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Experimental and survey-based evidences for effective biotic resistance by predators in ports

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Abstract Of the suite of species interactions involved in biotic resistance to species invasions, predation can have complex outcomes according to the theoretical and empirical framework of community ecology. In this study, we aimed to determine the likelihood of consumptive biotic resistance within fouling communities in four ports of central Chile. Notably, we examined the influence of micro- (> 1–2 mm, < 1–2 cm) and macro- (> 1–2 cm) predators, with a particular focus on their effects on non-indigenous species (NIS). Experimental and observational approaches were combined. An exclusion experiment was carried out over 4 months to examine predator effect on the early establishment of new assemblages on settlement panels. Later successional stages upon panels were examined over a total of 26 months and supported by rapid assessment surveys in the surrounding habitats. Community structure was significantly influenced by the exclusion treatments. Macropredators reduced the fouling biomass and abundance, although conflicting patterns emerged from the exclusion of both categories of predators. Altogether, predators reduced the abundance of most NIS and cryptogenic species, some of them being only observed when the two categories of predators were excluded—a pattern generally sustained over the long-term dynamics in community development. Our results show an effective consumptive biotic resistance, furthermore possibly dependent on predator size. Further work is however needed to determine the influence of the functional diversity of natural enemies on the efficiency of biotic resistance and its interplay with other biotic interactions (competition or mutualism). A comprehensive understanding of these processes should in turn help defining management strategies in a context of habitat modification and species loss.

Keywords Complementarity · Consumptive resistance · Enemy release · Exclusion experiment · Long-term survey · Marine infrastructure · Non-indigenous species · Species interactions

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Introduction

In ecological studies, the performance of native communities in buffering the establishment of newly introduced species has been experimentally unexplored until the 1990s (Kimbro et al. 2013; Levine et al. 2004; Papacostas et al. 2017), although this process caught attention much earlier (Elton 1958). The biotic resistance hypothesis predicts that the establishment and spread of introduced species can fail locally in diverse communities owing to interactions with native species (Bulleri et al. 2008; Lockwood et al. 2013). The most commonly explored mechanism underlying biotic resistance has been competition, where a more diverse assemblages of native species is assumed to utilize resources more completely than a less diverse community (Davis et al. 2000). Other mechanisms such as predation *lato* sensu (including herbivory and parasitism, Morin 2011) and mutualism may be particularly important (Lockwood et al. 2013). Beyond regulation of prey populations, predation may mediate competitive interactions and in turn affect species abundance and community structure (Morin 2011). Not surprisingly then, trophic interactions studies and food web theory were required to alleviate the diversity-stability debate and to understand the underlying mechanisms, such as productivity, connectance, interaction strength and opportunism (Connell and Ghedini 2015; Rooney and McCann 2012). Likewise, invasion ecology should benefit from a more comprehensive understanding of the direct and indirect effects of multiple enemies upon introduced species, especially in the framework of the biotic resistance paradigm (Caselle et al. 2018; Smith-Ramesh et al. 2017).

In the marine realm, bioinvasions are increasing in response to expanding transportation network, habitat losses and climate change (Chan et al. 2019; Giakoumi and Pey 2017; Seebens et al. 2016). Extra-range dispersal of NIS involve diverse vectors (crafts and ships, marine debris, cultivated and bait species) and pathways, such as mass dispersal shipping routes and physical corridors due to the 'ocean sprawl' (i.e., artificial structures built along natural shores) (e.g. Bishop et al. 2017; Seebens et al. 2016). Regarding these conspicuous risks of introduction and establishment of new species, the apparent resistance of particular habitats or regions to species invasions is particularly meaningful to examine in order to determine the underlying ecological processes (Freestone et al. 2013), and thus to build up relevant and effective management strategies (Caselle et al. 2018; Dafforn et al. 2015).

Beyond alteration of species connectivity, the ocean sprawl is directly associated with habitat degradation and loss (Bishop et al. 2017). Artificial habitats, such as ports, do not surrogate the diversity and community structure of neighbouring rocky reefs. These habitats constitute a haven for novel species interactions—notably involving non-indigenous species (NIS)—the direction and intensity of which are poorly understood (Chapman and Underwood 2011; Leclerc and Viard 2018; Rogers et al. 2016). Exclusion experiments in such habitats are fortunately flourishing and provide important insights into spatial and temporal variations in consumer effects upon fouling development (e.g. Dumont et al. 2011b; Giachetti et al. 2019; Lavender et al. 2017). Despite invasion theories which predict that a species predisposition to predation may depend on its evolutionary history (e.g. "enemy release" vs. "new association", Colautti et al. 2004; Hokkanen and Pimentel 1989), there has been seldom study that separated consumer effects upon marine native species and NIS (Leclerc and Viard 2018; Rogers et al. 2016). Likewise, there is mounting evidence that all predators do not equally influence fouling community development at local scales (notably depending on their functional/taxonomic group and size) (Lavender et al. 2014; Osman and Whitlatch 1998; Rogers et al. 2016) and yet the implications of such findings for consumptive biotic resistance are so far elusive in marine systems.

In the SE Pacific and more specifically along the Chilean coastline, the number and extent of artificial structures have dramatically expanded over the last decades in order to promote economic development through shipping trade, mining and aquaculture, but also to protect coastal populations from storms and tsunamis (Aguilera 2018). In this region, shipping pathways have been assumed to be responsible for the introduction of 30–38% of the NIS currently established (Castilla and Neill 2009). A recent study comparing local and international ports along 100s km in central Chile did not find any evidence that these ports differed in propagule pressure and NIS abundances, suggesting a role for other introduction vectors such as aquaculture or a rapid spread from international to local ports (Leclerc et al. 2018). This latter work also revealed that most NIS, albeit relatively diverse, were scarce, generally restricted to cryptic micro-habitats (out of the reach of large predators) and less effective in colonizing bare substrata than native taxa. In addition, diverse native predators were observed but their influence (indirectly inferred by the abundance of grazing marks) seemingly varied among sites. As such, the authors pointed out that biotic resistance due to consumptive interactions deserved further dedicated investigation.

The present study aimed to determine the influence of predators on the diversity and structure of fouling communities in Chilean ports. Combining both experimental and observational approaches, we gathered data from rapid assessment surveys, an exclusion experiment and a community development experiment within three sites (out of four, see methods) of central Chile. Exclusion experiments targeting either macro- (> 1–2 cm) and micro-predators (> 1–2 mm) or macro-predators only (i.e., leaving only micro-predators as putative consumers) were run to (1) quantify their influence on early community development, upon settlement panels and (2) whether their effects were concentrated upon specific prey categories (i.e., taxonomic and functional groups, native versus non-indigenous taxa). The outcomes (i.e., targeted preys) of this short term experiment were then compared with abundance data from field surveys and from the established panel community, in order to determine whether consumptive biotic resistance upon specific taxa/groups is sustained over time and to give insights into other presumable indirect effects (competition, facilitation) influencing the overall biotic resistance of the study communities.

Materials and methods

The study was performed along approximately 100 km of coastline in the Biobío region (Chile) within four ports: Coronel (37.0304°S, 73.1540°W), San Vicente (36.7591°S, 73.1551°W), Lirquén (36.7094°S, 72.9829°W) and Coliumo (36.5377°S, 72.9571°W).

Predator exclusion design

A series of settlement panels (black polypropylene, 15 × 15 cm) were deployed vertically upon two experimental units (110 × 100 cm) made of a plastic fence (mesh 2.5 × 2.5 cm) covered by a mosquito net (ca. 1–2 mm mesh), on two randomly selected pilings (in direct contact with the bottom) separated by 20–50 m within each port, at ca.—4 m. Each unit was composed of 15 panels, randomly organized as triplicates undergoing five treatments: caged, cage-control, screened, screen-control and open. Cages (20 × 20 × 12 cm) were constructed from galvanized steel fence (diagonal mesh of ca. 2 cm), previously protected by water based-anticorrosive paint. Cage-controls were cages lacking a roof and with two windows (40 × 60 mm) cut out on the sides (Leclerc and Viard 2018). Screened and screen-control treatments were respectively made of the same matrix used for caged and cage-control treatments, but covered by a nylon mosquito net (1–2 mm mesh). Open treatments were panels without cages.

The experiment was conducted over four months, between late December 2017 and late March 2018 (austral summer), a season favourable to the settlement of juveniles of many invertebrate species, including NIS, in the study area (Leclerc et al. 2018) and to the maintenance of the experimental units (a first trial was attempted in the previous winter but most cages were damaged by wave action). To prevent flow and recruitment disruption by fouling organisms, cages and meshes were cleaned every 4 weeks using a plastic brush. This cleaning frequency was chosen based on previous knowledge and observations made on pilings with this type of thin material (for instance, lower colonization than on floating pontoons; Leclerc et al. in revision; Leclerc and Viard 2018). It is noteworthy that at each cleaning occasion, only biofilm and a few vines (e.g. *Bougainvillia muscus*) were observed on the fences and meshes, suggesting that the flow had been properly maintained between consecutive cleaning dates (i.e., limited obstruction, JCL, pers. obs.). By the end of the trial, experimental units were retrieved by divers. On land, panels were quickly collected and cleared from cable tiles. A few screened cages were damaged (2 in Lirquén and 1 in Coliumo). Because mobile predators were recorded on the panels of these screened cages, these replicates were put aside. All the other panels were kept in individual plastic rubble-bags within seawater tanks until being processed in the laboratory.

Community development upon panels and predators characterization

The predator exclusion experiment targeted the early community development. In order to put the results into a longer term perspective, we analysed the community development upon panels deployed in the field over a 26 months-period. In August 2016 (first trial) and March 2017 (second trial), 20 settlement panels (cf., above) were deployed upon two experimental units (90 × 100 cm), at two plots (pilings) per port. After 1, 3, 7, 13 and 19 months, eight panels (four at random per plot) were retrieved using meshed bags, and transported within seawater to the laboratory.

In order to complement the settlement panel datasets and to get a more comprehensive list of putative macro-predators of the study communities, we used rapid assessment surveys conducted by the same diver (JCL) in November 2016 and June 2017 in all localities. During 30 min, all taxa (including fouling species and mobile taxa > 10 mm) encountered were given a score of semi-abundance according to the SACFOR scale (Superabundant, Abundant, Common, Frequent, Occasional, Rare). These surveys were conducted between ca. − 1.5 m and − 5 m, and usually over the horizontal distance between the experimental plots depending on the site conformation.

Data collection

In the laboratory, panels were weighted (corrected wet mass) and then left in seawater tanks until sessile fauna returned to their natural untense state. Sessile taxa (mostly fauna, see Results) were identified, under a dissecting microscope, at the lowest taxonomic level possible by the same observer (JCL). The same observer made at the same time a rough identification of the mobile fauna, incl. micro-predators: amphipods, annelids, shrimps, crabs (likely including macro-predator juveniles) were observed. To avoid edge effects, a 15 mm perimeter was excluded from the analysis. The abundances of the sessile taxa were assessed using cover. Species cover was estimated under 100 random intersection points out of 169 created between evenly spaced lengths of string of quadrat within the working area (120 × 120 mm). 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%. Voucher specimens were collected and preserved in 95% EtOH in order to fill in a local reference collection (cf., Leclerc et al. 2018). Taxa were assigned to functional groups based upon their morphology and space occupancy as these traits efficiently classify epibenthic assemblages (Woodin and Jackson 1979). Specimens were also categorized according to their status as 'native', 'non-indigenous' (NIS), 'cryptogenic' or 'unassigned' according to the literature and public databases (cf. Leclerc et al. 2018 and references therein). The cryptogenic species, from unknown/uncertain origin, found in this study displayed a cosmopolitan distribution and were potentially non-indigenous to the study area.

Statistical analyses

For the exclusion experiment, patterns in species richness, abundance and community structure as well as species-specific abundances (cover, number) of relevant response variables (selected on the basis of their contribution to community structure, see details below) were examined with a three-way design using PERMANOVAs with 4999 permutations. Factors were 'treatment' (fixed, 5 levels: open, cage-control, screen-control, caged and screened), 'site' (random) and 'piling' (random, nested with site). Panels, cages and controls were designed to allow sessile fauna colonization through settlement. However, in one site (Coronel), panel colonization was largely due to migration of adult mussels (*Semimytilus algosus*) from the edges of the experimental units—a behaviour strongly limited by cages. Settlement and migration of sessile fauna were thus confounded in this site, which we thus excluded from the analyses. By including the blocking term 'piling', part of the total variance was attributed to differences between blocks, thereby reducing the residual unexplained variation (Quinn and Keough 2002). The lowest interaction term 'treatment × piling (site)' was however excluded from the main model for two reasons. Firstly, a few screened replicates were damaged and thus excluded from the analyses. The number of replicates was then too small to robustly test for a possible interaction between treatments and pilings. Secondly, and more importantly, with two pilings per site, pairwise comparisons for the most important two-way interaction 'site × treatment' (see Results) were limited to a single degree of freedom for each denominator (vs. nine *den. d.f.*, when excluded) and thus could not be made (Anderson et al. 2008). The results of the analyses based on the full model are provided in Table S4, with discussions about their implications on spatial variability in biotic resistance. Univariate analyses were based on Euclidean distance matrices (analogous to the traditional ANOVA) whereas multivariate analyses were based on Bray–Curtis similarity matrices generated from either raw or transformed data. In the case of univariate analyses of the cover and number of individuals, response variables were selected upon their within-site contribution to multivariate structure (SIMPER) at a cut-off level of 90% within at least one treatment. For these response variables, a minimal occurrence of three within at least one Treatment × Site was considered. Otherwise, the corresponding site was dropped off from the analysis. The homogeneity in univariate or multivariate dispersion was checked among the levels of the interaction term Site × Treatment using PERMDISP (Anderson et al. 2008). In most cases, no transformation allowed homoscedasticity to be achieved in univariate data, therefore analyses were run on untransformed data (Underwood 1997).

As for the long-term survey, patterns in community assembly were examined, within each site, with a three-way design including the factors 'trial' (random, 2 levels: 1st vs. 2nd), 'piling' (random, nested within trial) and 'age' (fixed, 5 levels: 1, 3, 7, 13, 19 months). When appropriate, PERMANOVAs were followed by pairwise comparisons and *P*-values were estimated using a Monte Carlo procedure. To make these pairwise tests possible for the most relevant interaction (age × trial, see Results), the lowest interaction term [age × piling (trial)] had to be excluded from the model (increasing the degrees of freedom of each denominator from 1 to 13).

Univariate analyses were performed either on all variables combined (including unassigned taxa), natives, cryptogenics or NIS. For all multivariate data, PERMANOVA results were supported by ordination using principal coordinate (PCO) analyses and the main taxa or abiotic variables (e.g., bare surface, grazing marks, dead biota) explaining differences among treatments were identified according to their contribution to PCO axes (Anderson et al. 2008). The respective contributions of specific variables to community structure (SIMPER analyses) in each level of the exclusion experiment and of the field survey were determined and considered throughout. All analyses were performed using PRIMER 7 (Anderson et al. 2008).

Results

Diversity of native and non-indigenous sessile taxa across the study ports

Across sites, a total of 56 sessile taxa were identified upon settlement panels used in the exclusion experiment (4 months), largely dominated by fauna (48 taxa). These records included 11 non-indigenous and 11 cryptogenic species, of which 4 NIS and 2 cryptogenic taxa were exclusively found under predator exclusion (Table S1). Over the panel community assembly survey (26 months), a similar number of sessile taxa was observed (60 taxa), including 8 NIS and 13 cryptogenic species, of which only 1 NIS and 2 cryptogenic species were not observed during the 4 months of the exclusion experiment (Table S1). Complete lists and species authorities are provided for sessile taxa in Table S1.

Macro-predators reported in the study localities are mostly native

Diverse macro-predators were identified across sites (35 taxa), third of which locally classified as frequent to abundant (Table S2, Figure S5). Of these predators, a total of 18 taxa were observed upon experimental structures by the end of the experiment (Table S2). The vast majority of them (31 taxa; 89%) were native taxa while the four others were unassigned. Locally dominant predators were the native sea urchins *Arbacia dufresnii* and *Tetrapygus niger* in Lirquén, the sea snail *Tegula euryomphala* and the sea star *Patiria chilensis* in Coliumo as well as the barnacle shell-dwelling fish *Hypsoblennius sordidus* in San Vicente, whereas species commonly found across all locations were highly mobile crabs such as *Romaleon setosum, Cancer plebejus* and *Taliepus dentatus*.

Exclusion treatments influence the fouling community development

Exclusion treatments significantly affected community structure over the four months of the exclusion experiment, across all study sites (Tables 1, 2, Table S4). Caged and screened treatments differed from each other, and from all other treatments (Table 1). As compared to cage-control, the caged treatment (i.e., macro-predators exclusion) resulted in fouling assemblages reaching significantly greater biomass (Fig. 1a), and occupying more space in two of three sites (Fig. 1b, c). In contrast, the full exclusion of both macro- and micro-predators (screened treatment versus screen-control) led to variable results among sites (Fig. 1). In particular, the biomass increased significantly in Coliumo only (Fig. 1a). In addition, surprisingly, the screening negatively affected both the biomass (Fig. 1a) and the occupied space (Fig. 1c), concomitantly to an increase in bare space (Fig. 1b), in San Vicente. This later result suggested that the recruitment may have been limited locally by the complete envelopment of panels with the thin mesh used.

Taxa-specific responses to predator exclusion

The exclusion performed had an effect upon species richness, but the outcomes were ambiguous considering all taxa (Table 2, Table S4, Fig. 1d). Interestingly, only the non-indigenous species richness was influenced by the exclusion experiment (i.e., native and cryptogenic richness were not affected Table 2, Table S4, Fig. 2a–c). As mentioned above, it is noteworthy that 27% of the NIS and cryptogenic species were recorded only when predators were excluded (Table S1). As compared to open treatment, NIS richness was greater under macro-predators exclusion (i.e., caged treatment) in Lirquén and Coliumo (Fig. 2a, b, Fig. S6). Micro- and macro-predators exclusion (i.e., screen treatment) reduced NIS richness in all sites (Fig. 2a–c), though there may have been a screening effect in San Vicente (Fig. S6).

Irrespective of the group of taxa considered, total species abundances (covers) were affected by the exclusion experiment, though patterns differed among sites ('site × treatment' interactions, Table 2, Fig. 2a–c, see also Table S4 and Fig. S6). Overall, predator exclusion increased the cover of either NIS, cryptogenic taxa or both in the three sites (Fig. 2, Fig. S6). Altogether, the more drastic biotic resistance due to predation was observed in Lirquén, where macro- and micro-predator exclusions respectively led to 73% and 21% increases in NIS cover (Fig. 2a, Fig. S6). According to the principal component analysis (PCO, Fig. 2a), the respective effects of macro- and micro-predators could be responsible of ca. 56% (cf., ordination along axis 1) and 16% (axis 2) of variation in community structure in Lirquén. Examining the variables correlated to the different treatments suggests species-specific responses to predation across sites (Fig. 2a–c), which were further scrutinized by univariate analyses (Table S5, summarized in Fig. 3). Site-specific effects of treatments were detected on 3 natives (18%), 2 cryptogenics (18%) and 5 NIS (45%). The strongest biotic

Table 1 Results of PERMANOVA test for differences in fouling community structure among levels of the main factors (site and exclusion) and their interaction (PERMDISP T × S: $F_{14,72} = 6.587$, $P < 0.001$). Post-hoc pairwise tests are summarized

Factor	*df*	SS	MS	Pseudo-*F*	*P*
Site = S	2	123,510	61,756.0	25.890	< 0.001
Plot (site) = P	3	7161	2387.0	4.473	< 0.001
Treatment = T	4	23,011	5752.8	1.571	0.114
T × S	8	30,522	3815.2	6.628	< 0.001
Res	69	36,824	533.7		
Pairwise tests					
Lirquén	Op = CC = SC ≠ Cag ≠ Scr				
Coliumo	Op = CC = SC ≠ Cag ≠ Scr				
San Vicente	Op = CC = SC ≠ Cag ≠ Scr				

Table 2 Results of PERMANOVA tests for differences in response variables among levels of the main factors and their interaction

Response variable	Transf.	PERMANOVA Factor (*df* total = 86)				PERMDISP
		Site = S (2)	Plot (site) = P (3)	Treatment = T (4)	T × S (8)	
Community structure	SqRT	25.890***	4.473***	1.571^{ns}	6.628***	***
Biomass (g)	None	38.703***	1.169^{ns}	1.324^{ns}	10.394***	ns
Bare surface (%)	None	439.370***	0.191^{ns}	1.281^{ns}	9.897***	***
Abundances (%)						
All	None	50,888.0***	0.001^{ns}	1.537^{ns}	8.649***	ns
Native	None	2129.9***	0.193^{ns}	1.577^{ns}	12.189***	***
Cryptogenic	FoRT	6.360^{ns}	3.914*	0.174^{ns}	5.408***	ns
NIS	None	53.350*	1.523^{ns}	3.031^{ns}	4.434***	***
Richness S						
All	None	7.001*	4.732**	1.168^{ns}	2.814*	ns
Native	None	7.966^{ns}	1.955^{ns}	2.316^{ns}	1.557^{ns}	ns
Cryptogenic	SqRT	0.389^{ns}	11.300***	0.411^{ns}	0.855^{ns}	ns
NIS	None	8.658***	7.220***	4.771*	2.917**	ns

Transformations (Transf.) and PERMDISP tests (T × S) are summarized

SqRT square root transformed, *FoRT* fourth root transformed

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

^{ns}Non-significant at $\alpha = 0.05$

resistance played by macro-predators was observed in Lirquén, where 6–10 fold-reduction in the cover of colonial NIS, such as *B. muscus* and *Bugulina flabelatta*, were observed. In contrast, the effect of macro-predators was mainly concentrated upon native barnacles (up to fourfold-reduction) in San Vicente. Along with total cover and biomass reduction mentioned above (Fig. 1), exclusion of micro- and macro-predators led to an unexpected decrease in the cover of *Balanus laevis* in two sites and *Amathia* cf. *gracilis* in one of them (Fig. 3), suggesting that the full screened caged locally limited their recruitments, especially in San Vicente where the bare surface was greater in this treatment compared to the others. Nonetheless, the full predator exclusion had clearer effects (according to pairwise tests) on the abundance of diverse non-indigenous and cryptogenic species, such as the bryozoans *Bugula neritina, Exochella* sp. nov., and three ascidians: *Corella eumyota*, *Ciona robusta*, and *Diplosoma listerianum*—the latter two species being virtually absent from treatments exposed to predators in Lirquén and Coliumo, respectively (cf. infinite fold-change values, Fig. 3).

Panel community changes over time and under predation

Regarding the long-term settlement panel survey, the deployment date resulted in contrasting community development (significant two way-interaction "Trial × Age") in Lirquén (Pseudo-$F_{4,68} = 14.04$, $P < 0.001$, Fig. 4a), Coliumo (Pseudo-$F_{4,68} = 9.69$, $P < 0.001$, Fig. 4b) and San Vicente (Pseudo-$F_{4,68} = 17.56$, $P < 0.001$, Fig. 4c). Irrespective of the trial, important differences in community structure were however observed in all sites according to the time elapsed since the deployment (pairwise tests: $1 \neq 3 \neq 7 \neq 13 \neq 19$ months with P_{MC} values ranging from < 0.001 to 0.01). In all study sites, the first axis of the principal coordinate analyses—respectively responsible of 34.0, 39.3 and 30.0% of the total variation in Lirquén (Fig. 4a), Coliumo (Fig. 4b) and San Vicente (Fig. 4c)—illustrated the overall pattern of development of the panel communities from 1 month (dominated by bare surface, on the left in Fig. 4) to 19 months (dominated by native and a few cryptogenic species, on the right in Fig. 4).

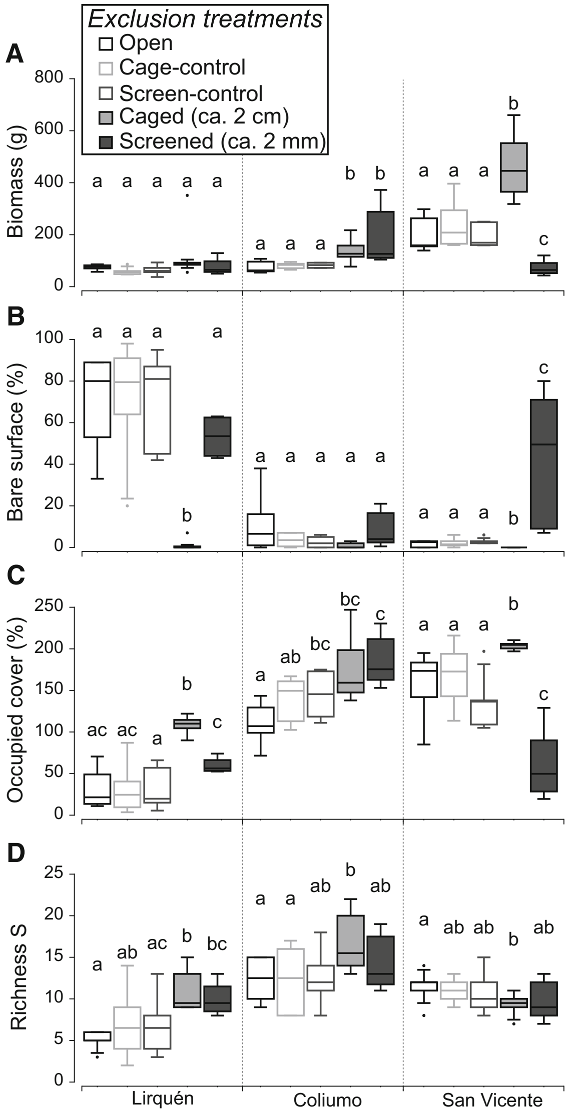


Fig. 1 Biomass (**a**), bare surface cover (**b**), occupied cover (**c**) and species richness (**d**) on experimental panels across treatments and sites. Upper letters indicate differences among treatments within each study site

Of the seven NIS that contributed to the multivariate structure in the exclusion experiment and were affected by predators (Fig. 2 and above), only two contributed to the long term community dynamics using the same criteria ($r > 0.5$): *B. muscus* in Lirquén and *Exochella* sp. nov. in Coliumo. It is noteworthy that in the very same sites, these two species were also the main NIS contributors to the community structure under predator influence after 4 months of the exclusion experiment (9.8 and 28.4% of total similarity, respectively, Fig. 3). In Lirquén, the maximal contributions of *B. muscus* to the community structure were observed on 3 months-old panels from the first trial (29.7%, SIMPER analyses) and 7 months-old panels from the second one (40.9%, axis 2 on Fig. 4a), respectively sampled at the same period (spring) of two consecutive years (November 2016 and October 2017). In this site, the cumulated contribution of other NIS ranged from 0% (12 months—trial 1) to 11.3% (12 months—trial 2) due to occasional emergence of *B. flabelatta* and *Hydractinia* sp. In Coliumo, maximal contributions of *Exochella* sp. nov. were observed after 13 (26.0%) and 7 months (15.7%) of the first and second trials, respectively (Fig. 4b). In this site, the cumulated contribution of other NIS remained < 3% all over the survey. Likewise, it is noteworthy that *Exochella* sp. nov. contributed up to 15.5% of the community structure in San Vicente on one occasion (7 months—second trial) while the cumulated contribution of all other NIS never exceeded 2% in this site. In parallel, the cumulated contribution of native species generally increased over community development: 60–70% at the end of the first trial and 28–31% at the end of the second one—when the contribution of either cryptogenic species (e.g. *Clytia linearis* in Lirquén, *Amphisbetia operculata* in San Vicente) or the bare space (in Coliumo) was locally important.

Discussion

While similar numbers of sessile taxa were observed during the short-term exclusion and the long-term panel experiments, a greater number of non-indigenous species (NIS) was observed upon settlement panels after the 4 months-duration of the exclusion experiment than over the 26 months of the colonization survey, during which the panels were left exposed to predators. Overall, the predation affected the early stage of community development. Micro- and macro-predators together reduced the abundance of most NIS and cryptogenic species, of which six taxa were only recorded under predator exclusion. This result, associated with the poor contribution of NIS to the community structure observed over more than 2 years of colonization experiment, strongly suggests consumptive biotic resistance in the study systems.

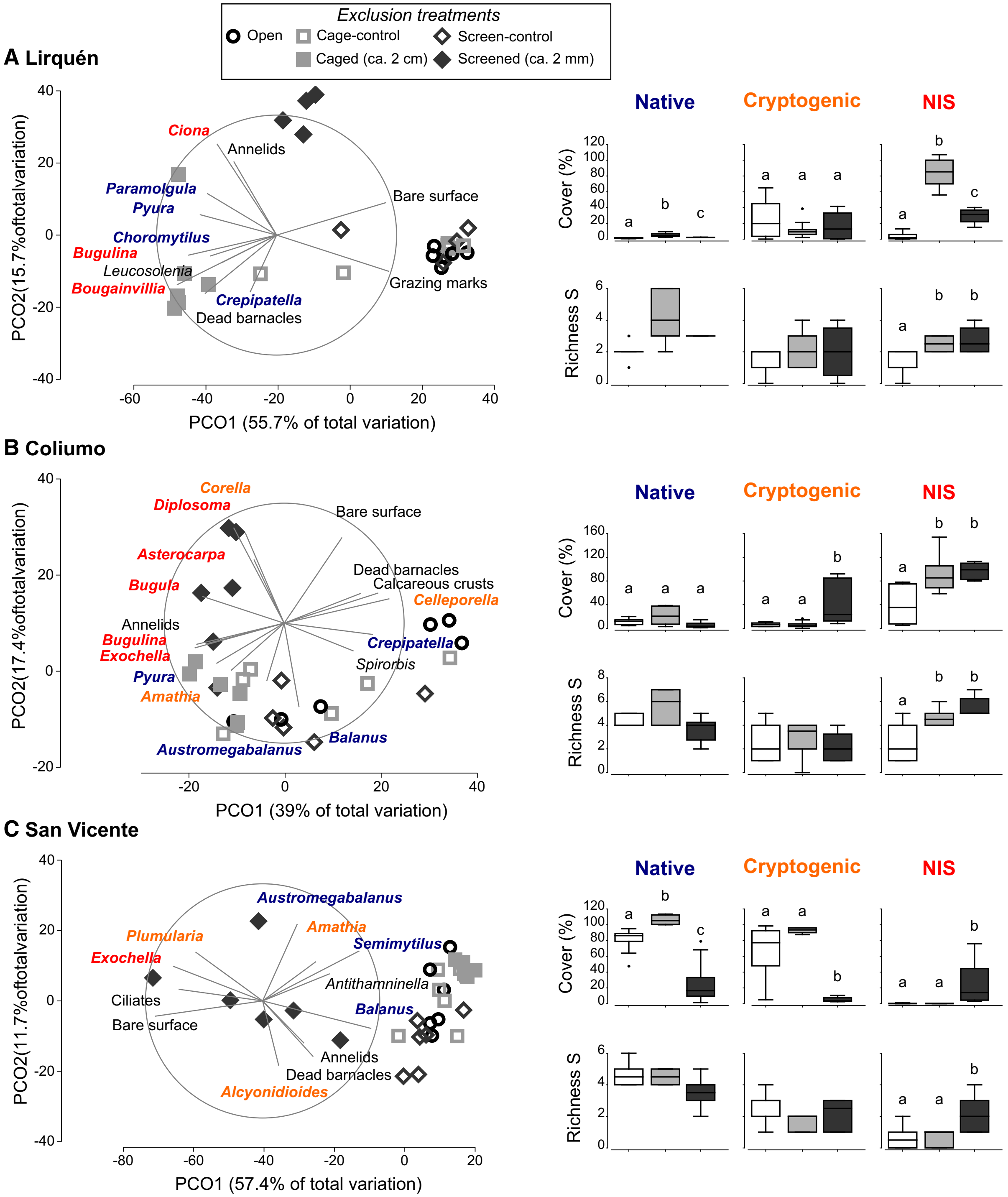
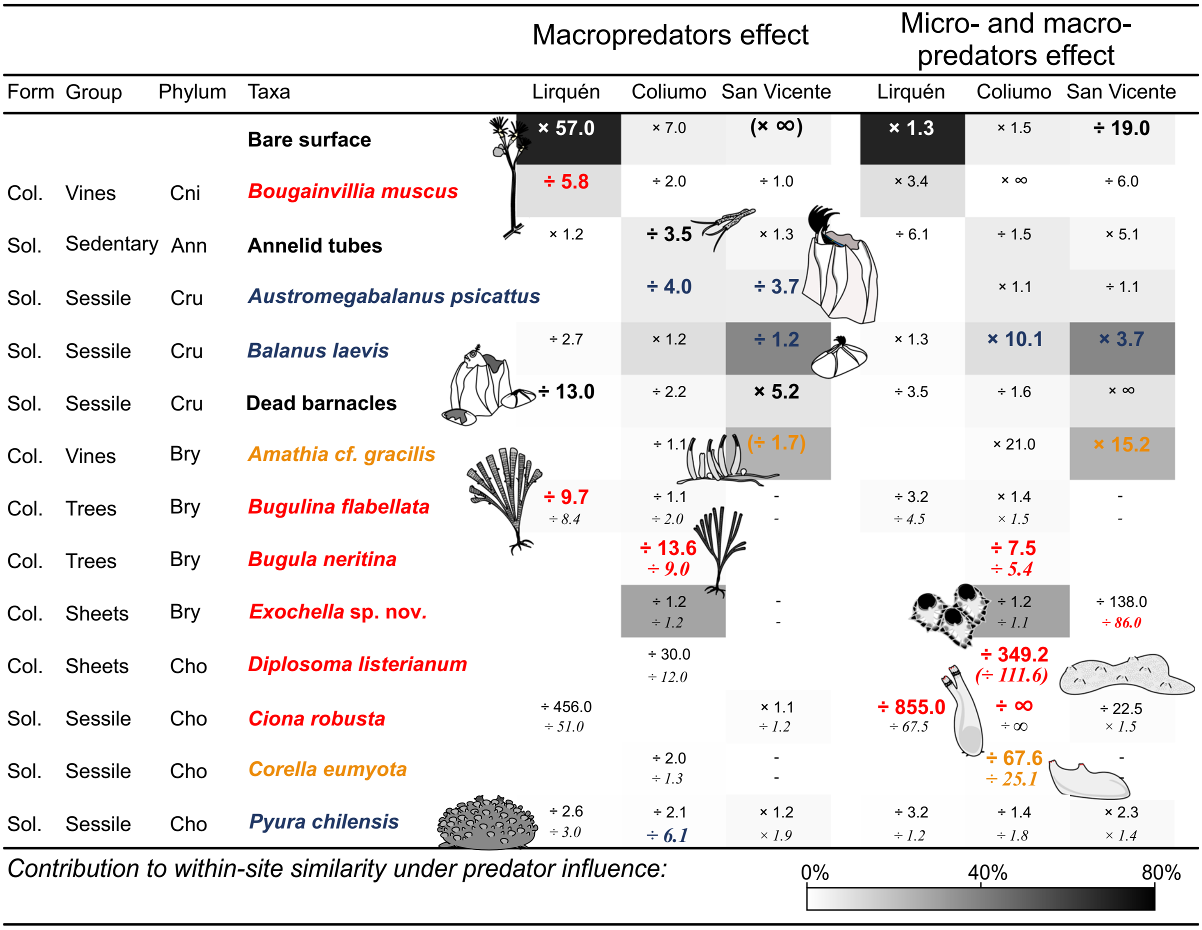


Fig. 2 Community structure (principal coordinate analyses, left panels), richness and abundances (right panels) of native (blue), cryptogenic (orange) and non-indigenous taxa (red) across treatments in each site. Detailed graphs, including control treatments (CC, SC), can be found in Figure S6. Vector plots of variables correlated with the PCO axes are indicated, with r > 0.5. Only the genus name is given. Note that scales differ among site plots



				Macropredators effect			Micro- and macro-predators effect		
Form	Group	Phylum	Taxa	Lirquén	Coliumo	San Vicente	Lirquén	Coliumo	San Vicente
			Bare surface	**× 57.0**	× 7.0	**(× ∞)**	**× 1.3**	× 1.5	**÷ 19.0**
Col.	Vines	Cni	***Bougainvillia muscus***	**÷ 5.8**	÷ 2.0	÷ 1.0	× 3.4	× ∞	÷ 6.0
Sol.	Sedentary	Ann	**Annelid tubes**	× 1.2	**÷ 3.5**	× 1.3	÷ 6.1	÷ 1.5	× 5.1
Sol.	Sessile	Cru	***Austromegabalanus psicattus***		**÷ 4.0**	**÷ 3.7**		× 1.1	÷ 1.1
Sol.	Sessile	Cru	***Balanus laevis***	÷ 2.7	× 1.2	**÷ 1.2**	× 1.3	**× 10.1**	**× 3.7**
Sol.	Sessile	Cru	**Dead barnacles**	**÷ 13.0**	÷ 2.2	**× 5.2**	÷ 3.5	÷ 1.6	× ∞
Col.	Vines	Bry	***Amathia cf. gracilis***		÷ 1.1	**(÷ 1.7)**		× 21.0	**× 15.2**
Col.	Trees	Bry	***Bugulina flabellata***	**÷ 9.7** *÷ 8.4*	÷ 1.1 *÷ 2.0*	- -	÷ 3.2 *÷ 4.5*	× 1.4 *× 1.5*	- -
Col.	Trees	Bry	***Bugula neritina***		**÷ 13.6** ***÷ 9.0***			**÷ 7.5** ***÷ 5.4***	
Col.	Sheets	Bry	***Exochella* sp. nov.**		÷ 1.2 *÷ 1.2*	- -		÷ 1.2 *÷ 1.1*	÷ 138.0 ***÷ 86.0***
Col.	Sheets	Cho	***Diplosoma listerianum***		÷ 30.0 *÷ 12.0*			**÷ 349.2** ***(÷ 111.6)***	
Sol.	Sessile	Cho	***Ciona robusta***	÷ 456.0 *÷ 51.0*		× 1.1 *÷ 1.2*	**÷ 855.0** *÷ 67.5*	**÷ ∞** *÷ ∞*	÷ 22.5 *× 1.5*
Sol.	Sessile	Cho	***Corella eumyota***		÷ 2.0 *÷ 1.3*	- -		**÷ 67.6** ***÷ 25.1***	- -
Sol.	Sessile	Cho	***Pyura chilensis***	÷ 2.6 *÷ 3.0*	÷ 2.1 ***÷ 6.1***	× 1.2 *× 1.9*	÷ 3.2 *÷ 1.2*	÷ 1.4 *÷ 1.8*	× 2.3 *× 1.4*

Contribution to within-site similarity under predator influence:

Fig. 3 Summary of the major species-specific and abiotic variable responses to predation (cf. Table S5). Color code is as in Fig. 2. Predation effects are presented as fold-changes of the species cover (normal font) and of the numerical abundance (italic). Fold-changes were obtained by comparing the average values of open and control panels with caged/screened treatments. Significant changes are in bold, with numbers in bracket indicating a possible caging effect. "-" indicates that the species is present but with an occurrence too small to make statistical inferences. Following the scale presented, backward shade indicates the average within-site contribution of each taxon/response variable to the community upon open and control panels (i.e., exposed to all predators)

Towards novel natural enemies

Besides the enemy release hypothesis which is the foundation of invasive species management by biological control, the new association principle emerged as a framework where biocontrol capitalizes on consumptive and "native" biotic resistance (Colautti et al. 2004; Hokkanen and Pimentel 1989). Such approach does not involve the introduction of a new species, albeit a natural enemy, and could thus avoid dramatic drawbacks (Elton 1958; Pearson and Callaway 2003). The new association principle stands on the hypothesis that a NIS is a naive prey/host, thus likely less efficient to defend itself against a novel predator/enemy.

In our exclusion experiment, all non-indigenous (and cryptogenic) ascidians were affected by predators in terms of cover and number of individuals whereas the native ascidians did not virtually suffer from any type of predators, except *Pyura chilensis* in Coliumo (Figs. 2, 3). This pattern was sustained over the long term survey where the native ascidian *P. chilensis* progressively became one of the dominant space occupiers in the other sites, i.e. Lirquén (cover: 20.6 ± 39.2% after 18 months, pooled for trials ± SD) and San Vicente (54.1 ± 46.8%), whereas

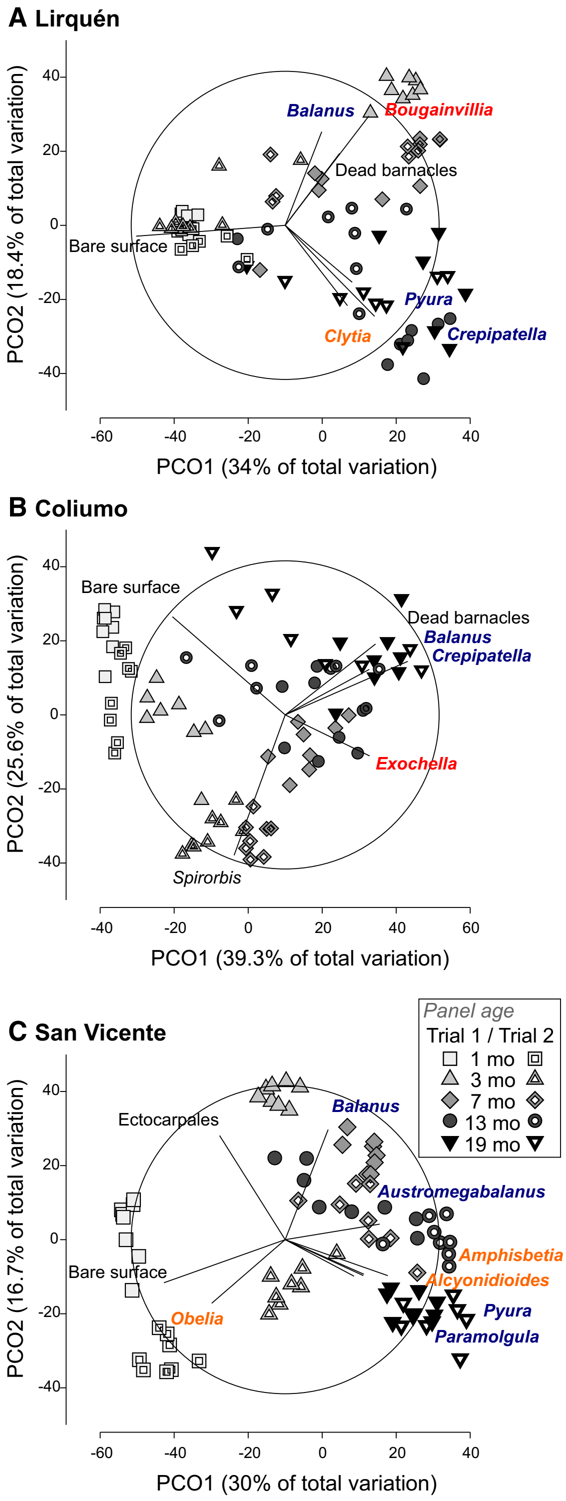


Fig. 4 Temporal variation in community structure (PCO) upon panels deployed in August 2016 (trial 1) and March 2017 (trial 2) in each study site. Color code in vector plots are as in Fig. 2

non-indigenous ascidians remained virtually absent upon panels (Fig. 4)—other than rare records of *Ciona robusta* juveniles in Lirquén.

While predators can efficiently regulate post-settlement survival of ascidians, they can also be quite selective (Nydam and Stachowicz 2007; Osman and Whitlatch 1995; Rius et al. 2014). In New England, both micro-gastropods *Cotonopsis lafresnayi* and *Astyris lunata* limited the survival of various non-indigenous or native ascidian recruits, such as *Botryllus schlosseri* and *Diplosoma* sp., although *Botrylloides diegensis* was generally avoided (Osman and Whitlatch 1995). In addition, traits that facilitates rapid colonization are often accompanied by costs to competitive advantage, such as defense against predators (Papacostas et al. 2017). As it grows, *P. chilensis* develops a thick cellulosic tunic and resist attacks from most local predators in northern Chile (Dumont et al. 2011a). Although other possible defense mechanisms may be involved (e.g. Stoecker 1980), non-indigenous and fleshy ascidians (e.g., *Ciona robusta*) were herein apparently more naive to predation than their native counterpart, and thus not released from their new enemies.

Consumptive biotic resistance might vary according to predator diversity

Although outcomes vary among habitats, the body of knowledge accumulated in terrestrial systems generally supports the hypothesis that the more diverse are natural enemies, the more likely they are to control pests (Letourneau et al. 2009). In the framework of the complementarity model, these previous results also suggest that additive (e.g., synchrony) and synergistic (e.g., facilitation) effects among enemies can be stronger than antagonistic effects (e.g., due to intraguild predation) at high richness. Notwithstanding all emergent implications of such finding for biotic resistance, the influence of predator diversity upon marine invaders has so far seemingly been generally overlooked (Byrnes and Stachowicz 2009; Dumont et al. 2011b for multiple-predator experiments in fouling communties; but see Nydam and Stachowicz 2007). Our caging experiment showed that macro-predators were responsible for a sharp reduction in abundance of the introduced vine hydrozoans (e.g., *Bougainvillia*) and tree-like bryozoans (e.g., *Bugulina*), in two of the three study sites. While this pattern is consistent with results from previous studies (Dumont et al. 2011b; Osman and Whitlatch 1998), further work would be needed to separate the effects of predator categories and to determine their interactions upon these focal invaders. Indeed, micro-predators could not be excluded without excluding also macro-predators. In addition, screening may have locally limited the recruitment of some taxa (as suggested by the variations of bare surface in San Vicente). Even so, we clearly observed a collapse of non-indigenous ascidians in the presence of micro-predators (i.e., in all treatments except screened) in every study sites. Because they presumably forage on different preys (and possibly on different life stages, Dumont et al. 2011a; Rius et al. 2014), it may be worth scrutinizing whether micro- and macro-predator effects are complementary at the assemblage level. This aspect may be critical for the biotic resistance in the study area, with regards to the non-indigenous status of most of these preys and their poor contribution to community structure over long-term community development and in established communities. A thorough examination of the associated food web would be needed to examine further this hypothesis which could have implications for impact mitigation and NIS establishment management strategies (Smith-Ramesh et al. 2017).

Biotic resistance is amongst the main targeted ecological services of eco-engineering in the context of ocean sprawl. Promoting the local abundance of invader-enemies seems a promising avenue (Bishop et al. 2017; Dafforn et al. 2015), as our results also mirror. The outcomes of consumptive biotic resistance may however be particularly conflicting in realistic food webs, especially interacting with species exploitation (Caselle et al. 2018). Both macro-predators (all crabs identified) and native sessile taxa (incl. ascidians, barnacles and mussels) are fished in the study region, including within ports. One can thus wonder whether the concomitant regional changes in habitats (ocean sprawl, e.g. Aguilera 2018) and fisheries (Andreu-Cazenave et al. 2017) may interact and to which extent it might decrease the biotic resistance herein reported—hypothesis that could be addressed through further experimental and modelling approaches.

Predator escapes and maintenance of discrete invader populations

Ecological interactions have the potential to limit the abundance of NIS, but rarely enable communities to fully resist biological invasions (Levine et al. 2004). While predators partially constrained the cover of the introduced hydrozoan *B. muscus* in Lirquén, they also liberated 10-fold as much bare surface (Fig. 3) available to colonization, notably by colonial species in place. Over the long term survey, *B. muscus* consistently bloomed over two subsequent springs (Fig. 4). Whether it was due to specific invader traits (e.g. phenology in reproduction and/or vegetative growth) or temporal variability in predator effects—here likely mainly due to the native sea urchin *Arbacia dufresnii* (Leclerc et al. 2018), our results suggest that biotic resistance may have complex dynamics in fluctuating environments (Stachowicz and Byrnes 2006). In the recently constructed jetty of Coliumo, where the bryozoan *Exochella* sp. nov. has been recorded for the first time within the study region (in March 2017, Leclerc et al. 2018), none of the exclusion treatments affected its abundance. This species became one of the dominant space-occupiers on well-developed panel assemblages (7–13 months) and established communities in surrounding habitats (Table S3). After a first record in September 2017, *Exochella* sp. nov. also contributed substantially to community structure in San Vicente, where the species apparently met at least one efficient enemy—likely absent from Coliumo—among micro-predators (Fig. 3). Although both our experiments suggest that the proliferation of this species may be limited locally by consumptive and/or competitive biotic resistance, they also suggest that none of these processes could have prevented its establishment. Besides, *Exochella* sp. nov. was also found as epibiont of *Crepipatella fecunda* and *B. laevis*, the dominant space occupiers at Coliumo in older assemblages (19 months, Fig. 4), suggesting that, even facultative, mutualistic interactions could impede biotic resistance processes over community assembly (Bulleri et al. 2008).

It is finally worth emphasizing that even non-indigenous ascidians, which experienced severe predation pressure on pilings at the depth investigated, were occasionally observed as few individuals or colonies (Table S3, Fig. S5) in diverse micro-habitats (ropes, buoys, cavities, jetty stairwells), likely out of the reach of most predators (Dumont et al. 2011a; Rogers et al. 2016). As our full model also suggested, exclusion treatments could have contrasting effects across pilings on community structure and on a few response variables (Table S4). Further work would be necessary to disentangle between within site-variability in predation and propagule pressures, among other confounding factors. While drastic abundance reduction can mitigate both the negative effects an invader may have on a focal habitat and its probability of spread (Levine et al. 2004), our results altogether suggest that, across multiple scales, both spatial and temporal variability in biotic resistance may provide invaders with escape opportunities to establish discrete and viable populations.

In conclusion, our experimental study showed that the abundance of most NIS was reduced under predation within the study region. Moreover, some NIS were only recorded when predators were excluded. In light of a two-year survey of panel colonization and field censuses, our results suggest that this consumptive biotic resistance is sustainable. Further work is however needed to determine whether this predation effect also influences biotic interactions (competition, mutualism) among fouling species. Our study also suggests some complementarity among predator categories (here according to their size). Unraveling local food webs and predator functions in urban areas might be helpful to develop NIS management strategies.

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Authors' contributions JCL, FV and AB conceived the idea and designed the study. JCL designed the methodology, collected the data and led the writing of the manuscript. FV and AB significantly contributed to manuscript writing and critical review.

Data accessibility Most of the data are provided in the supplementary material associated with the manuscript. Detailed data are available from the corresponding author upon reasonable request.

References

Aguilera MA (2018) Artificial defences in coastal marine ecosystems in Chile: opportunities for spatial planning to mitigate habitat loss and alteration of the marine community structure. Ecol Eng 120:601–610

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA + for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth

Andreu-Cazenave M, Subida MD, Fernandez M (2017) Exploitation rates of two benthic resources across management regimes in central Chile: evidence of illegal fishing in artisanal fisheries operating in open access areas. PLoS ONE 12:e0180012

Bishop MJ, Mayer-Pinto M, Airoldi L, Firth LB, Morris RL, Loke LH, Hawkins SJ, Naylor LA, Coleman RA, Chee SY (2017) Effects of ocean sprawl on ecological connectivity: impacts and solutions. J Exp Mar Biol Ecol 492:7–30

Bulleri F, Bruno JF, Benedetti-Cecchi L (2008) Beyond competition: incorporating positive interactions between species to predict ecosystem Invasibility. PLoS Biol 6:e162

Byrnes JE, Stachowicz JJ (2009) The consequences of consumer diversity loss: different answers from different experimental designs. Ecology 90:2879–2888

Caselle JE, Davis K, Marks LM (2018) Marine management affects the invasion success of a non-native species in a temperate reef system in California, USA. Ecol Lett 21:43–53

Castilla JC, Neill PE (2009) Marine bioinvasions in the Southeastern Pacific: status, ecology, economic Impacts, conservation and management. In: Rilov G, Crooks J (eds) Biological invasions in marine ecosystems. Springer, Berlin, pp 439–457

Chan FT, Stanislawczyk K, Sneekes AC, Dvoretsky A, Gollasch S, Minchin D, David M, Jelmert A, Albretsen J, Bailey SA (2019) Climate change opens new frontiers for marine species in the Arctic: current trends and future invasion risks. Glob Change Biol 25:25–38

Chapman MG, Underwood AJ (2011) Evaluation of ecological engineering of "armoured" shorelines to improve their value as habitat. J Exp Mar Biol Ecol 400:302–313

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecol Lett 7:721–733

Connell SD, Ghedini G (2015) Resisting regime-shifts: the stabilising effect of compensatory processes. Trends Ecol Evol 30:513–515

Dafforn KA, Glasby TM, Airoldi L, Rivero NK, Mayer-Pinto M, Johnston EL (2015) Marine urbanization: an ecological framework for designing multifunctional artificial structures. Front Ecol Environ 13:82–90

Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: a general theory of invasibility. J Ecol 88:528–534

Dumont C, Gaymer C, Thiel M (2011a) Predation contributes to invasion resistance of benthic communities against the non-indigenous tunicate *Ciona intestinalis*. Biol Invasions 13:2023–2034

Dumont CP, Harris LG, Gaymer CF (2011b) Anthropogenic structures as a spatial refuge from predation for the invasive bryozoan *Bugula neritina*. Mar Ecol Prog Ser 427:95–103

Elton CS (1958) The ecology of invasions by plants and animals. Methuen, London

Freestone AL, Ruiz GM, Torchin ME (2013) Stronger biotic resistance in tropics relative to temperate zone: effects of predation on marine invasion dynamics. Ecology 94:1370–1377

Giachetti CB, Battini N, Bortolus A, Tatián M, Schwindt E (2019) Macropredators as shapers of invaded fouling communities in a cold temperate port. J Exp Mar Biol Ecol 518:151177

Giakoumi S, Pey A (2017) Assessing the effects of marine protected areas on biological invasions: a global review. Front Mar Sci 4:49

Hokkanen HMT, Pimentel D (1989) New associations in biological control: theory and practice. Can Entomol 121:829–840

Kimbro DL, Cheng BS, Grosholz ED (2013) Biotic resistance in marine environments. Ecol Lett 16:821–833

Lavender JT, Dafforn KA, Johnston EL (2014) Meso-predators: a confounding variable in consumer exclusion studies. J Exp Mar Biol Ecol 456:26–33

Lavender JT, Dafforn KA, Bishop MJ, Johnston EL (2017) An empirical examination of consumer effects across twenty degrees of latitude. Ecology 98:2391–2400

Leclerc J-C, Viard F (2018) Habitat formation prevails over predation in influencing fouling communities. Ecol Evol 8:477–492

Leclerc J-C, Viard F, González Sepúlveda E, Diaz C, Neira Hinojosa J, Pérez Araneda K, Silva F, Brante A (2018) Non-indigenous species contribute equally to biofouling communities in international vs local ports in the Biobío region, Chile. Biofouling 34:784–799

Leclerc J-C, Viard F, González E, Diaz C, Neira J, Pérez K, Silva F, Brante A (in revision) Habitat type drives the distribution of non-indigenous species in fouling communities regardless of associated maritime traffic. Div Distrib

Letourneau DK, Jedlicka JA, Bothwell SG, Moreno CR (2009) Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annu Rev Ecol Evol Syst 40:573–592

Levine JM, Adler PB, Yelenik SG (2004) A meta-analysis of biotic resistance to exotic plant invasions. Ecol Lett 7:975–989

Lockwood JL, Hoopes MF, Marchetti MP (2013) Invasion ecology. Wiley, Hoboken

Morin PJ (2011) Predation and communities: empirical patterns. Community ecology. Wiley, Hoboken, pp 90–119

Nydam M, Stachowicz JJ (2007) Predator effects on fouling community development. Mar Ecol Prog Ser 337:93–101

Osman R, Whitlatch R (1995) Predation on early ontogenetic life stages and its effect on recruitment into a marine epifaunal community. Mar Ecol Prog Ser 117:111–126

Osman RW, Whitlatch RB (1998) Local control of recruitment in an epifaunal community and the consequences to colonization processes. Hydrobiologia 375:113–123

Papacostas KJ, Rielly-Carroll EW, Georgian SE, Long DJ, Princiotta SD, Quattrini AM, Reuter KE, Freestone AL (2017) Biological mechanisms of marine invasions. Mar Ecol Prog Ser 565:251–268

Pearson DE, Callaway RM (2003) Indirect effects of host-specific biological control agents. Trends Ecol Evol 18:456–461

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge

Rius M, Potter EE, Aguirre JD, Stachowicz JJ (2014) Mechanisms of biotic resistance across complex life cycles. J Anim Ecol 83:296–305

Rogers TL, Byrnes JE, Stachowicz JJ (2016) Native predators limit invasion of benthic invertebrate communities in Bodega Harbor, California, USA. Mar Ecol Prog Ser 545:161–173

Rooney N, McCann KS (2012) Integrating food web diversity, structure and stability. Trends Ecol Evol 27:40–46

Seebens H, Schwartz N, Schupp PJ, Blasius B (2016) Predicting the spread of marine species introduced by global shipping. Proc Natl Acad Sci USA 113:5646–5651

Smith-Ramesh LM, Moore AC, Schmitz OJ (2017) Global synthesis suggests that food web connectance correlates to invasion resistance. Glob Change Biol 23:465–473

Stachowicz JJ, Byrnes JE (2006) Species diversity, invasion success, and ecosystem functioning: disentangling the influence of resource competition, facilitation, and extrinsic factors. Mar Ecol Prog Ser 311:251–262

Stoecker D (1980) Chemical defenses of ascidians against predators. Ecology 61:1327–1334

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge

Woodin S, Jackson J (1979) Interphyletic competition among marine benthos. Am Zool 19:1029–1043