

# Biotic resistance and facilitation of a non-indigenous mussel vary with environmental context

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**ABSTRACT:** Expansion of invasive species in new host habitats is mediated by a combination of concurrent biotic and abiotic factors. *Xenostrobus securis* is an invasive mussel species that is spreading in the inner part of the Galician Rias Baixas, particularly in areas of low salinity. It co-occurs with the commercially important blue mussel *Mytilus galloprovincialis* in mixed patchy aggregations on intertidal shores. We asked whether biotic interactions, i.e. competition, facilitation and predation, could explain patterns of coexistence of the 2 species in an estuarine habitat. We also examined whether the effects of such interactions were dependent upon the environmental context. We manipulated predator access to synthetic aggregations of juvenile mussels at different sites using monospecific aggregations of *X. securis* and *M. galloprovincialis* and mixed aggregations at high and low densities. Performance of the species was measured as survivorship, growth and condition index. Predation played an important role in determining abundances of both species, especially in the case of the invader, although the effects on survivorship were context dependent. We did not find evidence of intra- or inter-specific competition. Growth rates of the invader were affected by environmental variability (i.e. significant site effect). Condition index of *M. galloprovincialis* improved in high density aggregations (monospecific and mixed), but only towards the sea (i.e. the outermost site). Results suggest that facilitative rather than competitive interactions contribute to the abundance patterns of the 2 species, at least during the juvenile stage. The environmental context was an important driver of such patterns. We emphasise the need to address the role of positive interactions regarding marine non-indigenous species.

**KEY WORDS:** Competition · Predation · Facilitation · Performance · Environmental context · *Xenostrobus securis* · *Mytilus galloprovincialis* · Non-indigenous species

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## INTRODUCTION

Many ecological mechanisms that modulate the invasion process are linked to the ability of native communities to resist the invader, i.e. biotic resistance (Kimbrow et al. 2013). Competition and predation are among the most important mechanisms conferring biotic resistance to the recipient communities (e.g. Parker & Hay 2005, Kimbro et al. 2013). Both mechanisms are connected with the capacity of the resident community to reduce performance and the

spread of non-indigenous species (NIS) in post-establishment phases (Levine et al. 2004).

However, in addition to biotic resistance from recipient communities, environmental context (i.e. gradients in physical properties, type of habitat and habitat heterogeneity) (e.g. Tamburello et al. 2013) and invader attributes are key in determining the success of invasions (Cheng & Hovel 2010, Kimbro et al. 2013). Recent research also shows that facilitation can enhance temporal variability and invasibility of communities (Bulleri et al. 2008). Facilitation is

believed to be particularly important where biotic and/or abiotic stress is high (Bertness & Callaway 1994). Multiple examples from the literature suggest that the ability of an invader to become established depends on the trade-off between negative and positive effects of extant species (Bulleri 2009, Rius & McQuaid 2009). Nevertheless, biotic interactions among species are not fixed because they can vary over space and time and can be influenced by a variety of factors (Rius & McQuaid 2006).

In the case of mussels, both competition (Steffani & Branch 2005, Rius & McQuaid 2006, Shinen & Morgan 2009) and facilitation (Rius & McQuaid 2009) between native and NIS may inhibit or promote invasion success. Similarly, predation may play an important role in limiting or facilitating the establishment of NIS depending on predator preferences. For instance, local predators could facilitate invasion of a NIS because they fail to recognize it as potential prey (Veiga et al. 2011). Strong preferences for NIS may also open up the possibility of associational susceptibility or defence within a community (Shinen et al. 2009). However, the final success of invasions seems to be mainly the result of the interplay between concurrent physical and biological drivers (Bownes & McQuaid 2010). For example, on rocky shores along the Californian coast, the abundance of 2 mussel species, the native *Mytilus trossulus* (Gould, 1850) and the NIS *Mytilus galloprovincialis* (Lamarck, 1819), is determined by the dynamic relationship between interspecific competition and species-specific tolerances to temperature (Lockwood & Somero 2011). In the warmer central and southern areas of the coast, the invader displaces the congener *M. trossulus*, whereas *M. trossulus* is able to outcompete the invader in the colder areas.

Estuaries are coastal ecosystems where biological invasions are frequent. These habitats usually suffer from several anthropogenic pressures and are exposed to some of the principal introduction vectors such as ship ballast waters or aquaculture production activities (Cohen & Carlton 1998). Moreover, strong environmental gradients in salinity and temperature make these systems especially interesting in the evaluation of how biotic resistance may be affected by environmental conditions (Cheng & Hovel 2010). Mussels are among the most common organisms to invade estuaries and have often been used as model species to investigate invasions (e.g. Shinen & Morgan 2009, Shinen et al. 2009, Lowen et al. 2013).

In this study, we focused on the mechanisms involved in the spread of the non-indigenous mussel *Xenostrobus securis* (Lamarck, 1819) in an estuarine

area on the Galician coast (NW of Spain). This species is spreading in the inner part of the Galician Rias Baixas (Garci et al. 2007, Gestoso et al. 2012), particularly in areas of low salinity where it dominates. It co-occurs with the commercially important blue mussel *M. galloprovincialis*, forming mixed patchy aggregations of differing densities on intertidal rocky shores and artificial substrates (Gestoso et al. 2013). While densities of *X. securis* decrease from areas close to the mouth of rivers to the middle part of rias, densities of *M. galloprovincialis* show an opposite trend (Gestoso et al. 2012). These species differ in their physiological and ecological traits (Babarro & Lassudrie 2011), and perform differently along the estuarine gradient (Babarro & Abad 2013). In addition, patterns of recruitment along the estuary differ between the 2 species (C. Olabarria pers. obs.). A combination of both biotic and abiotic factors that vary spatially within the study area is likely to explain the distribution patterns of the 2 species.

Several studies have addressed the invasion of *X. securis* from diverse perspectives, but few of them have focused on the processes affecting invasion success. Whereas an observational study suggested that *X. securis* might outcompete *M. galloprovincialis* in the inner part of the rias (Garci et al. 2007), a recent experimental study has indicated that the native mussel performed better when confronted with abiotic and biotic stress (Babarro & Abad 2013). A laboratory experiment showed that the crab *Carcinus maenas* (Linnaeus, 1758) exerted a greater predation pressure on *M. galloprovincialis* than on *X. securis* (Veiga et al. 2011). However, other native predators might be limiting the invasion success of *X. securis* via selective predation. Moreover, the presence of the invader among native mussels might change the native predator-prey interactions via apparent competition or associational defence (Menge 1995).

The aim of this study was to examine the role of competitive or facilitative interactions and natural predation on the coexistence of the 2 mussel species on rocky shores along an estuary. Additionally, we tested whether biotic interactions varied with environmental context (i.e. across sites) as has been previously suggested (Steffani & Branch 2003, Rius & McQuaid 2009). For that, we experimentally manipulated the access of predators to synthetic aggregations of mussels with varying densities (low vs. high) and species composition (monospecific vs. mixed) at 3 different sites. If predation affects mussels, then we would expect differences in survivorship rates among mussel aggregations exposed to and protected from predators. However, if interspecific facil-

itation via associational defence is relevant, then greater survivorship rates of mussels in high compared to low density aggregations would occur when exposed to predators (Menge 1995). Differential growth or condition among mussels from low and high density aggregations would indicate either competitive or facilitative interactions. Hence, a better performance of mussels in monospecific compared to mixed aggregations would suggest the existence of intense interspecific competition. However, if mussels from mixed aggregations performed better, it would indicate facilitation. Spatial variability, i.e. across sites, in the performance of mussels from either monospecific and/or mixed aggregations would indicate a major influence of the environmental context on those biotic interactions.

## MATERIALS AND METHODS

### Study area and experimental design

The experiment was conducted at the San Simon Inlet located in the inner part of the Ria de Vigo, NW Spain ( $42^{\circ} 18' 43''$  N,  $8^{\circ} 38' 9''$  W) (Fig. 1). The Ria de Vigo is mesotidal with a tidal range from 2 to 4 m and behaves as a partially mixed estuary with positive residual circulation (Prego & Fraga 1992). Three different sites along a salinity gradient were chosen on the mid-intertidal shore (i.e. approx. 0.4 m above mean low water spring tide). Site 1 (S1), where the invader reaches the largest abundances (*Xenostrobus securis*:  $2118 \pm 263.35$  ind.  $m^{-2}$ , *Mytilus galloprovincialis*:  $7.50 \pm 6.69$  ind.  $m^{-2}$ ,  $n = 30$ ; data from Gestoso et al. 2012 and reported as means  $\pm$  SE), is located next to the mouth of the Verdugo River and has, on average, low salinity, although values are highly fluctuating (from  $3.9 \pm 0.3$  to  $33.5 \pm 0.24$  psu). At this site, water flow is stronger than the other sites, but extremely variable due to the river influence and tidal regime. Site 2 (S2), where the invader and *M. galloprovincialis* form mixed patches on the rocky shore (*X. securis*:  $236.66 \pm 51.76$  ind.  $m^{-2}$ , *M. galloprovincialis*:  $190.8 \pm 41.94$  ind.  $m^{-2}$ ), is characterised by higher salinity values (from  $20.1 \pm 0.50$  to  $35.3 \pm 0.06$  psu). Site 3 (S3), where *X. securis* and *M. galloprovincialis* reach the smallest and the largest abundances, respectively (*X. securis*:  $39.06 \pm 3.32$  ind.  $m^{-2}$ , *M. galloprovincialis*:  $639.17 \pm 50.86$  ind.  $m^{-2}$ ), is characterised by more stable salinity values close to 35 psu (from  $25.4 \pm 0.30$  to  $35.3 \pm 0.03$  psu). The main potential predators in this area include the gastropods *Nucella lapillus* (Linnaeus, 1758) and *Ocenebra eri-*

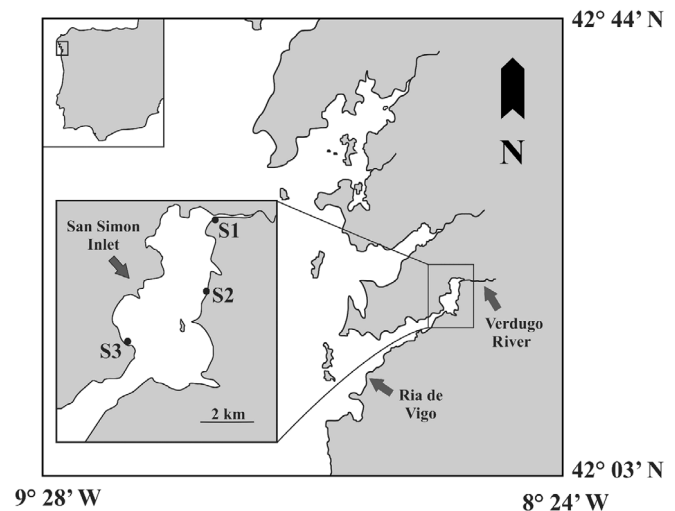


Fig. 1. Area of study and sites along the estuary from S1 to S3

*naceus* (Linnaeus, 1758), the decapod crustacean *Carcinus maenas*, the yellow-legged gull *Larus cachinnans* (Pallas, 1811) and fishes of the family Labridae and genus *Diplodus* Rafinesque 1810 (I. Gestoso pers. obs.).

Synthetic mussel aggregations of varying densities and species composition were constructed on previously sanded PVC plates of  $15 \times 15 \times 0.5$  cm, i.e. experimental units. Similar sized juveniles of each species (*M. galloprovincialis*:  $28.64 \pm 0.22$  mm,  $n = 1176$ ; *X. securis*:  $28.76 \pm 0.19$  mm,  $n = 840$ ) were collected from Site 2 and used to create the aggregations. After stripping biofouling from the shells and removing byssus from the ventral margin, individuals were allowed to establish primary attachment in the laboratory for 2 d prior to their transportation to the field. A biodegradable mesh was used to facilitate the attachment of mussels to the PVC plates and plastic cages were used to exclude predators when necessary.

Prior to the main experiment, a pilot experiment was conducted at site 2 from 16 March to 19 April 2011. In this study, we tested for potential artefacts caused by the manipulation of mussels and the effects of the biodegradable mesh and caging. We made synthetic mixed aggregations using specimens from both species collected from site 2, using low densities similar to those found in natural aggregations in the field, i.e. 7 individuals of *M. galloprovincialis* and 5 individuals of *X. securis* (Gestoso et al. 2013). Five treatments were included in the pilot study ( $n = 4$ ): (C) a control that consisted of a natural aggregation in the field with no manipulation; (PC1) procedural control for the mesh effect that consisted

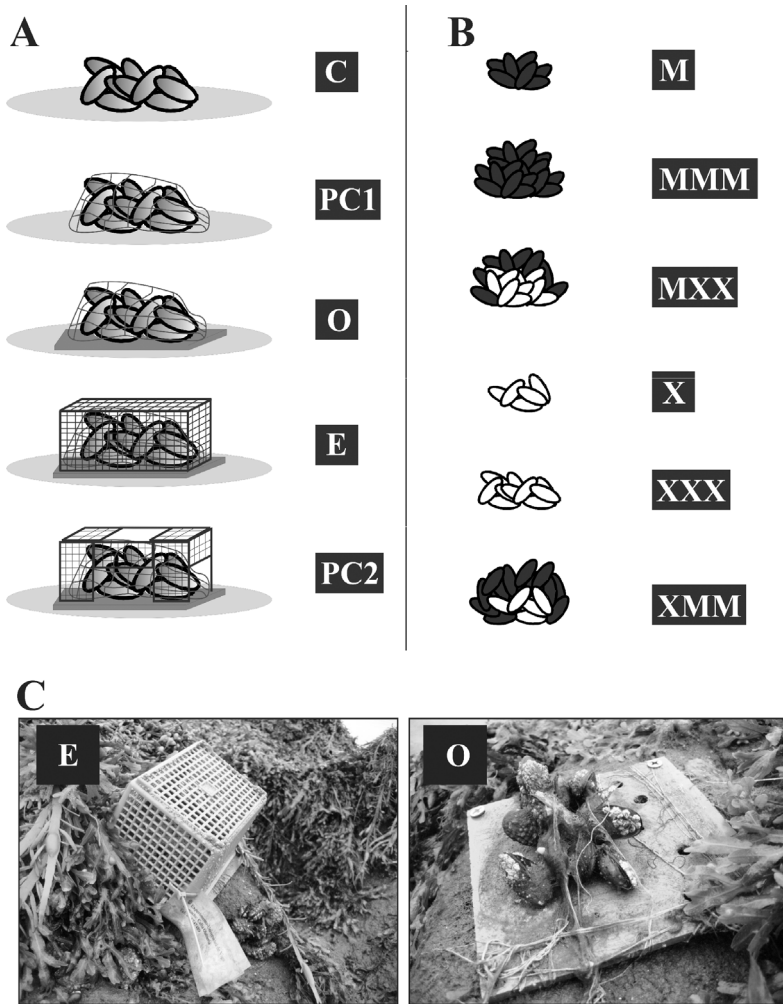


Fig. 2. Set up of the experiment. (A) Treatments used to control for the potential artefacts in the pilot experiment: C = control, PC1 = procedural control for the mesh, O = control of plate effects, E = exclusion treatment, PC2 = procedural control for the cage effects. (B) Artificial mussel aggregations to test competition for *Mytilus galloprovincialis* (M) and *Xenostrobus securis* (X). M/X and MMM/XXX correspond to low and high density monospecific aggregations, respectively, and MXX/XMM correspond to high density mixed aggregations. Black and white symbols represent *M. galloprovincialis* and *X. securis*, respectively. (C) Two aggregations on the rocky shore: one plate corresponds to the exclusion treatment (E) and the other to the open treatment (O)

of a natural aggregation wrapped in a biodegradable mesh; (O) open control plates that consisted of an artificial aggregation made of mussels attached to a PVC plate wrapped in a biodegradable mesh; (E) exclusion treatment that consisted of an artificial aggregation similar to that of O, but protected from predation with an exclusion cage; and (PC2) a procedural control for the cage that consisted of an artificial aggregation similar to that of E, but with 3 holes (on the top and lateral sides of cages) in the exclusion cages to allow access to predators (Fig. 2A). Mussels

from treatments C and PC1 were never detached from the shore, whereas mussels for treatments O, E, and PC2 were detached, transported to the laboratory and artificially assembled and then returned to the field.

The main experiment was set up in May 2011 and lasted for 3 mo, from 17 May to 17 August 2011. One orthogonal random factor (Site) and 2 orthogonal fixed factors (Type of aggregation and Predation) were included in the experimental design (number of replicates per combination of treatments  $n = 4$ ). The factor Type of aggregation included different densities of mussels in monospecific and mixed aggregations assembled in 6 treatments: (M) with low density of *M. galloprovincialis*, 7 individuals; (MMM) with the highest density of *M. galloprovincialis*,  $7 + 14 = 21$  individuals; (MXX) with low density of *M. galloprovincialis* and high density of *X. securis*,  $7 + 10 = 17$  individuals; (X) with low density of *X. securis*, 5 individuals; (XXX) with the highest density of *X. securis*,  $5 + 10 = 15$  individuals and (XMM) with low density of *X. securis* and high density of *M. galloprovincialis*,  $5 + 14 = 19$  individuals (Fig. 2B). Comparisons between monospecific aggregations at low density (i.e. M/X) and monospecific and mixed aggregations at high density (i.e. MMM and MXX/XXX and XMM) allowed us to test for density dependence. In addition, comparisons between monospecific and mixed aggregations at high density (i.e. MMM and MXX/XXX and XMM) evaluated whether the magnitude of intra- and inter-specific interactions were similar. Densities were chosen based on

natural densities of both species when co-occurring in aggregations at site 2 (Gestoso et al. 2013). Despite the overall higher density of *X. securis* at this site, naturally assembled mixed aggregations had a greater proportion of *M. galloprovincialis* by approximately 25% (*X. securis*:  $4.64 \pm 0.47$  ind. per aggregation, *M. galloprovincialis*:  $7.74 \pm 1.39$  ind. per aggregation,  $n = 54$ ). We decided to maintain this proportion in synthetic assemblages to resemble natural aggregations as much as possible. Consequently, experimental assemblages were not fully equivalent

in number of mussels, but the magnitude of differences was similar across the different density treatments. To test for predation, all levels of the factor Type of aggregation were replicated with and without exclusion cages (Fig. 2C).

Once assembled, experimental units were transported from the laboratory to the field (over 3 consecutive days, one day for each site) and screwed to rocky platforms using stainless steel screws. They were randomly placed at the same height on the shore and separated by a minimum distance of ~1 m. To test for the effect of environmental conditions, the experimental units were placed at the 3 different sites (S1, S2 and S3). A total of 144 assemblages with a total of 2016 mussels were built and deployed in the field.

Species performance was measured as survivorship, growth and condition index. Survivorship was evaluated by calculating the percentage of individuals found alive at the end of the experiment from the total randomly selected and marked at the beginning of the experiment (i.e. 4 focal individuals in all experimental units). In the open experimental units, survivorship was corrected by natural mortality ( $9.6 \pm 0.03\%$  for *M. galloprovincialis*,  $13.7 \pm 0.04\%$  for *X. securis*) obtained from those plates where predators were excluded (Menge et al. 2004, Shinen et al. 2009 for similar approach). In each experimental unit, the antero-posterior lengths from the 4 marked individuals were measured with a digital calliper (resolution 0.01 mm). Growth for each individual was estimated as the change in antero-posterior length during the experiment and calculated as:

$$G = AP_e - AP_s \quad (1)$$

where G is the growth and  $AP_e$  and  $AP_s$  are the antero-posterior lengths at the end and the start of the experiment, respectively.

Condition index (CI) was calculated as:

$$CI = DTW \times 1000/DSW \quad (2)$$

where DTW is the dry soft-tissue weight, and DSW is the dry shell weight (Walne & Mann 1975). To obtain body weights of mussels from experimental units, 30 extra individuals of each species from site 2 were collected at the start and the end of the experiment. Individuals were measured and dried at 60°C for 48 h for tissue and shell dry weight determination. The relationship of AP on DSW was adjusted by a simple linear regression (Eq. 1; see Table S1 in the Supplement at [www.int-res.com/articles/suppl/m506p163\\_supp.pdf](http://www.int-res.com/articles/suppl/m506p163_supp.pdf)). Then, the relationship of DTW on DSW was adjusted by another linear regression (Eq. 2; Table S1).

By using both equations, we could estimate those parameters needed to calculate CI of individuals in experimental units.

### Statistical analyses

Survivorship was analysed as percentage of survivorship per plate, whereas growth and CI were analysed using mean values per plate. All the analyses considering the 2 species simultaneously found interactions of the factor Species with the other experimental factors. Consequently, to simplify results and reduce high order interactions, we explored the effects of competition, predation and site for each species separately.

In the pilot experiment, changes in survivorship were tested with 1-way ANOVAs, with Treatment as a single fixed factor with 5 levels (Fig. 2A). Changes in growth and CI were evaluated with ANCOVAs with the same design as that used in the ANOVA analysis. AP length and CI of mussels at the start of the experiment were used as covariates in the ANCOVAs.

In the main experiment, differences in survivorship were evaluated using a 3-way orthogonal analysis of variance including factors (1) Site (S, 3 levels: S1, S2 and S3, random), (2) Predation (P, 2 levels: Exclusion and Open, fixed) and (3) Type of aggregation (Aggr, 3 levels: monospecific aggregations at low density, i.e. M/X; monospecific aggregations at high density, i.e. MMM/XXX and mixed aggregations at high density, i.e. MXX/XMM, fixed) ( $n = 4$ ). Changes in growth and CI were evaluated with ANCOVAs, this time including only data from the exclusion plates because of the low survivorship rates in some experimental units. Site (S, 3 levels) and Type of aggregation (Aggr, 3 levels) were random and fixed factors, respectively, and AP length and CI at the start of the experiment were covariates in the ANCOVAs.

Homogeneity of variances was examined using Cochran's C-test and normality was examined through visual inspection of residual and probability-probability plots. Data were transformed when necessary. When heterogeneity persisted, analyses were done on untransformed data but the p-values for the F-ratios were considered significant at  $p < 0.01$  (Underwood 1997). For the ANCOVAs, the interaction terms of each factor with the covariate were included in the design to test whether slopes of the regression lines were significantly different. No significant interactions with the covariate indicated homogeneity of slopes and the analysis was re-run

without interactions with the covariate (McDonald 2009). To test for density dependence and examine the magnitude of both intra- and inter-specific interactions, comparisons on significant sources of variation involving the factor Type of aggregation were done using *a priori* contrasts using *t*-tests (Tables S2 & S3 in the Supplement). When significant differences of the main factors Predation or Site, or their interaction (i.e.  $P \times S$ ) were found, Student–Newman–Keuls (SNK) tests were used as *a posteriori* comparisons. All data are reported as means  $\pm$  SE.

ANOVAs were performed with GMAV 5 statistical software for Windows (Underwood & Chapman 1997), ANCOVAs were done with the SPSS software (version 19.0; SPSS Inc) and *t*-tests were performed with the Statistica software (StatSoft).

## RESULTS

### Pilot experiment

Survivorship of both species did not vary significantly among treatments (ANOVA, *Mytilus galloprovincialis*:  $F_{4,15} = 1.11$ ,  $p = 0.389$ ; *Xenostrobus securis*:  $F_{4,15} = 0.17$ ,  $p = 0.949$ ). Similarly, growth and CI were not influenced by treatments (ANCOVA, *M. galloprovincialis*: Growth,  $F_{4,14} = 0.452$ ,  $p = 0.769$  and CI,  $F_{4,14} = 1.014$ ,  $p = 0.433$ ; *X. securis*: Growth,  $F_{4,14} =$

$0.537$ ,  $p = 0.711$  and CI,  $F_{4,14} = 0.915$ ,  $p = 0.482$ ). This means that the manipulation of mussels and the effects of mesh and caging were negligible and, therefore, the main experiment was run without any procedural control experimental units.

### Experiment on competition and predation

Natural mortality of the 2 species was similar across sites, although survivorship of *X. securis* was slightly lower than that of *M. galloprovincialis* (overall natural mortality of *M. galloprovincialis*:  $9.60 \pm 1.87\%$ ; overall natural mortality of *X. securis*:  $23.60 \pm 1.70\%$ ,  $n = 36$ ). Predation affected both species, although its effects were context dependent (i.e. significant interaction Site  $\times$  Predation; Table 1). While predation had a significant effect on survivorship rates of *M. galloprovincialis* at sites 2 and 3, the effect of predation was only detected at site 3 for *X. securis* (Fig. 3A). Although not formally analysed, mortality due to predation appeared to be greater for *M. galloprovincialis* than *X. securis* (overall mean mortality of *M. galloprovincialis* in MMM and MXX:  $37.24 \pm 7.73\%$ ; overall mortality of *X. securis* in XXX and XMM:  $26.30 \pm 7.30\%$ ,  $n = 24$ ). Survivorship of *M. galloprovincialis* was not affected by the Type of aggregation (Table 1). The survivorship of the invader was enhanced in the presence of either conspecifics or native mussels when aggregations were exposed to predators (significant interaction  $P \times$  Aggr; Table 1), suggesting a positive density-dependent effect (i.e. significant *a priori* test  $X < XXX$  & XMM, *t*-test:  $-3.287$ ,  $p = 0.002$ ; Table S2), irrespective of the species identity (i.e. nonsignificant *a priori* test  $P \times XXX$  vs. XMM, *t*-test:  $-0.728$ ,  $p = 0.470$ ; Table S2).

Growth of *M. galloprovincialis* was not affected by any of the factors included in the model (Table 2). However, growth of *X. securis* varied spatially, with individuals growing up to  $\sim 2$  times slower at site 3 (Fig. 4A, Table 2). CI of *M. galloprovincialis* varied depending on the Type of aggregation, but the pattern was not consistent across space. Condition was greater in high density aggregations, i.e. MMM and MXX, but

Table 1. Univariate analyses of variance (ANOVA) for survivorship of mussels in experimental treatments ( $n = 4$ ). Site (S) was a random orthogonal factor, and Predation (P) and Type of aggregation (Aggr) were fixed orthogonal factors. SNK *posteriori* tests or *a priori* contrasts to evaluate main effects or interactions are also given (only significant results are shown; see Fig. 2 for treatment abbreviations). Significant *p*-values are indicated in **bold**. Cochran's tests were considered significant at  $p < 0.05$ ; ns = not significant

Source	df	<i>Mytilus galloprovincialis</i>		<i>Xenostrobus securis</i>	
		F	p	F	p
S	2	14.21	<b>0.000</b>	3.85	0.027
P	1	2.58	0.250	2.17	0.278
Aggr	2	0.09	0.914	3.15	0.151
S $\times$ P	2	18.83	<b>0.000</b>	5.35	<b>0.008</b>
S $\times$ Aggr	4	0.83	0.514	0.51	0.731
P $\times$ Aggr	2	0.15	0.867	33.13	<b>0.003</b>
S $\times$ P $\times$ Aggr	4	0.78	0.545	0.14	0.965
Error	54				
Total	71				
Cochran's test		0.3171 ( $p < 0.05$ )		0.1564 (ns)	
Transformation		None		None	
SNK		SNK of: S $\times$ P S2: E > O S3: E > O		SNK of: S $\times$ P S3: E > O <i>a priori</i> of: P $\times$ Aggr O: X < XXX & XMM <i>t</i> -test, $t = -3.287$ , $df = 54$ , $p = 0.002$	

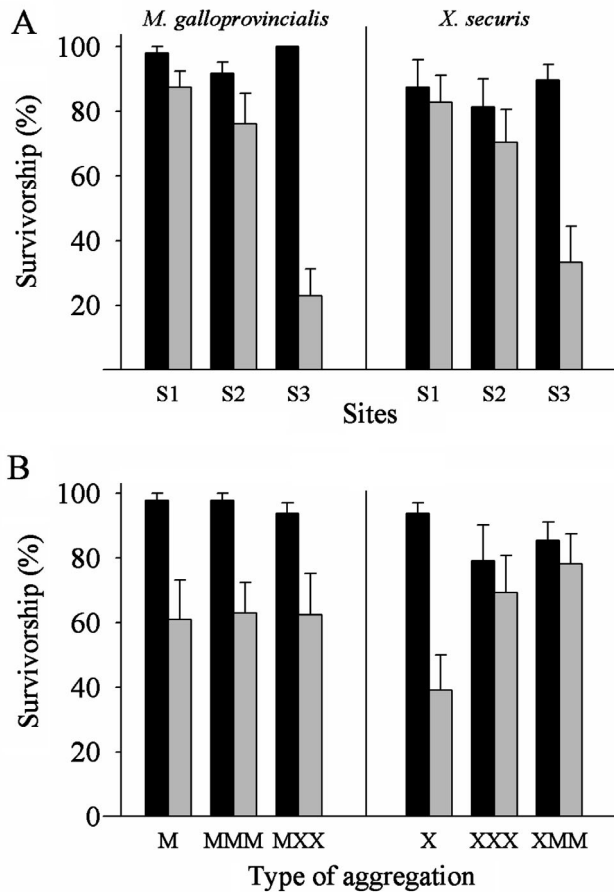


Fig. 3. Mean (+SE) survivorship of *Mytilus galloprovincialis* and *Xenostrobus securis* that were exposed (grey bars) and not exposed (black bars) to predators, (A) at 3 sites (n = 12) and (B) in the different types of aggregation (n = 24; see Fig. 2)

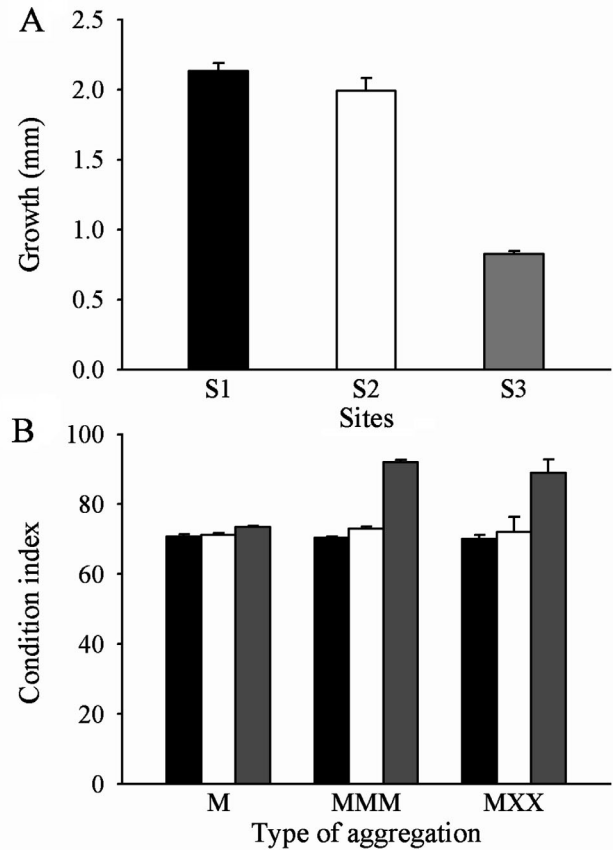


Fig. 4. Mean (+SE) of (A) growth of *Xenostrobus securis* across sites (n = 12) and (B) condition index of *Mytilus galloprovincialis* across different types of aggregation (see Fig. 2) and sites (n = 4). Black, white and grey bars correspond to sites 1 (S1), 2 (S2) and 3 (S3), respectively. Data correspond to exclusion treatments, where mussels were not exposed to predators

Table 2. Univariate analyses of covariance (ANCOVA) for growth and final condition index (CI) of mussels in experimental treatments (n = 4). Site (S) and Type of aggregation (Aggr) were orthogonal random and fixed factors, respectively, and antero-posterior length and CI at the start of experiment were used as covariates (Cov) for growth and CI, respectively. SNK *posteriori* tests or *a priori* contrasts to evaluate main effects or interactions are also given (only significant results are shown; see Fig. 2 for treatment abbreviations). Significant p-values are indicated in **bold**. Cochran's tests were considered significant at p < 0.05; ns = not significant

Source	df	<i>Mytilus galloprovincialis</i>				<i>Xenostrobus securis</i>				
		Growth		CI		Growth		CI		
		F	p	F	p	F	p	F	p	p
S	2	1.870	0.222	5.462	0.072	9.778	<b>0.030</b>	0.564	0.612	
Aggr	2	0.711	0.542	1.177	0.396	0.044	0.958	0.520	0.635	
Cov	1	0.990	0.329	0.115	0.737	3.058	0.092	11.673	<b>0.002</b>	
S × Aggr	4	1.028	0.411	7.373	<b>0.000</b>	1.249	0.315	0.789	0.543	
Error	26									
Total	36									
Cochran's test		0.3135 (ns)		0.5016 (p < 0.01)		0.2656 (ns)		0.6565 (p < 0.01)		
Transformation		None		None		None		None		
SNK		<i>a priori</i> of: S × Aggr				SNK of: S				
		S3: M < MMM & MXX				S1 > S3				
		t-test, t = -6.511, df = 26, p < 0.001				S2 > S3				

only at site 3 (Fig. 4B, Table 2). In contrast, CI of the invader was not affected by any of the factors included in the model, although it was influenced by the CI of individuals at the start of the experiment (Table 2).

## DISCUSSION

This experiment suggests that predation might be contributing to the patterns of abundance of both species, although its magnitude was context dependent. Indeed, the strongest effect of this factor was detected in the outermost area where salinity is close to seawater levels. Moreover, survivorship of predation by the invader was density dependent because it was enhanced by the presence of high densities of either conspecifics or native mussels. There was no effect of predation and/or competition on growth of *Mytilus galloprovincialis*, and only growth of the invader was affected by the environmental conditions. Furthermore, condition of *M. galloprovincialis* was better in high density aggregations irrespective of species composition, but only at site 3. Overall, we did not find any evidence of competition between the 2 species because their performance was not negatively affected when comparing mixed vs. monospecific assemblages. Thus, the results suggest that facilitative rather than competitive interactions might affect the success of this invader, although the effect of such interaction was context dependent and varied depending on the response variable considered. A growing body of literature suggests that native species frequently facilitate non-indigenous species through diverse mechanisms (see Bulleri et al. 2008 and references therein).

When individuals were not exposed to predators, both species showed similar survivorship rates, indicating that juveniles of the 2 species are adapted to cope with different environmental conditions within the area of study, particularly with strong fluctuations of salinity (Gestoso et al. 2012). However, when exposed to predators, patterns of survivorship changed, especially at site 3, where both species registered the greatest mortality. Benthic predators such as the crab *Carcinus maenas* and the dogwhelk *Nucella lapillus* are more abundant at this site ( $4.58 \pm 1.39$  ind.  $m^{-2}$ ,  $n = 60$ ) compared to site 1 ( $2.05 \pm 0.89$  ind.  $m^{-2}$ ,  $n = 60$ ) (I. Gestoso unpubl. data). These results partially agree with the observed patterns of abundance in the field. Whereas the invader presents the smallest abundances at site 3, *M. galloprovincialis* shows the opposite pattern (Gestoso et

al. 2012). More favourable environmental conditions together with a weaker ecological resistance (i.e. lower predation pressure) in the inner part of the ria compared to the outermost part could explain, to some extent, the invader's abundance patterns. Predation may be more important in constraining the abundance of the invader in its range limit than in repelling it from the native community, i.e. biotic containment rather than biotic resistance, sensu Levine et al. (2004). In fact, predation has been pointed out as an important force controlling recruitment, distribution, abundance and coexistence of diverse mussel species, although it rarely inhibits establishment of invaders (Shinen et al. 2009, Lowen et al. 2013).

From the initial stage of invasion to the current situation, the invader has spread and continues to expand along the estuarine area towards the sea. Interestingly, the survivorship of the invader was affected by the type of aggregation and the density of individuals, suffering greater mortality in monospecific aggregations at low density. This result suggests the occurrence of positive interactions among conspecifics and facilitation by the native mussel via associational defence (Bertness & Callaway 1994, Menge 1995). In fact, the aggregation of mussels in clumps has been demonstrated to diminish risk of predation (Côté & Jelnikar 1999, Kobak & Kakareko 2011). Limited accessibility, difficulty in handling individuals, a confusion effect or immobilization of the predator by byssal threads are some of the advantages of forming aggregations (Kobak & Kakareko 2011). In the outermost areas, the natural occurrence of *Xenostrobus securis* with *M. galloprovincialis* in mixed aggregations might release the invader from predation, favouring its spread. Although survivorship of *M. galloprovincialis* was not significantly affected by the type of aggregation, this species appeared to suffer more mortality due to predation than *X. securis*, except for monospecific aggregations at low density (M vs. X; Fig. 3B), in which the invader was more vulnerable to predation. These results partially agree with those reported by Veiga et al. (2011) that found the native crab *C. maenas* consumed larger numbers of *M. galloprovincialis* over *X. securis*. This experiment was, however, a laboratory experiment with only one predator species in which the crabs were offered single mussels. Nevertheless, predator-prey interactions may vary over the distributional range of the 2 mussel species because prey preferences and predation rates can vary with prey availability (Wieters & Navarrete 1998, Nakao 2000). Survivorship of *M. galloprovincialis* was



not greater at high density aggregations with or without conspecifics suggesting that clumping is a mechanism not efficient enough to prevent predation in the area of study. In contrast, *X. securis* benefited in the presence of conspecifics (XXX), but also in the presence of *M. galloprovincialis* (XMM). In mixed aggregations, mussels were distributed in 2 distinct layers, with individuals of *X. securis* at the bottom and individuals of *M. galloprovincialis* on the top. This division between 2 layers, which has been previously observed in the same area of study (Babarro & Abad 2013), might provide mechanical protection for the invader by limiting the access of predators or provoking a confusion effect on predators (Kobak & Kakareko 2011).

Performance of both species, measured as growth rate and CI, varied across the environmental conditions, but in different directions. Growth rate of *X. securis* decreased towards the outermost area. Greater predation pressure at this site might force the invader to reduce filter feeding and, thus, allocate less energy to shell growth (Nakaoka 2000, Czarnoleski et al. 2011). These non-lethal effects of predation have been demonstrated to affect population dynamics of bivalves and are likely when prey exhibit trade-offs between energy acquisition and predator defences (Nakaoka 2000). On the other hand, the invader is a brackish water species which tolerates a wider salinity range than *M. galloprovincialis* (Wilson 1969, Babarro & Lassudrie 2011), although in its native range it occurs predominantly where salinity is low (Colgan & da Costa 2013). Less favourable environmental conditions, particularly an increase in salinity, might negatively affect its growth rates. Alternatively, differences in the quality and quantity of food within the study area might also affect its growth rates (Moore & Suthers 2005). A greater availability of land-derived organic matter and microphytobenthos (through resuspension) in inner areas might positively affect growth rates of the invader. Recent work indicated that the contribution of terrestrial matter through dissolved matter might be important in the inner part of the study area (Filgueira & Castro 2011).

In contrast, CI of *M. galloprovincialis* increased in the outermost site, but only at high density aggregations which suggests an indirect positive effect of assembling at high densities. As a fully marine species, *M. galloprovincialis* might find more favourable environmental conditions at site 3, even though predation pressure is higher. High density aggregations of either conspecifics or the invader would reduce its vulnerability to predators at this site and, thus, *M.*

*galloprovincialis* could allocate more energy to soft-tissue growth than to protective tissues (Babarro & Carrington 2013). In mixed aggregations, *M. galloprovincialis* could also obtain benefit from the invader by being on the top of aggregations (Babarro & Abad 2013). We suggest that *M. galloprovincialis* might benefit from better access to food resources.

Despite the logistical constraints, i.e. lack of replication of sites along the environmental gradient, the study highlights the importance of environmental context in altering biotic resistance generated by native communities. The study also points out the importance of considering positive interactions when analysing biological invasions. Contrary to results found by Babarro & Abad (2013) that reported interference competition between both species, we did not find any effect of competition. These contrasting outcomes might result from the fact that the design used by Babarro & Abad (2013) did not disentangle the effect of competition from predation. Differences in the prey preference of predators may confound competition experiments when predation is not properly included in the design (Gurevitch et al. 2000). One intriguing unsolved question is why the invader has completely invaded site 1 almost excluding *M. galloprovincialis* from this area. Earlier works indicated the presence of both species at this site (Garci et al. 2007), but currently the presence of *M. galloprovincialis* at this site is almost insignificant. From our results, one feasible explanation is that, at the beginning of the invasion, optimal environmental conditions, i.e. low salinity conditions for larval development of *X. securis* (Wilson 1969), could have favoured successful recruitment, and eventually conspecific attraction arising from Allee effects would have positively influenced the establishment of a large population at this site (Stephens & Sutherland 1999). Also, competition at the earliest larval stage in optimal environmental conditions might have favoured the invader over the native species (Rius et al. 2009). Further investigation would be necessary to separate abiotic and biotic impacts on recruitment of both species to clarify the biotic resistance mechanisms in the early life history, a next key step to improve predictions about this invasion process (Rius et al. 2014). Nevertheless, whatever the mechanisms, it is obvious that under favourable conditions, the introduced mussel may exclude native mussel populations as has occurred at site 1.

Invasion ecology is just beginning to address the role of positive interactions on the invasibility of marine communities (Bulleri 2009). Here, we found that facilitative interactions between *X. securis* and the

native mussel may benefit the invader, at least under particular environmental conditions and predation pressures. Although predation may be important in reducing abundance of the invader in its range limit, facilitation by the native species, i.e. associational defence, could partially offset its negative effect. Biotic resistance and facilitation may operate at different points along an environmental gradient to determine the distributional patterns of an invader. Because spatial variation of biotic interactions may only explain, in part, the observed patterns of abundance of the 2 species, recruitment is very likely another key factor to take into account. Predictions of future invasion scenarios and management strategies will require a greater understanding of the role of recruitment and post-recruitment processes (both negative and positive interactions), and how the direction and magnitude of such interactions vary across environmental gradients.

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