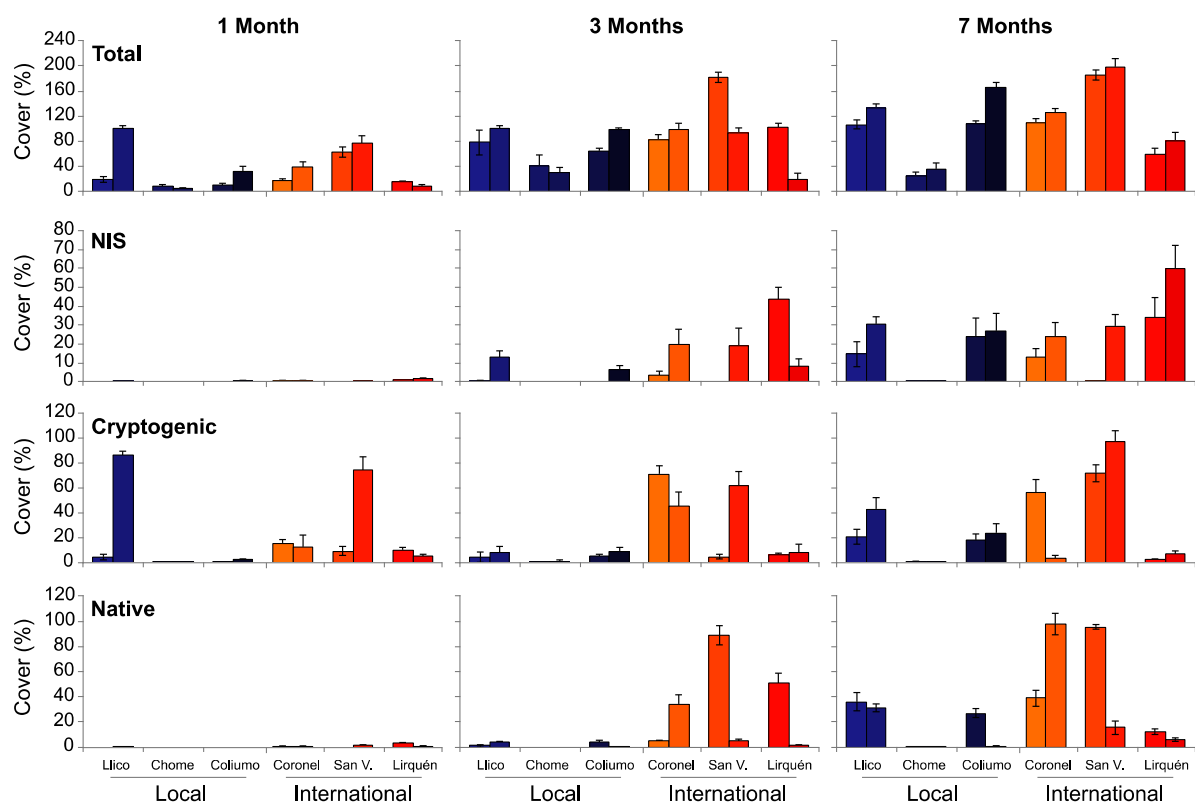


Figure S7. Sessile taxa (total, NIS, cryptogenic and native) cover (%) on the experimental panels collected after 1, 3 and 7 months in all study sites at each sampling occasion (\pm S.E.). Legend is the same as in figure S5.

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

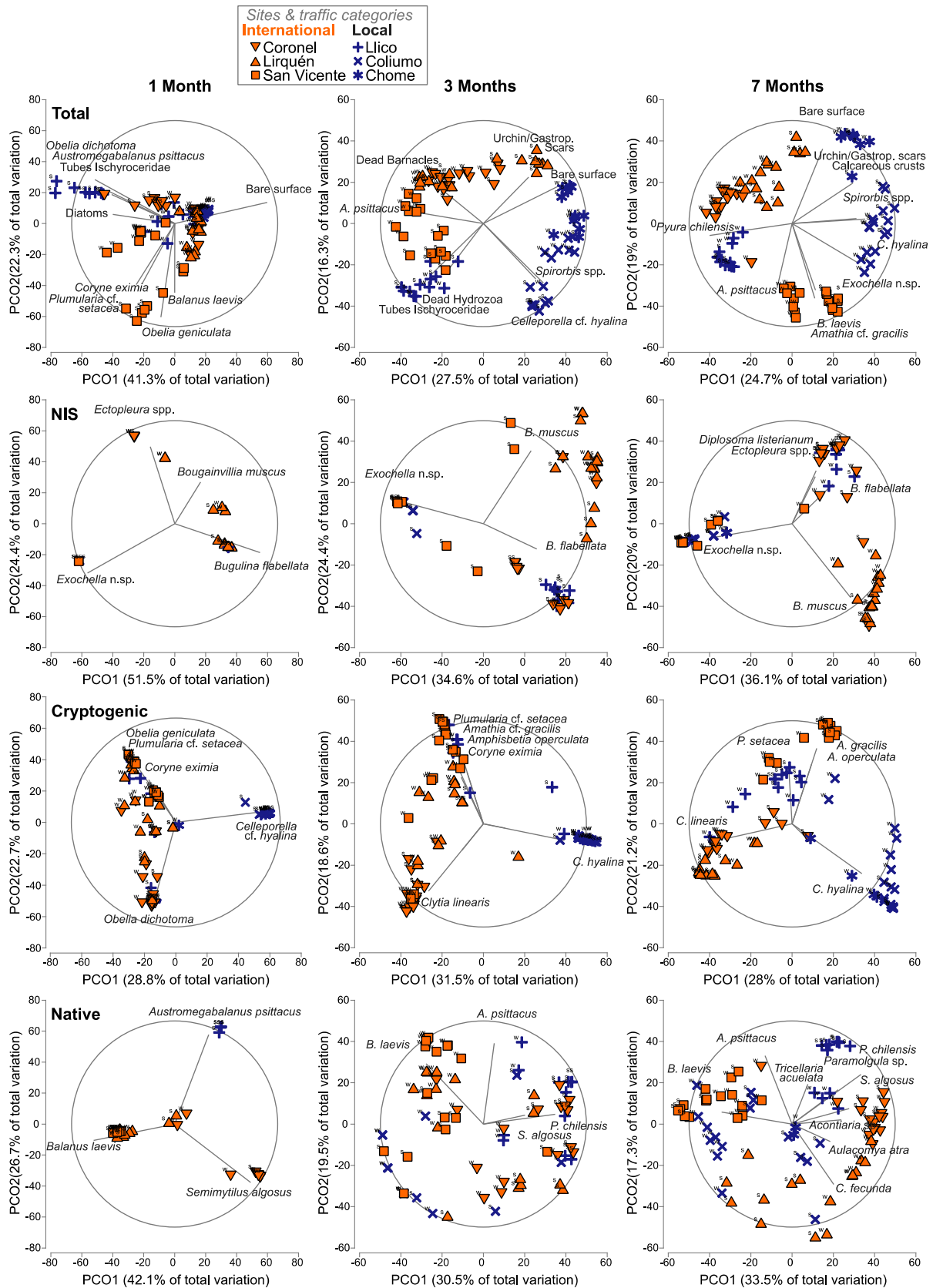


Figure S8. Principal coordinate analyses describing the assemblage structure of all, non-native, cryptogenic and native species on the panels collected after 1, 3 and 7 months in all study sites for each trial (w: winter and s: summer). Vector plots of variables correlated with the PCO axes are indicated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

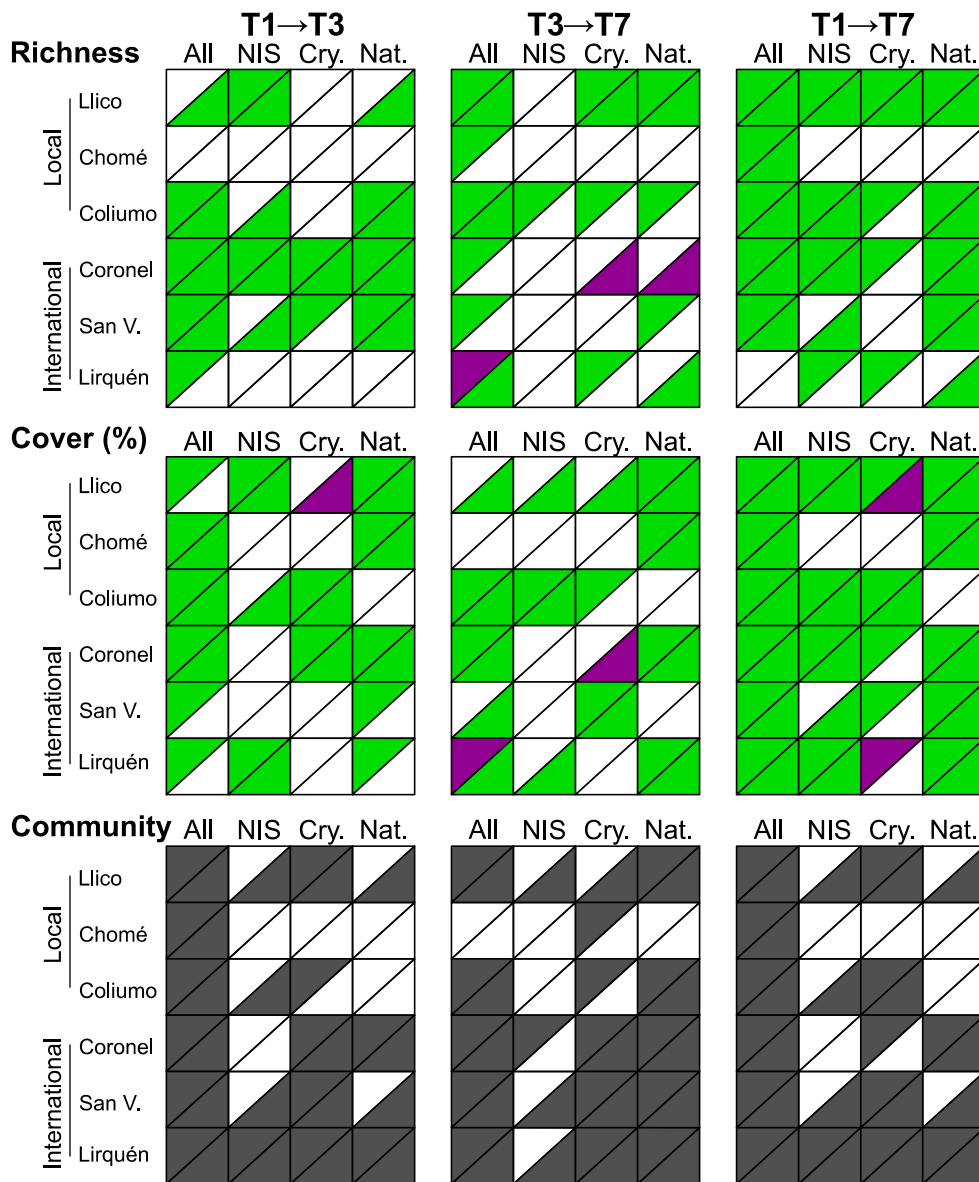


Figure S9. Summary of pairwise tests following univariate and multivariate PERMANOVAs testing for differences in sessile taxa richness, cover and community structure. Tests are presented for all, non-indigenous (NIS), cryptogenic (Cry.) and native (Nat.) species, separately. Results are illustrated as 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 $\alpha = 0.05$ (based on Monte Carlo procedure).