

# Invasive ascidians: How predators reduce their dominance in artificial structures in cold temperate areas

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## ABSTRACT

Artificial structures can provide suitable space for invasive species through a colonisable surface and refuge against predators. Besides several physical factors, invasive species can present different colonization success and dominance on structures that are connected to the sea floor (i.e. fixed) compared to structures that are suspended, depending on the differential predation pressure that affect both structures. Benthic and nektonic predators can reach fouling communities on fixed structures, while only nektonic predators can affect communities growing on suspended structures, depending on the distance to the sea floor. Only few studies tested both communities of predators at the same time in different artificial structures. In this study, we evaluated the effect of benthic and nektonic predators on the cover, composition and diversity on fouling communities and on the dominance of invasive ascidians on these communities, in two different types of artificial structures. We performed an experiment in the port area of Puerto Madryn (Southwestern Atlantic, Argentina) to compare the fouling community development between fixed and suspended structures and among different predator exclusion treatments. Results showed that benthic predators exerted a higher predation pressure than nektonic predators on the cover, composition and diversity of the fouling communities. In the absence of benthic predators, and even where nektonic predators were not excluded, communities were greatly dominated by two invasive ascidians, *Ascidella aspersa* (Müller, 1776) and *Ciona robusta* Hoshino and Tokioka, 1967. Our results suggest that in this type of cold temperate ports, fouling organisms find refuge from benthic predators growing on suspended structures, where nektonic predators exert low predation pressure. We propose that, since benthic predators in temperate latitudes have an essential role decreasing invasive ascidians dominance and the cover of fouling communities, future studies should consider both predator communities, i.e. nektonic and benthic, in experimental designs performed to test biotic resistance and predation pressure.

## 1. Introduction

The construction of marine artificial structures, such as jetties, buoys, harbours or breakwaters largely modified the coastal geomorphology worldwide providing new habitats and new “niche opportunities” for invasive species (Airoidi and Bulleri, 2011; Dafforn et al., 2012; Airoidi et al., 2015). Indeed, these structures can host a higher number of invasive species compared to the nearby natural rocky reefs (Glasby et al., 2007). Artificial structures can provide new limiting resources, such as colonisable space, refuge from natural enemies, and favourable physical and chemical conditions for the settlement of invasive species (Dafforn, 2017). In this way, artificial structures are “stepping stones” for invasive species, acting as “corridors” for the

establishment and dispersal of these species in a new area, particularly when those structures are placed in sandy or muddy habitats (Bulleri and Airoidi, 2005; Airoidi et al., 2015; Soares et al., 2020). However, different types of artificial structures can provide different physical and biological conditions that can determine the kind of community that will colonize these structures. Floating or suspended structures greatly differ from those that are connected to the sea floor, i.e. fixed structures, in terms of current velocity, water flow, shading and proximity to sea floor (Glasby, 1999; Glasby, 2001; Perkol-Finkel et al., 2006). Moreover, the regime of disturbance can be different because of the maintenance of artificial structures or as an effect of wind or sea force (Airoidi and Bulleri, 2011). Also, there are evidences of a differential predation pressure among artificial structures (Kremer and da Rocha,

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**Table 1**

Effects of predation on fouling communities of different artificial structures. We considered only experimental studies from 2008 to the present that excluded predators in at least one treatment. Studies are ordered according to the latitude, from north to south.

Study region	Latitude	Type of structure	Evaluated predators	Variable negatively affected by predation	Source
UK, Angola and Brazil	53° N to 12° S	Suspended	Nektonic	Diversity and species composition at the Tropics	Dias et al., 2020
Vancouver Island, Canada	48° N	Fixed	Benthic and nektonic	Survival of <i>Botrylloides violaceus</i>	Simkanin et al., 2013
Hampton Marina, USA	42° N	Floating	Benthic and nektonic	Recruitment of mussels in presence of benthic predators	Auker et al., 2014
Gulf of Maine, USA	42° to 43° N	Fixed	Benthic and nektonic	None	Miller and Etter, 2008
NW Brittany, France	48° N	Suspended	Nektonic	None	Leclerc and Viard, 2018
Northwestern Atlantic (several locations)	9° to 41° N	Fixed	Benthic and nektonic	Diversity and species richness at the Tropics (9° and 14° N)	Freestone et al., 2011
Connecticut, USA	41° N	Fixed	Benthic and nektonic	Cover of an exotic solitary ascidian	Freestone et al., 2013
Caribbean, Panama	9° N			Richness of exotic tunicates	
Northwestern Atlantic	9° to 41° N	Fixed	Benthic and nektonic	Survival of <i>B. neritina</i> and/or <i>Didemnum</i> spp.	Cheng et al., 2019
Connecticut, USA	41° N	Fixed	Benthic	Abundance of small predators, increasing indirectly the abundance of ascidians	Papacostas and Freestone, 2019
Florida, USA	27° N				
Bodega Harbor, USA	38° N	Fixed and floating	Benthic and nektonic	Recruitment and cover of sessile organisms in fixed structures	Rogers et al., 2016
Madeira, Portugal	32° N	Suspended	Benthic and nektonic	Cover of native species	Gestoso et al., 2018
Hong Kong	22° N	Suspended	Nektonic	Community assemblages only during summer	Astudillo et al., 2016
Brazil (several localities)	3° to 27° S	Floating	Nektonic	Abundance of ascidians.	Kremer and da Rocha, 2016
Praia do Segredo, Brazil	23° S	Suspended	Nektonic	Diversity and dominance of ascidians. Species richness only the first 2 months	Vieira et al., 2012
São Sebastião, Brazil	23° S	Floating	Nektonic	Post-settlement stages of ascidians and their dominance in plates.	Oricchio et al., 2016
Cabo Frío Island, Brazil	23° S	Fixed	Nektonic	Community structure	Pereira Masi et al., 2016
La Herradura Bay and Tongo Bay, Chile	29° S	Fixed and suspended	Benthic and nektonic	Recruitment and cover of <i>Ciona robusta</i> (as <i>C. intestinalis</i> ) in fixed structures	Dumont et al., 2011a
La Herradura Bay, Chile	29° S	Fixed	Benthic	Recruitment of <i>Bugula neritina</i>	Dumont et al., 2011b
Sydney Harbor, Australia	33° S	Suspended	Nektonic	Cover of sessile community	Bolton et al., 2017
Biobío Region, Chile	36° to 37° S	Fixed	Benthic and nektonic	Community structure	Leclerc et al., 2020
Blackwood Bay, New Zealand	41° S	Fixed and suspended	Benthic and nektonic	Richness and total cover of exotic species	
Puerto Madryn, Argentina	42° S	Fixed	Benthic and nektonic	Cover and recruitment of <i>Didemnum</i> sp. in fixed structures	Forrest et al., 2013
Comodoro Rivadavia, Argentina	45° S	Fixed	Benthic and nektonic	Abundance of ascidians and diversity of sessile communities	Giachetti et al., 2019
				Cover of ascidians (until 90 days). Abundance of benthic predators	Rico et al., 2015

2016; Leclerc and Viard, 2018).

There is a variety of experimental approaches testing the effect of predators on fouling communities growing on artificial structures. However, the way in which these experiments are designed directly affects the pool of predators that are able to access these communities. While in some studies the artificial panels were deployed on piers (fixed structures), in others the panels were attached to floating or suspended structures (Table 1). While fixed structures are connected to the sea floor, enabling the access of benthic predators, structures that are not connected to the sea floor (floating or suspended) are only accessible to nektonic predators. At the same time, the predation pressure on fouling communities might be different at different latitudes (Table 1). In tropical and warm waters, predators have a strong effect on abundance, richness and diversity of fouling communities (Freestone and Osman, 2011; Freestone et al., 2013; Kremer and da Rocha, 2016; Oricchio et al., 2016; ; Dias et al., 2020), while at higher latitudes predation pressure appears to decrease (Freestone et al., 2013; Leclerc and Viard, 2018; Dias et al., 2020). These studies support the hypothesis that tropical communities present a stronger biotic resistance to new invasive species, through strongest predation (Freestone and Osman, 2011; Freestone et al., 2013). However, some of these studies only focus on suspended structures (Kremer and da Rocha, 2016; Oricchio et al., 2016; Leclerc and Viard, 2018; ; Dias et al., 2020), and there are evidences that benthic predation strongly affects the composition and diversity of fouling communities of fixed structures (Dumont et al., 2011a; Simkanin et al., 2013; Rico et al., 2015; Cheng et al., 2019; Giachetti et al., 2019; Leclerc et al., 2020). At high latitudes suspended structures could act as refuges for invasive species, as predation

pressure over artificial suspended structures is significantly low (Dumont et al., 2011a; Kremer and da Rocha, 2016; Leclerc and Viard, 2018; Dias et al., 2020). Unfortunately, only few studies evaluated predation in both types of structures (fixed and suspended) considering the whole community of predators (benthic and nektonic) at the same time (Dumont et al., 2011a; Forrest et al., 2013; Rogers et al., 2016). Then, different experimental approaches could be biasing the conclusions about the effects of predation on fouling communities, even at similar latitudes (Table 1).

Among invasive species, ascidians appear to be the most successful sessile filter feeders in colonizing artificial structures, and one of the most important components in the fouling communities, even with strong predation pressure (Lambert and Lambert, 1998; Lambert, 2007; Zhan et al., 2015). This observed predation could depend not only on the type of structure and the community of predators, but also on the ecology and biology of each ascidian species (Rico et al., 2015; Giachetti et al., 2019; Leclerc et al., 2020). For example, differences in the attachment strategies, the number and timing of larvae release, and their mobility and resistance may influence the vulnerability of each species to predation or to a bulldozing effect by benthic organisms (Osman and Whitlatch, 1996; Rajbanshi and Pederson, 2007). Consequently, differences in predation pressure on species growing on different artificial structures, i.e. fixed vs. floating/suspended, might affect the colonization success and dominance of each species of ascidian differently. For all these reasons, the aim of this study is to evaluate the effect of different predators on (1) the cover, composition and diversity of the fouling communities, and (2) the dominance of two invasive solitary ascidians, *Ascidella aspersa* and *Ciona robusta*, on these

communities on two different artificial structures, fixed and suspended. We chose the term “suspended” to distinguish our non-floating structures from floating marinas used in other studies. Moreover, these suspended structures allows us to avoid any bias due to difference in depth between fixed and suspended structures (see Section 2). Our hypothesis is that the effect of predation on the fouling communities and on the dominance of invasive ascidians depends on the type of artificial structure, as it determines the predator community associated to it. Because of the effect that benthic predators had on fouling communities in similar cold temperate areas (Dumont et al., 2011a; Rico et al., 2015; Giachetti et al., 2019; Leclerc et al., 2020), we expect that benthic predators will exert a higher predation pressure on the fouling communities than nektonic predators. This effect will lead to (1) a lower cover and diversity of the fouling communities on fixed artificial structures than in suspended structures, and (2) a decrease of invasive ascidians dominance on fixed structures.

## 2. Methods

### 2.1. Study area

We conducted this study over a 6 month period from late spring to early fall (November 2015–April 2016), in Puerto Madryn port, situated inside the Nuevo Gulf (Southwestern Atlantic, Argentina, 42°49' S; 65°04' W, Fig. 1). This port is characterized by having calm and clear waters, and a semidiurnal tidal regime (Servicio de Hidrografía Naval, 2019) with an amplitude of approximately 5 m. During the experimental period, sea water temperature was measured with data loggers placed in the study area, and we registered a mean of 16.4 °C, with a minimum of 11.7 °C during November and a maximum of 20.4 °C during January and February. We performed the experiments in an area with restricted ship movements (white arrow, Fig. 1), to avoid potential damage or replicates loss and to reduce the effect of ship propellers on the fouling communities, with a maximum depth of 9 m.

The pool of benthic predators present in the study area consisted of

native sea urchins such as *Arbacia dufresnii* (Blainville, 1825); native sea stars *Allostichaster capensis* (Perrier, 1875), *Anasterias antarctica* (Lütken, 1857), *Cycethra verrucosa* (Philippi, 1857) and *Cosmasterias lurida* (Philippi, 1858); gastropods such as the invasive *Pleurobranchaea maculata* (Quoy and Gaimard, 1832) and the natives *Trophon geversianus* (Pallas, 1774) and *Tegula patagonica* (d'Orbigny, 1835); and crabs such as the invasive *Carcinus maenas* (Linnaeus, 1758) and the natives *Leucippa pentagona* H. Milne Edwards, 1834 and *Halicarcinus planatus* (Fabricius, 1775), among others. In the fouling communities of the port, the abundance of these predators is constant among seasons (Giachetti, 2020). Regarding nektonic predators, it is common to find a small native fish, *Helcogrammoides cunninghami* (Smitt, 1898), associated to the fouling communities. Other bigger native fishes, such as *Acanthistius patachonicus* (Jenyns, 1840), *Pseudopercis semifasciata* (Cuvier, 1829) and *Pinguipes brasilianus* Cuvier, 1829, are present in the Nuevo Gulf but are rare in the study area. Although the fouling communities of the pilings are dominated by mussels like *Aulacomya atra* (Molina, 1782) and *Mytilus* spp., three species of solitary ascidians are also common in these fouling communities: the invasives *Ascidella aspersa* (Müller, 1776) and *Ciona robusta* Hoshino and Tokioka, 1967, and the cryptogenic *Asterocarpa humilis* (Heller, 1878) (Tatián et al., 2010; Schwindt et al., 2014). Since the effect of predation on the diversity, total cover of sessile organisms and abundance of invasive ascidians in the fouling communities are independent of the seasons (Giachetti et al., 2019), we performed the experiment during spring and summer when the recruitment and growth of sessile organisms in cold temperate regions is higher and faster than in other seasons (see Rico et al., 2010; Dumont et al., 2011a, 2011b; Leclerc and Viard, 2018; Giachetti et al., 2019). For a detailed description of the fouling communities, see Giachetti et al. (2019).

### 2.2. Experimental design

We evaluated the effect of predation on sessile communities by excluding predators in two types of artificial structures: one connected

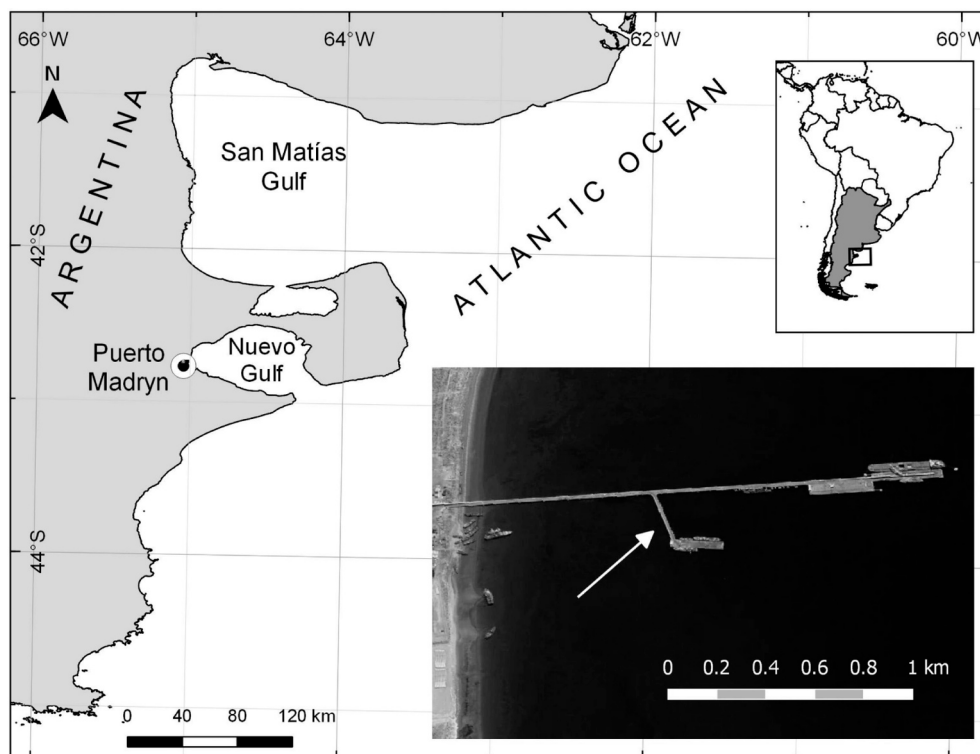
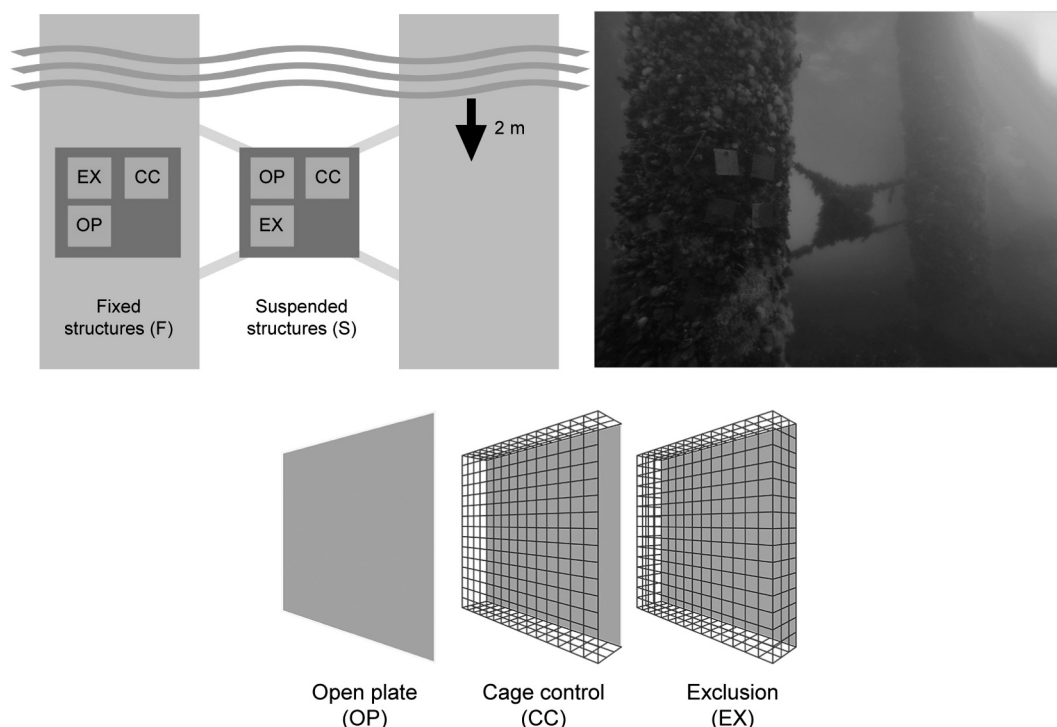


Fig. 1. Map of South America and a close up of the northern Patagonia, showing the location of Puerto Madryn port in the Nuevo Gulf. Satellite image (Google Earth) with a white arrow pointing at the experimental site.



**Fig. 2.** Experimental design, its position under the sea (only one row of pilings is represented) and a detail of predation treatments (OP, open plate; CC, cage control; EX, exclusion). Photo: N. Battini.

and another one unconnected to the sea floor (hereafter referred to as fixed and suspended, respectively). Each structure consisted of a nylon net ( $60 \times 60$  cm) to which three grey PVC recruitment plates ( $15 \times 15$  cm) were firmly attached with cable ties. Plates had a rough surface to facilitate the settlement of organisms and were separated from each other at least 12 cm. Ten of these structures were then tied vertically to the port pilings (fixed structures, one per piling) using nylon ropes (diameter 6 mm), while another ten were firmly tied vertically between two pilings (suspended structures, also with nylon ropes) (Fig. 2). One of the following predation treatments was assigned to each plate following a randomly ordered design: (1) Predator exclusion (EX), a plastic cage of  $1 \times 1$  cm mesh covered the plate at a distance of 10 cm approximately; (2) Cage control (CC), a similar cage with two open sides was attached to the plate allowing free motility of predators; and (3) Open plate (OP), plates without a cage (Fig. 2). Thus, one fixed and one suspended structure were deployed in the same row of pilings (i.e. 10 rows of pilings, 10 fixed structures and 10 suspended structures, each structure with three recruitment plates). Since the suspended structures were attached between two pilings and not to buoys in the surface, they did not float or move along with the tide, maintaining the same depth, luminosity and wave exposure than the fixed structures throughout the experiment. In all cases, the orientation of the plates was perpendicular to the sea floor and facing the same cardinal direction (south) to avoid potential effects due to differences in current exposure, turbulence or shading (Glasby, 1999). We did not remove the fouling on the pilings before the experiment because previous observations indicated that recruitment on bare plates is faster than secondary growth of organisms already present on the pilings (Schwindt et al., 2014; Giachetti et al., 2019).

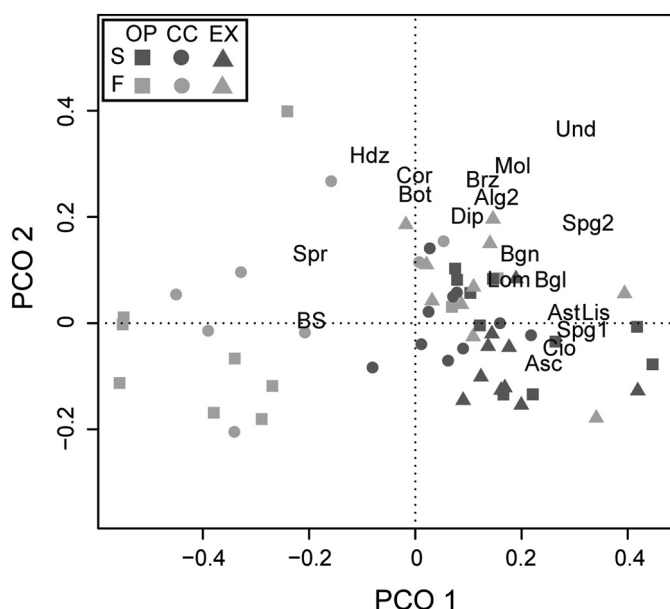
After six months, plates were carefully removed, labelled, placed in separate plastic bags with sufficient sea water and transported to the laboratory in insulated containers within an hour. We paid special attention to preventing the loss or escape of mobile and sessile organisms. In the laboratory, we removed the cages and registered the abundance of mobile species in all treatments. Plates were maintained independently in containers in the Experimental Aquarium of the CCT

CONICET-CENPAT. We measured the cover of all sessile species, i.e. the proportion of the plate cover by the vertical projection of the organisms, using a 2 cm grid in the totality of the central area ( $10 \times 10$  cm) of each plate to avoid edge effects. We considered both organisms attached directly to the plate and the organisms growing as epibionts, thus accumulated total cover could reach values higher than 1. We identified organisms to the lowest taxonomic level possible using the appropriate reference material of the Invertebrate Collection of IBIOMAR-CONICET (CNP-INV) and taxonomic keys (Van Name, 1945; Lichtschein de Bastida and Bastida, 1980; Carlton, 2007; Häussermann and Försterra, 2009; Piriz, 2009; da Rocha et al., 2012). Expert taxonomists identified algae, crabs, ophiuroids and new records of ascidians (see Acknowledgements section). We classified species as invasive, native or cryptogenic (see Carlton, 1996; Richardson et al., 2000; Blackburn et al., 2011). We considered as invasive those exotic species with established populations beyond the site where they were introduced by first time (Richardson et al., 2000; Blackburn et al., 2011), according to previous literature (Orensanz et al., 2002; Schwindt et al., 2014; Schwindt et al., 2020). We deposited the new records of ascidians species in the Invertebrate Collection of IBIOMAR-CONICET (CNP-INV) (see Section 3 for collection numbers).

### 2.3. Statistical analyses

To evaluate the effect of predators and the type of structure, i.e. fixed vs. suspended on the composition and species cover of the fouling communities, we performed a principal coordinates analysis (PCO). Cover data of sessile organisms, including bare space, was fourth root transformed and a Bray Curtis similarity matrix was used in all the analyses (Clarke and Warwick, 2001). We analysed differences in the percentage cover of sessile species with a crossed factorial PERMANOVA test (999 permutations) with two fixed factors, “type of structure” (fixed and suspended) and “predation treatment” (exclusion, cage control and open plate), and one random factor, “piling row” (1 to 10), using package *vegan* v. 2.5–6 (Oksanen et al., 2019) in R. When pairwise tests were needed, *p*-values were adjusted with Bonferroni correction to





**Fig. 3.** PCO for sessile organisms (percent cover) comparing all predation treatments (OP, open plate; CC, cage control; EX, exclusion) and type of structure (S, suspended; F, fixed). Some dots might be overlapped. Species abbreviations: Und, *Undaria pinnatifida*; Lom, *Lomentaria clavellosa*; Spg1 and Spg2, Porifera indet. 1 and 2; Hdz, *Eudendrium ramosum*; Bgl, *Bugula neritina*; Bgn, *Bugulina flabellata*; Brz, Bryozoa indet.; Spr, Spirorbinae; Asc, *Asciidiella aspersa*; Cio, *Ciona robusta*; Ast, *Asterocarpa humilis*; Cor, *Corella eumyota*; Mol, *Molgula manhattensis*; Bot, *Botryllus schlosseri*; Lis, *Lissoclinum fragile*; BS: bare space.

control global alpha (Zar, 2009). Finally, when significant differences among pairs were observed, we performed a SIMPER test (vegan package) to determine which taxa contributed the most to those differences (Clarke and Warwick, 2001). We also compared total cover and diversity (Shannon Index) of sessile organisms among type of structure and predation treatments fitting a general linear mixed effects model, using the package *nlme* v. 3.1–147 (Pinheiro et al., 2017) in R. In both cases, “type of structure” and “predation treatment” were considered fixed factors, and “piling row” a random factor. We calculated diversity from the percentage cover data, including the organisms attached directly to the plate and the organisms growing as epibionts, considering or not the cover of invasive ascidians *Asciidiella aspersa* and *Ciona robusta* separately. We tested normality and homogeneity of variance with Shapiro-Wilks test and through visual inspection of the residuals plots tests, respectively. When considering the cover of ascidians, it was necessary to incorporate a variance structure function (*varIdent*), applying a different variance coefficient to each type of structure account for the heterogeneity of variance and include it in the model.

Finally, to evaluate the effects of both types of structure and the different predation treatments on the dominance of both invasive ascidians (*A. aspersa* and *C. robusta*) we compared the cover of each species separately using a general linear mixed effects model (package *nlme*, Pinheiro et al., 2017). We also measured the cover of epibiont ascidians, thus the accumulated total cover of each species could be higher than 1. The model for each species included “type of structure” and “predation treatment” as fixed factors and “piling row” as a random factor. For both analyses, we tested normality and homogeneity of variance with Shapiro-Wilks test and through visual inspection of the residuals plots tests, respectively. When we observed deviations from the homoscedasticity, we incorporated a power variance structure function to the model, using the cover of each ascidian species as a covariate (*varPower*).

In all cases, we selected the final linear model according to the

minimum AIC criteria and parsimony when  $\Delta AIC \leq 2$ . We performed likelihood ratio tests of the full model vs. the model without the interaction term to evaluate the significance for the interaction. When pairwise tests were needed, *p*-values of Tukey test were adjusted with Bonferroni correction factor (Zar, 2009). All the statistical analyses were performed with the software R (R Core Team, 2020).

### 3. Results

We identified a total of 42 taxa: 12 (28.5%) were invasive species, 16 (38%) were native, two (5%) were cryptogenic and 12 (28.5%) needed further taxonomic studies (Table S1). The richness of invasive species was characterized by a larger number of sessile species (11 species), while the richness of the native species showed the opposite pattern with a large number of mobile fauna (12 species). Two species were first records for the area: the invasive ascidian *Molgula manhattensis* (De Kay, 1843) (CNP-INV 3043 and 3044) and the cryptogenic ascidian *Corella eumyota* Traustedt, 1882 (CNP-INV 3035).

#### 3.1. Effect of predators on fouling communities of different artificial structures

Benthic predators negatively affected the cover and composition of species of the sessile community. Fouling communities developed without these predators (exclusion treatment on fixed structures and all predation treatments in suspended structures) were similar in terms of total cover and species composition, and differed to the communities developed in presence of benthic predators (PCO, Fig. 3). In the presence of these predators, total cover was greatly reduced and Spirorbids were more abundant. When benthic predators were absent, the species richness increased driven by the presence of colonial and solitary ascidians, bryozoans, sponges and algae (Fig. 3). The PERMANOVA showed that the interaction between type of structure and predation treatment was significant, i.e. the effect of predation varied between the two types of structures (Table 2A). Thus, we performed pairwise tests between predation treatments for each type of structure. The species composition of sessile communities developed in the absence of predators was significantly different from the communities developed with predators, in both types of structures (Table 2B). The taxa that most contributed to this dissimilarity among predation treatments depended on the type of structures (Table 3). On fixed structures, solitary ascidians contributed more to the dissimilarity, due to their higher abundance in exclusion treatments (Table 3), whereas bryozoans and bare space contributed the most to the dissimilarity among predation treatments of suspended structures (Table 3).

Total cover of sessile organisms was high only in the absence of

**Table 2**

(A) Results of the main effects tests of the two-way PERMANOVA for sessile organisms cover (including bare space) between type of structure (fixed and suspended) and among predation treatments. (B) Results of pairwise test for sessile organisms cover (including bare space) among treatments (EX, exclusion; CC, cage control; OP, open plate) for each structure. Significant *p*-values are in bold.

(A)	Df	SS	MS	Pseudo F	P (perm)
Type of structure (S)	1	1.45	1.45	22.3	<b>0.001</b>
Treatment (T)	2	0.90	0.45	6.92	<b>0.001</b>
Piling	9	0.70	0.08	1.20	0.254
S x T	2	0.67	0.33	5.12	<b>0.001</b>
Error	45	2.94	0.06		
<hr/>					
(B)	Fixed structure		Suspended structure		
	Pseudo F	P adjusted	Pseudo F	P adjusted	
EX x CC	10.9	<b>0.003</b>	4.37	<b>0.006</b>	
EX x OP	11.5	<b>0.003</b>	3.27	<b>0.006</b>	
CC x OP	0.27	1.000	1.88	0.333	

**Table 3**

SIMPER analysis of cover data comparing pairs of levels of predation treatment factor (EX, exclusion; CC, cage control; OP, open plate) for each level of type of structure factor with significant differences in PERMANOVA pairwise tests.

Taxa	Average abundance	Average abundance	Contribution (%)	Cumulative contribution (%)
FIXED				
	EX	CC		
<i>Ascidella aspersa</i>	0.87	0.25	20.0	20.0
Bare space	0.60	0.94	11.1	31.1
<i>Ciona robusta</i>	0.51	0.19	11.3	42.4
<i>Asterocarpa humilis</i>	0.46	0.11	11.0	53.4
	EX	OP		
<i>Ascidella aspersa</i>	0.87	0.38	16.0	16.0
<i>Ciona robusta</i>	0.51	0.10	14.0	30.0
<i>Asterocarpa humilis</i>	0.46	0.09	12.6	42.6
Bare space	0.60	0.93	11.7	54.3
SUSPENDED				
	EX	CC		
Bare space	0.57	0.80	13.5	13.5
<i>Bugula neritina</i>	0.10	0.30	12.6	26.1
<i>Bugulina flabellata</i>	0.71	0.25	11.0	37.1
<i>Ascidella aspersa</i>	1.00	0.79	11.0	48.1
<i>Asterocarpa humilis</i>	0.46	0.39	9.80	57.9
	EX	OP		
<i>Bugula neritina</i>	0.10	0.47	18.9	18.9
Bare space	0.57	0.53	15.2	34.1
<i>Asterocarpa humilis</i>	0.46	0.38	10.8	44.9
<i>Bugulina flabellata</i>	0.71	0.20	9.00	53.9

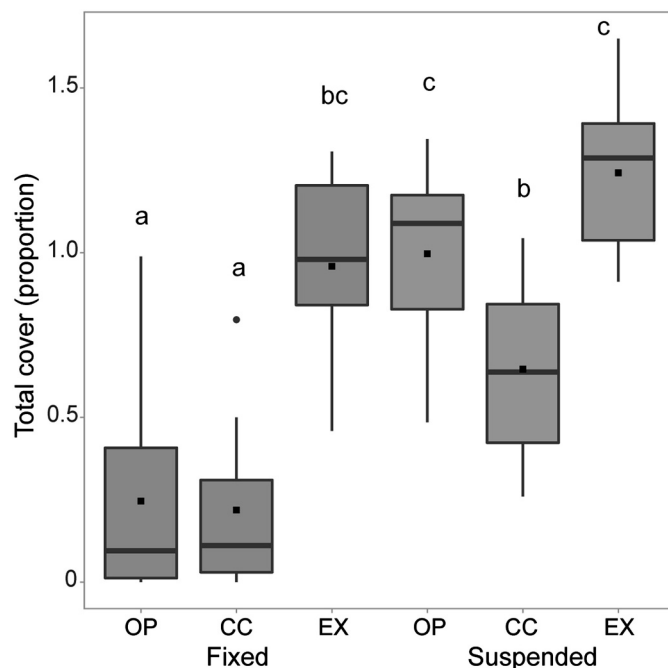
benthic predators, i.e. exclusion treatment of fixed structures and the three predation treatments of suspended structures (interaction term: predation treatment\*type of structure,  $p = 0.010$ ; Fig. 4; Table S2). In the presence of benthic predators, i.e. open plate and cage control treatments of fixed structures, total cover was similar between predation treatments and showed the lowest values among all predation treatments (Fig. 4; Table S2). Interestingly, diversity (Shannon Index) appeared to be affected not only by predation but also by the cover of invasive ascidians. When we ignored the cover of *Ascidella aspersa* and *Ciona robusta* in the calculation of the Shannon index, diversity was lower in the presence of benthic predators, i.e. open plate and cage control of fixed structures (interaction term: predation treatment\*type of structure,  $p = 0.001$ ; Fig. 5A; Table S3). In contrast, when we considered ascidian cover in the calculation of the diversity index, the pattern related to predation disappeared, and diversity in cage control and open plate treatments of fixed structures was as low as in the exclusion treatment of suspended structures (interaction term: predation treatment\*type of structure,  $p = 0.001$ ; Fig. 5B; Table S3).

### 3.2. Effect of predators on the dominance of invasive solitary ascidians on fouling communities of different artificial structures

The effect of predation on the dominance of invasive ascidians, measured as the percentage cover of each species, varied with the type of structure (interaction term predation treatment\*type of structure: *A. aspersa*,  $p = 0.023$ ; *C. robusta*,  $p = 0.026$ ). In both cases, the dominance of ascidians on the communities of fixed structures was low in presence of predators, whereas the dominance of ascidians was significantly higher in all predation treatments of suspended structures (*A. aspersa*, Fig. 6A; *C. robusta*, Fig. 6B; Table S4). Indeed, all communities developed in the absence of benthic predators presented similar cover of each species of ascidian regardless of the type of structure, i.e. exclusion of fixed structures and all predation treatments of suspended structures (Fig. 6A and B). Although the dominance of both species varied in a similar way among predation treatments and structures, *C. robusta* presented a lower cover than *A. aspersa* (Fig. 6).

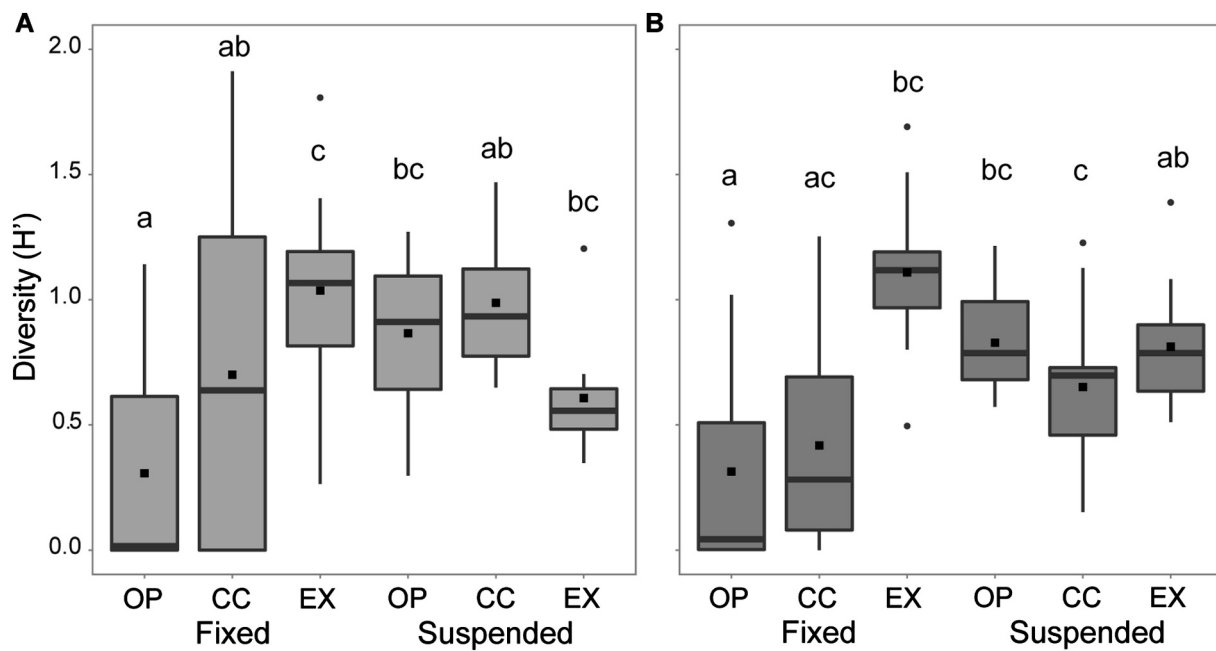
## 4. Discussion

We found that benthic predators have an important role in shaping



**Fig. 4.** Box plot showing total cover of sessile species per predation treatment (OP, open plate; CC, cage control; EX, exclusion) and type of structure. Different letters denote significant differences ( $p < 0.05$ ). The superior and inferior ends of the box correspond to the third (Q3) and the first (Q1) quartiles, respectively. The difference between them is the interquartile range (IQR), used to calculate the extreme lines of the box: from  $Q3 + 1.5 \times IQR$  (superior) to  $Q1 - 1.5 \times IQR$  (inferior). Inside the box, the black line represents the median of the data and the black square is the mean of the data (package *ggplot2* (Wickham, 2009)).

fouling communities of cold temperate ports, particularly constraining the dominance of the invasive ascidians *Ascidella aspersa* and *Ciona robusta*. These predators alter the composition of species and significantly reduce the total cover and diversity of the sessile communities in which they are present. Moreover, the effect of predators on the diversity appears to be masked by the dominance of these invasive



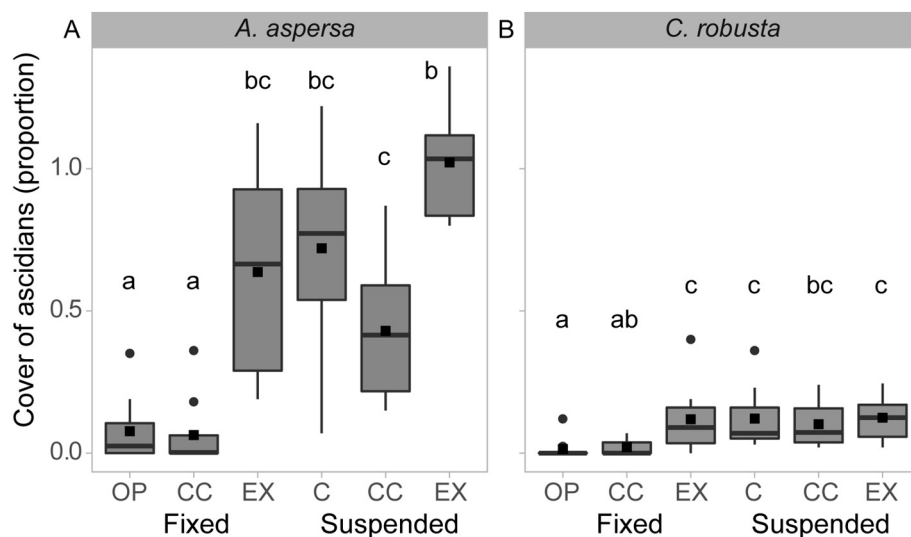
**Fig. 5.** Box plot of diversity (Shannon Index,  $H'$ ) per predation treatment (OP, open plate; CC, cage control; EX, exclusion) and type of structure, without invasive ascidians (a) and with them (b). Different letters denote significant differences ( $p < 0.05$ ). See Fig. 4 legend for box plot references.

ascidians in the absence of benthic predation. Only when the cover of both species was not considered in the analyses, we detected a negative effect of benthic predation on the diversity of the fouling communities.

In this cold temperate region, benthic predators such as echinoderms, gastropods, chitons and crabs exert a larger predation pressure than nektonic predators, such as fish. The most common fish in the study area, *Helcogrammoides cunninghami*, reaches a small adult size and has an amphipod-based diet (Muñoz and Ojeda, 1997). Other species, such as *Acanthistius patachonicus*, *Pseudoperca semifasciata* and *Pinguipes brasilianus*, are larger but are rarely observed associated to the fouling communities because they use reefs as a refuge and feed on adjacent environments (Galván et al., 2009). These behaviours could explain the poor or even null influence of fish on the fouling communities, as it was evidenced by the lack of differences between predation treatments in suspended artificial structures. Moreover, sessile communities developed in the absence of benthic predators presented a similar cover and

composition of species, clearly different to the communities developed in their presence that presented a high percentage of calcareous polychaetes and bare space.

The strong predation pressure observed in our study differs from the pattern observed previously at high latitudes, where predation pressure has no effect on fouling communities of artificial structures (Miller and Etter, 2008; Freestone et al., 2011; Freestone et al., 2013; Leclerc and Viard, 2018; Dias et al., 2020). Indeed, in warmer areas of the Western Atlantic, fish are active predators of fouling communities of artificial structures (Freestone et al., 2013; Kremer and da Rocha, 2016; Dias et al., 2020). Thus, predation pressure appeared to be higher in lower latitudes leading to the hypothesis that tropical and subtropical communities present a high biotic resistance (Freestone et al., 2013). However, a thorough review of scientific articles from 2008 to the present (Table 1) shows that these patterns might not be so evident worldwide. Several studies evaluated the effect of nektonic predators,



**Fig. 6.** Box plot showing the cover of (A) *Ascidiella aspersa* and (B) *Ciona robusta* per predation treatment (OP, open plate; CC, cage control; EX, exclusion) and type of structure interaction. Different letters denote significant differences ( $p < 0.05$ ). See Fig. 4 legend for box plot references.

i.e. using suspended structures (Vieira et al., 2012; Astudillo et al., 2016; Kremer and da Rocha, 2016; Oricchio et al., 2016; Leclerc and Viard, 2018; Dias et al., 2020). However, experiments including both nektonic and benthic predators, i.e. performed in fixed structures, showed that predation pressure could have significant effects on fouling communities even at high latitudes (Dumont et al., 2011a, 2011b; Forrest et al., 2013; Simkanin et al., 2013; Rico et al., 2015; Cheng et al., 2019; Leclerc et al., 2020). These effects vary from a decrease in the cover and/or recruitment rate of sessile organisms, including invasive species, to changes in composition and diversity of the entire fouling community (Table 1). Not only the community of predators able to access each type of artificial structures may be responsible for these effects (Dumont et al., 2011a; Giachetti et al., 2019), but also the diversity of predators and their seasonal abundance pattern (Byrnes and Stachowicz, 2009; Cheng et al., 2019). Unfortunately, very few studies evaluated both nektonic and benthic communities of predators at the same time in different types of structures (Dumont et al., 2011a; Forrest et al., 2013; Rogers et al., 2016). In this regard, our results greatly imply that the spatial variations of predator communities could strongly influence the biotic resistance of a community to the settlement and dominance of invasive species. Therefore, experimental approaches aiming to understand the role of predation over marine fouling communities must carefully consider both nektonic and benthic predator communities, to achieve a comprehensive understanding on the complete scenario of the processes driving the community patterns. Otherwise, it should be clearly stated which predator community is involved in the predation treatment and results should be interpreted accordingly.

The differential access of predators can also lead to a differential biotic resistance among fixed or suspended structures, as suggested by our results. This effect could be driven by the suspended structures acting as a refuge and source of propagules for invasive species. In fact, this differential predation pressure between both types of artificial structures was beneficial for both invasive ascidians, *A. aspersa* and *C. robusta*. In the absence of benthic predators, *A. aspersa* reached the largest cover and dominated the fouling communities. In the case of *C. robusta*, it was also affected by benthic predators, although it did not dominate the fouling communities as expected based on previous studies. This species is a worldwide invader, known for thriving in marinas, ports and shellfish aquaculture facilities (Carver et al., 2003; Dumont et al., 2011a; McKenzie et al., 2016). However, as its congener *C. intestinalis*, it can present poorer attachment abilities and settlement strategies than *A. aspersa* (Osman and Whitlatch, 1996; Rajbanshi and Pederson, 2007), suggesting that interspecific interactions such as competition could play an important role in the fouling communities. In addition, species of *Ciona* might have a better performance in calm waters such as those occurring in marinas, enclosed ports and aquaculture facilities, compared to ports in open waters like the Puerto Madryn port (Nuñez Velazquez et al., 2017). Therefore, a combination of environmental conditions and interspecific interactions might have decreased the abundance of *C. robusta* relative to *A. aspersa*, even when predators were excluded. Clearly, more studies are needed to elucidate the role of interspecific competition between *A. aspersa* and *C. robusta* and the influence of environmental conditions in the dominance of these species on fouling communities. Despite these differences between the two ascidians species, and although benthic predators cannot prevent the establishment of invasive ascidians in fixed structures, these predators could still exert biotic resistance reducing the abundance and dominance of invasive ascidians (Rius et al., 2014).

Benthic predators also reduced the diversity of the fouling community, in agreement with previous observations (Giachetti et al., 2019). However, this effect only became evident when the estimators did not consider the dominance of *A. aspersa* and *C. robusta*. It is known that invasive ascidians can reduce the diversity, sometimes in a density-dependent manner, because of their high settlement and colonization rates (Blum et al., 2007; Zhan et al., 2015; Robinson et al., 2017). In

this study, we observed that solitary ascidians, particularly *A. aspersa*, were able to cover the entire plate surface forming a dense and compact structure. This dominance may decrease the diversity as it reduces the availability of free space, hampering the settlement of other sessile organisms with lower rates of colonization (Osman, 2015; Oricchio et al., 2016). As predation pressure on suspended structures was low, recruits of ascidians were not consumed or bulldozed from the substrate, and there was no generation of new bare space that could be colonized by pioneer organisms. However, further studies are necessary to evaluate if the high cover of ascidians could effectively reduce the diversity compared to communities with a low cover of ascidians. On the other hand, communities with presence of benthic predators presented high dispersion in the values of diversity, compared to those without benthic predators. These results suggest that benthic predators have a spatially heterogeneous effect depending on their size and distribution pattern (Nydam and Stachowicz, 2007; Oricchio et al., 2016). Some predators such as large gastropods or sea stars might have strong but scattered effects, i.e. because of their low density and patchy distribution. In contrast, other smaller predators such as crabs or sea urchins, which are more abundant and broadly distributed predators, can have a more homogeneous but weaker effect (Giachetti, 2020). Due to their size, they affect mainly ascidians recruits and have little effect once they reach an adult size (Giachetti, 2020). Thus, the timing of prey recruitment and growth, coupled with temporal variation in predator abundance are important factors determining the dominance of sessile species (Cheng et al., 2019).

#### 4.1. Conclusions

In contrast to other studies, we used suspended structures instead of floating structures, thus they presented analogous physical characteristics to the fixed structures, i.e. similar depth, luminosity and level of exposure to currents and waves during all the experimental period. This allows us to assume that the differences between the types of structures were mainly caused by differences in the associated predator communities. The similarities observed between the exclusion treatments of both types of structures, where communities of sessile organisms presented a similar composition and cover, supports this assumption. The difference in the dominance of invasive ascidians between different types of structures evidences the role of suspended structures as a refuge for invasive species from benthic predators. In this way, benthic predation at higher latitudes could be replacing nektonic predation observed at lower latitudes. In this regard, this study shows how different communities of predators, i.e. nektonic and benthic, could drive different effects on the fouling communities growing on artificial structures and evidences their importance during the design of experiments that aim to compare biotic resistance and predation pressure. Finally, although benthic predators cannot prevent the establishment of invasive species, they may be able to strongly reduce the impacts of some species, such as invasive ascidians and other soft sessile organisms. Enabling the access of benthic predators to suspended artificial structures (Dafforn et al., 2009; Airolidi et al., 2015; Johnston et al., 2017) appears to be a promising strategy to reduce the establishment, spread and impact of invasive species, and to achieve a sustainable use of coastal areas.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jembe.2020.151459>.

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