

Research Article

Dynamics and processes influencing recruitment of the invasive mussel *Xenostrobus securis* and the coexisting indigenous *Mytilus galloprovincialis* in north-western Spain

Agar Montes¹, Elsa Vázquez¹, Laura G. Peteiro^{1,2} and Celia Olabarria^{1,*}

¹Centro de Investigación Mariña, Universidade de Vigo, EcoCost, Facultade de Ciencias del Mar, Edificio CC Experimentais, Campus de Vigo, As Lagoas, Marcosende 36310 Vigo, Spain

²Instituto de Investigaciones Marinas IIM-CSIC, Eduardo Cabello 6, 36208 Vigo, Spain

Author e-mails: agarmontes@gmail.com (AM), eotero@uvigo.es (EV), lauragpeteiro@gmail.com (LGP), colabarria@uvigo.es (CO)

*Corresponding author

Citation: Montes A, Vázquez E, Peteiro LG, Olabarria C (2021) Dynamics and processes influencing recruitment of the invasive mussel *Xenostrobus securis* and the coexisting indigenous *Mytilus galloprovincialis* in north-western Spain. *Aquatic Invasions* 16(3): 391–414, <https://doi.org/10.3391/ai.2021.16.3.02>

Received: 11 September 2020

Accepted: 30 January 2021

Published: 19 April 2021

Handling editor: Philippe Gouletquer

Thematic editor: Charles Martin

Copyright: © Montes et al.

This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International - CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).

OPEN ACCESS

Abstract

The local extent of populations of invasive species is dependent on coupled dispersal–recruitment dynamics and subsequent interactions with the recipient community and the local environment. In this study several field experiments were conducted in order to investigate the main factors controlling larval supply (settlement and recruitment time-series), substrate selection (7 different treatments) and post-recruitment mortality (predation exclusion experiment) of the invasive mussel *Xenostrobus securis* and the coexisting, commercially important native mussel *Mytilus galloprovincialis*. The study aimed to evaluate differences between the two species in spatio-temporal patterns of recruitment along an estuarine gradient in the Ría de Vigo (NW Spain). Settlement and recruitment of *X. securis* were coupled to the distribution of the adult populations and were mainly restricted to the innermost part of the ria, in direct contrast to the patterns displayed by the native *M. galloprovincialis*. The recruitment period of the invader in the study area lasted 5 months, partly overlapping with that of *M. galloprovincialis*. *Xenostrobus securis* larvae did not display a substrate preference, whereas *M. galloprovincialis* larvae preferred substrates with the presence of conspecifics. Although predation pressure was greater on *M. galloprovincialis* than on the invader, size-dependent predation pressure was observed in both species, suggesting that smaller individuals are more vulnerable to predators. Density-dependent processes may also account for recruitment density, especially under low predatory pressure. Despite having life-history traits characteristic of a successful invader, *X. securis* populations have remained stable since 2012 and are mainly restricted to the inner part of the Ría de Vigo. This observation suggests that the species spread may be controlled by seasonal dispersal barriers associated with topographic features.

Key words: larval supply, substrate selection, post-recruitment predation, dispersal barriers, recruitment patterns

Introduction

Recruitment of benthic invertebrates in coastal areas is spatially and temporally variable, with large differences (by orders of magnitude) in cohorts of diverse species on intertidal rocky shores (Roughgarden et al. 1985; Scrosati and Ellrich 2016) and on artificial substrates (Smith 2010). This variability strongly influences population dynamics and community

structure through changes in abundance, richness and diversity (Connell 1985). In addition, the spread and impact of invasive species, an ecologically and economically important phenomenon, depends on recruitment (Grosholz 1996). This is a serious problem because, once established, invasive species are often impossible to eradicate (Vitousek et al. 1996).

Recruitment is determined by larval supply, settlement and post-settlement mortality (Karlson and Hurd 1999), which depend on the influence of several abiotic and biotic factors. Most benthic invertebrates have a pelagic larval stage, which contributes to the uncertainty of recruitment. Mortality rates during the planktonic stage of invertebrates can be up to 0.14 loss day⁻¹ or $\geq 1.5\%$ survival during a 30-day larval period (White et al. 2014). However, post-recruitment mortality may be even higher (Gosselin and Qian 1997) often leading to benthic-pelagic decoupling (Hunt and Scheibling 1997) and changes in the size of populations, with further implications for community structure and functioning (Connell 1985; Pineda et al. 2010).

Settlement depends on factors such as larval survival and supply as well as the availability and selection of optimum substrata, all of which are modulated by behavioural and hydrodynamic processes (Nielsen and Franz 1995; Rodríguez et al. 1993; Queiroga and Blanton 2005; Pineda et al. 2010). Regarding larval survival and supply, large-scale oceanographic processes such as wind-driven upwelling and downwelling events are important in regulating the settlement of invertebrates in coastal areas because they influence food availability as well as along- and cross-shore larval transport (Queiroga et al. 2007). In general, larvae are carried far from the shore during upwelling events, thus hindering their return to settle on available substrata and resulting in high rates of larval wastage. The reverse occurs during downwelling events, resulting in high rates of larval settlement. Nonetheless, many species have developed different behavioural strategies to prevent displacement far from suitable habitats, so that relationship between upwelling and settlement magnitude is not consistent in many species (Shanks and Brink 2005; Shanks and Shearman 2009). Small-scale hydrodynamic processes and wave exposure can also regulate larval supply and larval advection (McKindsey and Bourget 2000), but they are usually related to the substrate selection capacity of larvae (Pernet et al. 2003) and to post-settlement mortality (Denny and Wethey 2001).

Temperature, salinity, quantity and quality of food supply and predation are also important factors affecting recruitment rates via the survival of marine larvae and settlers (e.g. Young and Chia 1987; Rodríguez et al. 1993; Leonard et al. 1999; Vázquez and Young 2000). These factors can interact with the settlement substrate to determine the survival rate, and substrate selection is therefore a key evolutionary process influenced by several physical and/or chemical cues. Substrate selection is influenced by physical cues such as the complexity of substrata, roughness and thermal

capacity of surfaces, elemental and organic composition of substrata, the orientation of the settlement surface and light incidence (Vázquez and Young 1998; Palomo et al. 2007; Carl et al. 2012). In the case of mussels, larvae initially attach as pediveligers and actively explore the substratum being capable of discriminating between different substrata and showing preferences on textured and complex surfaces (Carl et al. 2012 and references therein). In addition, chemical cues associated with specific prey and predators, biofilms, macroalgae or conspecific individuals (either waterborne or absorbed on surfaces) also play an important role in driving larval settlement (Tamburri et al. 2008; Gribben et al. 2009; Whalan et al. 2012; Morello and Yund 2016). For example, in the case of mussel beds, larval responses to chemical cues released by adults of the same species often lead to dense aggregations of conspecifics (Chiba and Noda 2000; Morello and Yund 2016), whose presence enhances post-metamorphic survival and/or mating success (Tamburri et al. 2008).

Mechanisms explaining the high level of short-term variability observed in the early phase of benthic life are poorly understood, because of the complexity of factors involved. In addition to the factors determining larval supply and substrate selection, post-settlement processes such as physical and/or biological disturbance, competition and predation strongly influence the survival and growth rates of settlers (Menge 1991; Hunt and Scheibling 1997; Leonard et al. 1999; McQuaid and Lindsay 2005).

Intra- and inter-specific interactions are often disregarded, although they strongly determine population dynamics of some species. Negative interactions such as predation and competition for food/space are important drivers influencing recruitment success. For example, predation is one of the main factors, together with salinity, that determines the recruitment of the invasive mussel *Musculista senhousia* (Benson, 1842) (Cheng and Hovel 2010). Nevertheless, positive interactions and facilitative processes are also important for post-settlement stages (Cáceres-Martínez et al. 1994; Leonard et al. 1999). For example, survival of barnacle recruits is enhanced when the larvae settle within adult clumps because they are buffered from thermal stress (Leonard et al. 1999).

In north-western Spain, the invasive “black-pygmy mussel” *Xenostrobus securis* (Lamarck, 1819), which is originally from brackish waters of Australia and New Zealand (Wilson 1968; Colgan and da Costa 2013), first became established in the Galician Rías Baixas in 1995 (Garci et al. 2007; Gestoso et al. 2012; Montes et al. 2018). The more generally accepted hypothesis to explain the arrival of this species into Galician waters is that larvae might have been transported onto shells of other bivalves destined to aquaculture (Gestoso et al. 2012). This species coexists with the commercially important native mussel *Mytilus galloprovincialis* Lamarck, 1819 forming mixed aggregations of different densities on intertidal rocky shores (Gestoso et al. 2012). The tolerance of *X. securis* to a wide range of environmental conditions,

together with an almost continuous breeding season, during which planktotrophic larvae are present in the water column for almost 10 months, suggests that this species has the ability to spread to different habitats along estuarine gradients (Wilson 1969; Montes et al. 2018, 2020), although the distribution of adults is almost entirely restricted to the inner part of the rias (Gestoso et al. 2012; Montes et al. 2018).

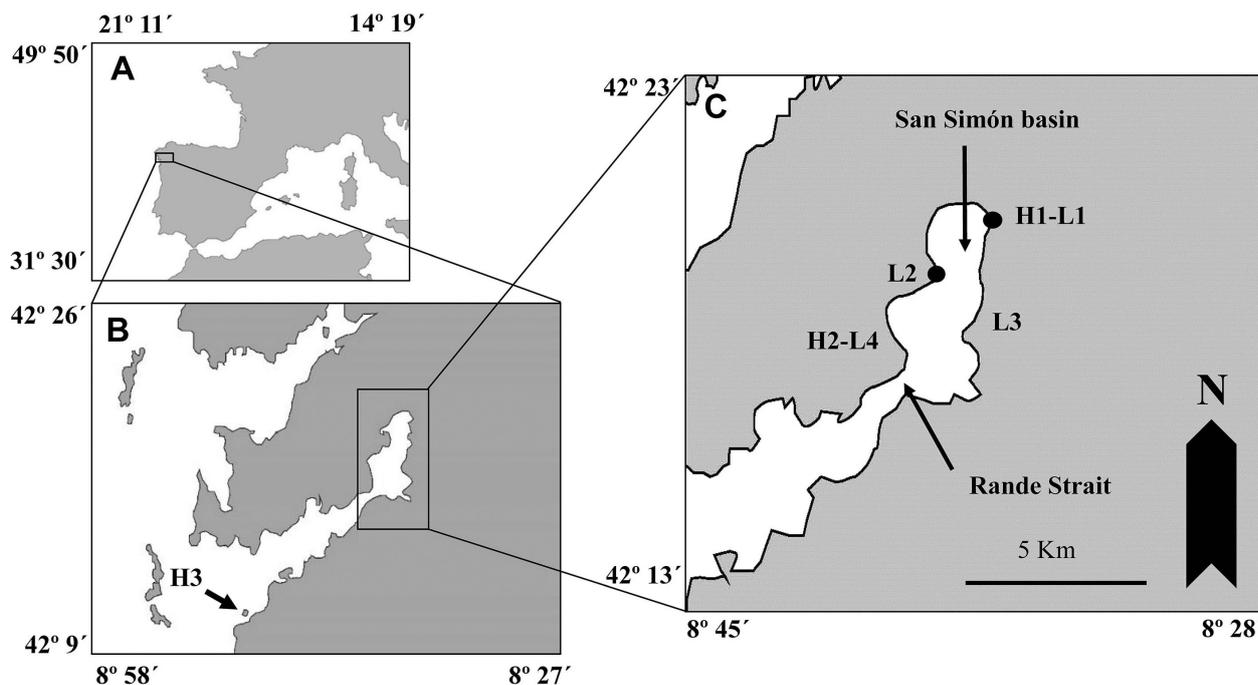
Studies on this invader outside its native range suggest that predation may play an important role in regulating juvenile and adult populations, especially at salinity levels higher than 30 (Gestoso et al. 2014), although competitive and facilitative interactions with the closely related *M. galloprovincialis* may also play an important role (Babarro and Abad 2013; Gestoso et al. 2014). Factors affecting larval supply and survival, substrate selection for settlement and post-settlement mortality are poorly understood, although they may be critical in determining the distribution and dynamics of adult populations of this invasive species, which can negatively impact native brackish water ecosystems (Iwasaki and Yamamoto 2014).

The aim of the present study was to elucidate the main factors determining *X. securis* larval supply, substrate selection and post-settlement mortality in order to explain the spatial and temporal variation in recruitment of the invader and the coexisting mussel species *M. galloprovincialis* across an estuarine gradient. To achieve this goal, high frequency settlement time-series (every other day) were examined during the peak period of the reproductive season of *X. securis* (Montes et al. 2020) in order to evaluate the possible influence of upwelling on larval supply. The temporal and spatial variability in settlement and recruitment was evaluated as monthly settlement on plates over one year. The influence of temperature and salinity on these patterns was also examined. In addition, the effect of predation on post-settlement mortality was evaluated in an exclusion experiment. Finally, the influence of physical structure and/or biological cues on larval substrate selection and post-settlement survival of the invader was assessed by comparing recruitment on a range of different substrates.

Materials and methods

Study area

The experiments were carried out on rocky intertidal shores in the Ría de Vigo (NW Iberian Peninsula, Figure 1). The Ría de Vigo, which occupies an area of 176 km², is V-shaped, with its central axis lying in a southwest–northeast direction and with a marked strait in the middle (Rande Strait). The water is generally less than 50 m deep and is particularly shallow after the Rande Strait, where the ria widens to form a well-differentiated, very shallow basin, the Ensenada de San Simón (Nombela et al. 1995), which is strongly influenced by fluvial discharges (Perez-Arlucea et al. 2005). The ria is influenced by upwelling (Alvarez et al. 2008), which mainly occurs



Location	GPS	Salinity (mean \pm SD; n= 370)	Density (ind. m ⁻² ; mean \pm SE; n= 20)
H1-L1	42° 20' 43'' N	13.57 \pm 9.18	<i>X. securis</i> 2897.5 \pm 487.18
	8° 36' 27'' W	to 24.25 \pm 7.58	<i>M. galloprovincialis</i> 0
L2	42° 19' 39'' N	22.49 \pm 7.24	<i>X. securis</i> 12.5 \pm 4.54
	8° 38' 12'' W	to 23.10 \pm 7.42	<i>M. galloprovincialis</i> 327.5 \pm 68.19
L3	42° 18' 55'' N	28.22 \pm 3.00	<i>X. securis</i> 123.75 \pm 24.76
	8° 37' 10'' W	to 30.20 \pm 2.02	<i>M. galloprovincialis</i> 165 \pm 44.98
H2-L4	42° 17' 39'' N	29.03 \pm 2.90	<i>X. securis</i> 1.25 \pm 1.25
	8° 39' 20'' W	to 32.63 \pm 2.55	<i>M. galloprovincialis</i> 197.5 \pm 38.59
H3	42° 12' 07'' N	33.43 \pm 1.14	<i>X. securis</i> 0
	8° 47' 57'' W		<i>M. galloprovincialis</i> 569.60 \pm 53.55

Figure 1. Sampling locations in the Ría de Vigo (Galicia, NW Spain). (A) Map of Spain situating Galicia. (B) Map of Ría de Vigo showing San Simón basin and Toralla (H3). (C) Map of San Simón basin showing the sampling locations. All locations indicated by black circles (H1–L1: Pontesampaio, L2: Punta Cabalo, L3: Cesantes, H2–L4: Rande, H3: Toralla). San Simón basin and Rande Strait are indicated by black arrows. Table shows coordinates, range of salinity fluctuations and density of the two mussel species at the different sampling locations.

during spring and summer under favourable wind conditions (northerly winds) along the coast. This seasonal wind pattern is, however, intermittently altered by winds that favour downwelling, i.e. south – southwesterly winds (Alvarez et al. 2003).

Several experiments were established at different locations along the Ría de Vigo: high-frequency settlement time-series were collected at 3 locations along the length of the ria (H1, H2 and H3), while low-frequency time-series were concentrated in the San Simón basin (L1, L2, L3 and L4, Figure 1). These locations are characterized by a salinity gradient and by a high though variable density of the invader except for L4.

Environmental variables

Seawater temperature and salinity were recorded daily, every 30 minutes, between March 2014 and March 2015 by using mini data storage tags (DST, Star Oddi) placed in the lower intertidal zone at each location of the inner part of the ria (H1–L1, L2, L3, and H2–L4). The weekly average,

minimum and maximum temperature and salinity levels were calculated from these data. Weekly temperature and salinity values are displayed in Supplementary material Figure S1. The data recorded during the low-frequency settlement time series were used to analyse the influence of environmental variables on recruitment.

The daily upwelling index (UI; $\text{m}^3 \text{s}^{-1} \text{km}^{-1}$) was calculated for the Ría de Vigo from 6-hourly data collected in the period between 20 June and 20 July 2015 (Instituto Español de Oceanografía; www.indicedeafloramiento.ieo.es). This index was calculated for the Iberian Peninsula according to Lavín et al. (1991), by using sea level pressure from the Meteogalicia WRF atmospheric model (www.meteogalicia.es/modelos). Average UI values for the two days prior to the collection of the high-frequency time series samples were calculated in order to analyse the influence of UI on larval supply at each location for *X. securis* and *M. galloprovincialis*.

Field sampling

High-frequency settlement time-series: larval supply along the estuarine gradient and influence of upwelling

To study the larval supply along the estuarine gradient and the influence of upwelling on settlement, artificial collectors made of green nylon scourers ($8 \times 10 \text{ cm}$; $n = 5$) were attached to rocks in the mid intertidal zone at locations H1–L1, H2–L4 and H3, during the peak period of the *X. securis* reproductive season (June–August; Montes et al. 2018), i.e. for one month between 20 June and 20 July 2015 (Figure S2). The collectors were placed at random locations on the shore, at the same height, separated by a distance of $\sim 1 \text{ m}$, and were replaced every other day. The removed collectors were transported in individual plastic bags to the laboratory, where they were frozen until required. Prior to analysis, the scourers were gently scraped with a palette knife and rinsed with water through a $500 \mu\text{m}$ sieve. *Mytilus galloprovincialis* and *X. securis* larvae were separated, identified, counted and measured (antero-posterior shell length) under a stereomicroscope. The scourers were also examined under the stereomicroscope to verify that all recruits had been removed.

Low-frequency settlement time-series: spatial and temporal variability of recruitment and the effect of predation

Artificial collectors made of PVC plates ($15 \times 15 \times 0.5 \text{ cm}$) covered with green nylon scourers ($9 \times 14 \text{ cm}$) were used to estimate the variability in recruitment (survival 1 month after settlement) and the effects of predation at locations H1–L1, L2, L3 and H2–L4. The collectors were randomly distributed at 1 m intervals and attached to rocks with stainless steel screws in the mid intertidal zone of the four locations, between March 2014 and March 2015. The experimental design consisted of three “treatments” ($n = 4$): a) control (C), PVC plate covered with scourer; b) predator exclusion (WP),

PVC plate with scourer protected from predation with a plastic exclusion cage ($14.5 \times 11.5 \times 8.5$ cm; mesh size: 0.5 cm); and, c) procedural control (PC) for the cage, consisting of a PVC plate with scourer with a plastic exclusion cage with three holes (on the top and lateral sides of the cage) to allow free access to predators (Figure S2). The collectors were replaced monthly, and the removed collectors were transported to the laboratory. Sample processing was as described above.

Substrate selection and the effect on post-settlement mortality: role of physical structure and biological cues

To identify possible cues related to the presence of conspecifics (*X. securis*) or the potential competitor/facilitator (*M. galloprovincialis*) on recruitment success of the invader, a substrate selection experiment was established in two locations (H1–L1 and L3). Artificial collectors to which adults of *X. securis* or *M. galloprovincialis* were previously attached were used. To distinguish physical and biological cues, which could determine post-settlement mortality or substrate selection, plates with “dead” (i.e. shells only) *M. galloprovincialis* and *X. securis* were also included. Finally, three additional experimental “treatments”, consisting of plates with Velcro® previously submerged for at least 48 h in a mixture of seawater and crushed adults (10 gL^{-1}) of *X. securis* or *M. galloprovincialis*, and plates with only Velcro® were established (Figure S2). Artificial dead or live mussel aggregations each comprising 12 individuals of similar size (*X. securis*: 3 ± 0.3 cm; *M. galloprovincialis*: 4 ± 0.2 cm, mean \pm S.D.) were constructed on previously sanded PVC plates ($15 \times 15 \times 0.5$ cm). In the treatments with live mussels, biofouling was cleaned from the shells and byssal threads were removed from the ventral margin; the mussels were then placed on the PVC plates and held in the laboratory in running seawater for 2–3 days to enable attachment, before being transported to the field. We assumed that handling, caging and the biodegradable mesh used for attachment did not significantly affect mussel survival (Gestoso et al. 2014). For the treatments with shells, the individuals were previously frozen, and the flesh was then removed and the shells were cleaned. Finally, both valves were filled and glued to the PVC plate with neutral silicone forming an aggregation of 12 individuals, as in treatments with live mussels. Plastic cages ($14 \times 12 \times 8$ cm) were used to exclude predators in all treatments and to isolate predation from the post-settlement mortality associated with the substrate. Once assembled, the experimental plates were located in Pontesampaio (H1–L1) and Cesantes (L3) ($n = 5$) and randomly screwed to rocks on the low intertidal zone, where the density of *X. securis* was maximal. The plates were left in the field for three months, between June and August 2015, when larvae of *X. securis* are most abundant in the water column (Montes et al. 2018). The collected plates were frozen in the laboratory until further analysis, following the

previously described protocol. Again, all bivalves were separated and *M. galloprovincialis* and *X. securis* recruits were identified, counted and measured.

Statistical analysis

High-frequency settlement time-series: larval supply along the estuarine gradient and influence of upwelling

Cross-correlations were determined in order to evaluate settlement synchrony between locations and species (*M. galloprovincialis* and *X. securis*) at the peak of the reproductive season of the invader (June–July 2015), as well as to evaluate the impact of UI along the estuarine gradient for each species.

Low-frequency settlement time-series: spatial and temporal variability in recruitment and the effect of predation

Changes in abundance of *X. securis* and *M. galloprovincialis* recruits were established separately for each species by applying linear models to log-transformed data. As recruitment was very low outside the peak season (≤ 3 individuals), only the temporal windows between July and September and between May and August were formally analysed for *X. securis* and *M. galloprovincialis*, respectively. In addition, due to the low number of recruits of *X. securis* and *M. galloprovincialis* at some locations, recruitment was only formally analysed and represented graphically for two locations (H1–L1 and L2) for *X. securis* and for three locations (H1–L1, L2, H2–L4) for *M. galloprovincialis*.

The model for *X. securis* included three fixed orthogonal factors: Location (2 levels: H1–L1 and L2), Treatment (3 levels: control [C], exclusion [WP] and procedural control [PC]) and Time (3 levels: July, August, September, i.e. the months when recruitment was the highest, see results). For *M. galloprovincialis*, the Location factor included 3 levels (H1–L1, L2 and H2–L4), the Time factor included 4 levels (May, June, July, August), and the Treatment factor included the same 3 levels as for *X. securis*.

Changes in size of *X. securis* and *M. galloprovincialis* were modelled by fitting linear models to log-transformed data, where only *X. securis* and *M. galloprovincialis* individuals from respectively H1–L1 and H2–L4 were analysed. The model included two fixed orthogonal factors: Treatment (3 levels: control [C], exclusion [WP] and procedural control [PC]) and Size class (2 levels: early recruits smaller than 1 mm and late recruits ranging between 1 and 3 mm) as fixed factors in the case of *X. securis* and only the Treatment factor in the case of *M. galloprovincialis* (due to the low number of recruits found in the artificial collectors during the peak recruitment period for *X. securis*). Recruitment of *M. galloprovincialis* in the Ría de Vigo is known to be maximal in May (Cáceres-Martínez et al. 1993). Monthly data over the peak recruitment period were pooled for these analyses.

In addition, cross correlations were used to evaluate the effects of temperature and salinity, i.e. mean, minimum and maximum values, on the total monthly recruitment of *X. securis* and *M. galloprovincialis* at each location. The total monthly number of recruits at each location was calculated by pooling all recruits across C, WP and PC treatments over the whole recruitment period.

Substrate selection and its effect on post-settlement mortality: role of physical structure and biological cues in recruitment

Changes in abundance of *X. securis* and *M. galloprovincialis* recruits were modelled separately by fitting linear models to log-transformed data. The model of abundance included two orthogonal fixed factors: Location (2 levels: H1–L1 and L3) and Treatment (7 levels: plates with live *X. securis*, plates with dead *X. securis*, plates with Velcro® previously embedded in a liquid composed of water and crushed *X. securis* residues, plates with live *M. galloprovincialis*, plates with dead *M. galloprovincialis*, plates with Velcro® previously embedded in a liquid composed of water and crushed *M. galloprovincialis* residues, and plates with Velcro® only).

Prior to all analyses, the normality and homogeneity of variances were checked by visual inspection of Q–Q and residual plots. A posteriori pairwise tests were used to compare levels of significant factors or interactions ($P < 0.05$) on the response variables and Bonferroni correction was used when multiple comparisons were done. All analyses were performed with the open-source RStudio software, version 6.3.1 (R Core Team 2014). LMs and cross correlations were calculated using the *lm*, *glm* and *ccf* functions of the *stats* package.

Results

Environmental variables

The highest fluctuations in sea surface temperatures (SST) were recorded at L2 (largest standard deviations), followed by H1–L1, L3, H2–L4, and then H3, where SST was more stable. Mean surface temperature followed a seasonal cycle, peaking between August and September 2014, when temperature ranged between 19.8 and 21 °C, and decreasing to minimum values between January and February 2015 (range 10.3–11.9 °C) (Figure S1). However, at H3, the minimum temperature was delayed until March 2015 (range 9.15–10.96 °C).

The strongest fluctuations in salinity were recorded at H1–L1, due to the influence of the Verdugo river (larger standard deviations), followed by L2, L3, H2–L4 and H3. Salinity peaked between June–July and September 2014, with average values of 25.29, 30.22, 30.54, 33.67, and 34.56 at H1–L1, L2, L3, H2–L4 and H3, respectively (Figure S1). In July, a decrease in average salinity was observed at L3 and, in August, an important decrease

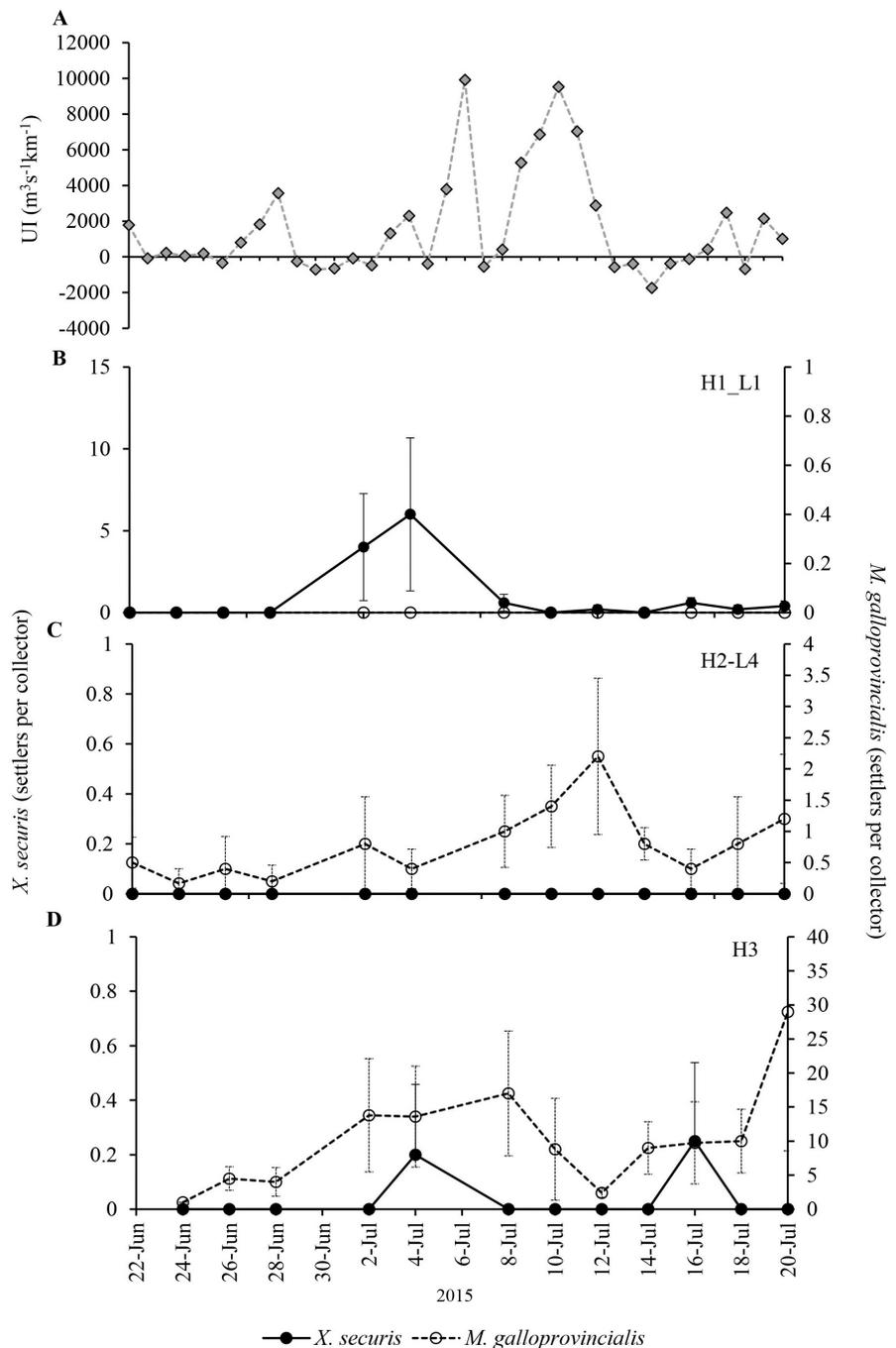


Figure 2. A) Daily upwelling index (UI; $\text{m}^3 \text{s}^{-1} \text{km}^{-1}$) for the Ría de Vigo, and mean (\pm SE) abundance of *X. securis* and *M. galloprovincialis* settlers per collector ($n = 5$). B) *X. securis* and *M. galloprovincialis* settlers at H1–L1. C) *X. securis* and *M. galloprovincialis* settlers at H2–L4. D) *X. securis* and *M. galloprovincialis* settlers at H3.

was observed at H1–L1 (with values close to 11 recorded) and to a lesser extent at L2. However at H3, salinity reached minimum values ~ 25 in March 2014.

Two important peaks in the upwelling index (Figure 2A) were recorded: one in the first week of July, and (after a short period of relaxation) another of the same intensity, but which lasted longer, in the second week of July. During the period considered (22 June to 20 July), no downwelling event was recorded.

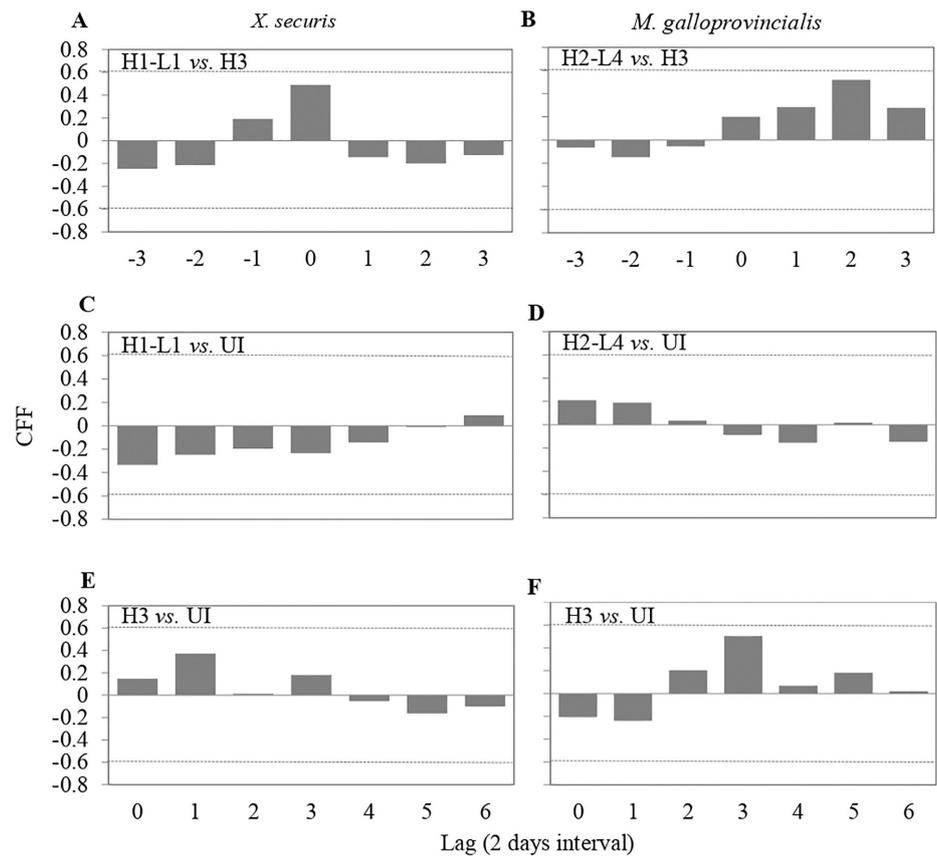


Figure 3. Cross correlations between different locations (H1–L1, H2–L4 and H3) for settlers of the two different species under study (A) *X. securis* and (B) *M. galloprovincialis*. In addition, cross correlations between different locations (H1–L1, H2–L4 and H3) and the upwelling index (UI), calculated separately for *X. securis* and *M. galloprovincialis*, are shown. C) H1–L1 vs UI for *X. securis*. D) H2–L4 vs UI for *M. galloprovincialis*. E) H3 vs UI for *X. securis*. F) H3 vs UI for *M. galloprovincialis*. The horizontal dashed lines represent the 95% confidence intervals of the correlation values. The values outside the two horizontal (unbroken, dashed) lines indicate significant correlations. Time lag, 2 days.

High-frequency settlement time-series: larval supply along the estuarine gradient and influence of upwelling

A clear settlement gradient was observed along the estuary for both species, but with opposite patterns (Figure 2). Thus, while *X. securis* settlement was concentrated in the inner part of the ria (H1–L1) (Figure 2B), with no or low settlement recorded at H2–L4 and H3 (Figure 2C, D), settlement of *M. galloprovincialis* was highest at the outermost location (H3) (Figure 2D) and was much lower in the middle location (H2–L4) (Figure 2C), with no record of settlers at the innermost location (H1–L1) (Figure 2B).

Although not significant, CCF analysis between sampling sites for each species revealed some differences between *X. securis* and *M. galloprovincialis* on their spatio-temporal settlement patterns (Figure 3A, B). Settlement of *X. securis* was fairly synchronous in the outermost and the innermost locations ($r = 0.490$ for lag 0, Figure 3A), while in the middle part of the ria (H2–L4) settlement of *M. galloprovincialis* tended to be delayed by ~ 4 days relative to that in the outermost location (H3) ($r = 0.519$ for lag 2, Figure 3B).

Concerning the effect of UI on settlement, no significant relationship was observed at any location for either mussel species (Figure 3C–F). Nonetheless, settlement of *X. securis* at the innermost location (H1–L1) was negatively correlated with UI, suggesting an increase in settlement at this location with periods of relaxation of upwelling (Figure 3C). By contrast, settlement of *M. galloprovincialis* at the outermost location was positively correlated with UI, but with a delay of ~ 6 days (lag 3, $r = 0.505$; Figure 3F).

Low-frequency settlement time-series: spatial and temporal variability in recruitment and the effect of predation

Recruitment of *X. securis* and *M. galloprovincialis* varied widely between locations (Figure 4). Recruitment of *X. securis* was concentrated during summer-early autumn, specifically between June and October and mainly occurred at two locations, H1–L1 and L2 (Figure 4A, C). Recruitment of the invasive species was much higher at the innermost location (H1–L1) (Table 1; Figure 4A, C) during the whole recruitment season (pairwise tests; $P < 0.001$), whereas it was almost anecdotal at locations L3 and H2–L4 (total number of recruits: 5 for L3 and 3 for H2–L4 between July and October). In general, recruitment of *M. galloprovincialis* was low (Figure 4B, D, E) and peaked at H2–L4 (the outermost location) in July (Figure 4E), while at the other locations, recruitment was irrelevant (a total of 5 recruits at L2 and 16 at H1–L1) (Figure 4D, E). Recruitment of *M. galloprovincialis* mainly occurred between late spring and summer (between May and August).

The recruitment of *X. securis* and *M. galloprovincialis* was very variable at small spatial scales, i.e. between replicates at the same location. For instance, the largest and the smallest number of *X. securis* recruits recorded at L1 in July were respectively 1150 and 28 (± 310.75 , SD) (Figure 4A). The location-time interaction reflects the displacement of the peak period of *X. securis* recruitment between H1–L1 and L2 (Table 1). While the peak of recruitment for *X. securis* occurred during July and August in the innermost location (H1–L1), the maximum number of recruits was recorded at L2 in August and September (Table 1; Figure 4C).

With regard to the effect of predation on recruitment, no significant effect of treatment was observed on the density of *X. securis* recruits, whereas in *M. galloprovincialis*, recruitment varied significantly between treatments, although it was dependent on space and time (i.e. significant Location \times Time \times Treatment interaction; $P = 0.015$, Table 1). Nonetheless, post-hoc tests only detected a significantly higher density of recruits in the exclusion treatment relative to the control in Rande Strait in July (pairwise tests, $P < 0.001$). The high variability between replicates precluded detection of more significant differences between predator exclusion, procedural control or control (Figure 4A, C).

The effect of treatment on the mean size of *X. securis* varied depending on the size class considered (Table 2; Figure 5A). Early recruits followed a

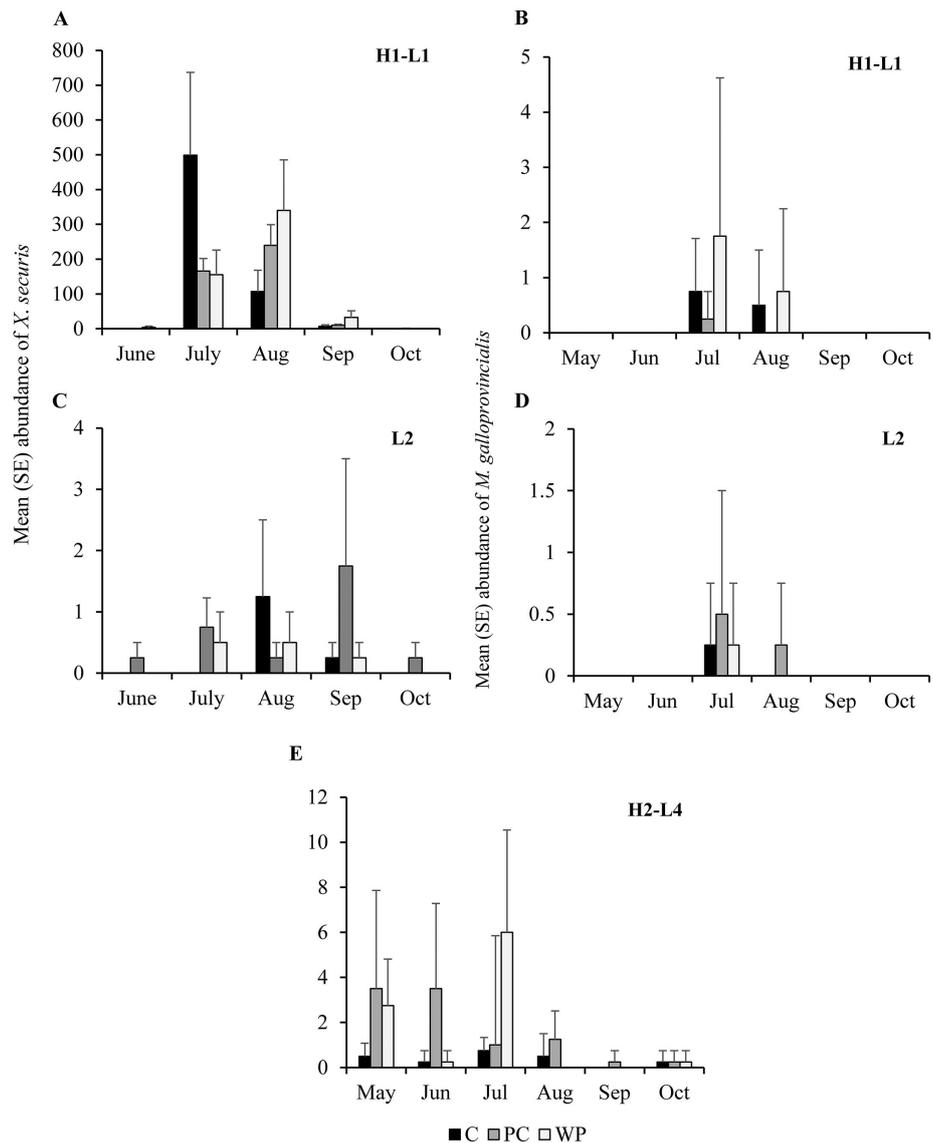


Figure 4. Mean (+SE) abundance of *X. securis* and *M. galloprovincialis* recruits (individuals/plate; n = 4). A) *X. securis* recruits at H1-L1. B) *M. galloprovincialis* recruits at H1-L1. C) *X. securis* recruits at L2. D) *M. galloprovincialis* recruits at L2. E) *M. galloprovincialis* recruits at H2-L4. Treatments: Control (C): PVC plate covered with a scourer with no manipulation; exclusion (WP): a PVC plate covered with a scourer protected from predation with a plastic exclusion cage; procedural control (PC) PVC plate covered with a scourer with a plastic exclusion cage with three holes to allow free access to predators. Recruitment at locations L3 for both species and at H2-L4 for *X. securis* is not shown because it was almost anecdotal. Only the data collected between July and September 2014, for *X. securis*, and between May and August 2014, for *M. galloprovincialis*, were analysed.

similar pattern to that of *Mytilus* (Table 2; Figure 5B), with significantly smaller sizes for the exclusion treatment than for the control treatment, but similar to that of the procedural control (pairwise tests, $P < 0.001$). Late recruits showed the opposite pattern, with larger mean sizes for the procedural control and exclusion treatments (pairwise tests, $P < 0.001$).

CCF analysis revealed that the increase in minimum temperature tended to favour *X. securis* recruitment at H1-L1 and L2 (lag 0, $r = 0.841$ at H1-L1, $P > 0.05$ and $r = 0.988$ at L2, $P < 0.05$). Although not significant, the increase in minimum salinity seemed to enhance the recruitment at H1-L1 (lag 0;

Table 1. Summary of results of LM testing the effect of Location, Treatment and Time on the abundance of *X. securis* and *M. galloprovincialis* recruits, separately (individuals/plate; n = 4). The model for *X. securis* the factor Location included 2 levels (H1–L1 and L2), Treatment (3 levels: control [C], exclusion [WP] and procedural control [PC]) and Time (3 levels: July, August and September). For *M. galloprovincialis*, the factor Location included 3 levels (H1–L1, L2 and H2–L4) and the factor Time included 4 levels (May, June, July and August).

	df	F	P
Abundance of <i>X. securis</i>			
Location	1	200.74	< 0.001
Treatment	2	0.32	0.724
Time	2	13.92	< 0.001
Location x Treatment	2	0.124	0.868
Location x Time	2	46	< 0.001
Treatment x Time	4	0.51	0.771
Location x Treatment x Time	17	0.98	0.425
Residuals	54		
Abundance of <i>M. galloprovincialis</i>			
Location	2	10.96	< 0.001
Treatment	2	3.42	0.036
Time	3	1.73	0.164
Location x Treatment	4	2.95	0.023
Location x Time	6	1.06	0.389
Treatment x Time	6	2.88	0.012
Location x Treatment x Time	12	2.21	0.015
Residuals	108		

Table 2. Summary of results of LM testing the effect of Treatment (3 levels: control [C], exclusion [WP] and procedural control [PC]; plates n = 4) and Size class (2 levels: recruits smaller than 1 mm and larger than 1 mm) on the size of *X. securis* and *M. galloprovincialis* recruits (separately) from H1–L1 and H2–L4, respectively.

	df	F	P
Size of <i>X. securis</i>			
Treatment	2	4.25	0.014
Class size	1	3803.61	<0.001
Treatment x Class size	2	11.22	<0.001
Residuals	2076		
Size of <i>M. galloprovincialis</i>			
Treatment	2	6.91	<0.001
Residuals	70		

$r = 0.792$, $P > 0.05$) while at L2 the correlation was not as clear. Likewise, an increase in maximum salinity seemed to favour *M. galloprovincialis* recruitment at H2–L4 (lag 0, $r = 0.948$, $P < 0.05$).

Substrate selection and the effect of substrate on post-settlement mortality: role of physical structure and biological cues on recruitment

Recruitment of *X. securis* was only significantly affected by location, but not by the type of treatment, as opposed to *M. galloprovincialis* (Table 3; Figure 6A, B). As expected, more *X. securis* recruits were found at H1–L1 than at L3, where the rate of recruitment was very low. The number of recruits was also very variable within treatments, as indicated by high values of standard deviation (e.g. H1–L1, *X. securis*: maximum SD: 18.08), revealing the patchy nature of recruitment (Figure 6A). *Mytilus galloprovincialis* recruits were mainly observed in the presence of conspecific individuals

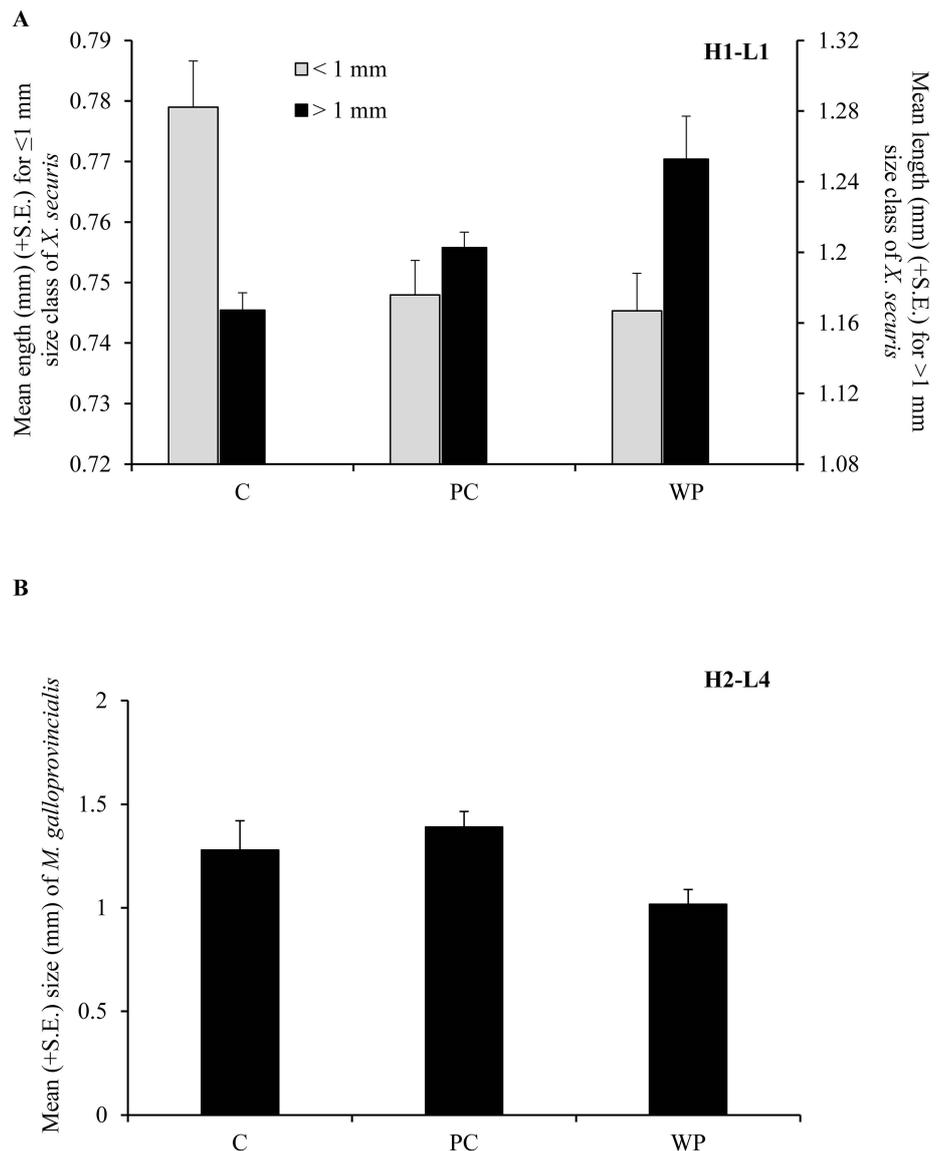


Figure 5. Mean (+SE) size of recruits (individuals/plate; $n = 4$) for (A) *X. securis* at H1–L1 between July and September 2014 and (B) *M. galloprovincialis* at H2–L4 between May and August 2014. Recruits of *X. securis* were grouped into two size classes: larger than 1 mm and smaller than 1 mm. Experimental treatments: Control (C): PVC plate covered with a scourer with no manipulation; exclusion (WP): a PVC plate covered with a scourer protected from predation with a plastic exclusion cage; procedural control (PC) PVC plate covered with a scourer with a plastic exclusion cage with three holes that allowed free access to predators. Plates were collected monthly and recruits per experimental treatment were pooled.

(pairwise tests, $P < 0.01$; Figure 6B), which appeared to avoid the presence of live *X. securis* at both locations.

Discussion

The results of the settlement and recruitment time-series revealed inverse distribution patterns for *X. securis* and *M. galloprovincialis* in the Ría de Vigo, which were closely linked to the distributions of adult populations (Gestoso et al. 2012; Montes et al. 2018). Although *X. securis* populations were almost entirely restricted to the San Simón basin in the inner part of the Ría de Vigo, and populations of *M. galloprovincialis* were mainly found

Table 3. Summary of results of LM testing the effects of Location (2 levels: H1–L1 and L3) and Treatment on the abundance of the two species (*X. securis* and *M. galloprovincialis*), separately (individuals/plate; n = 5). Location included two levels (H1–L1 and L3), Treatment included 7 levels: plates with live *X. securis*; plates with dead *X. securis*; plates with Velcro® previously embedded in a liquid composed of water and crushed *X. securis* residues; plates with live *M. galloprovincialis*; plates with dead *M. galloprovincialis*; plates with Velcro® previously embedded in a liquid composed of water and crushed *M. galloprovincialis* residues; and plates with Velcro® only.

	df	F	P
Abundance of <i>X. securis</i>			
Location	1	83.732	< 0.001
Treatment	6	1.085	0.382
Location x Treatment	6	57.963	0.555
Residuals	56		
Abundance of <i>M. galloprovincialis</i>			
Location	1	0.936	0.337
Treatment	6	3.622	0.004
Location x Treatment	6	2.002	0.080
Residuals	56		

in the middle and outer parts of the ria, the recruitment pattern along the estuary did not match the expected gradient corresponding to a linear longitudinal dispersion model (Bilton et al. 2002). Recruitment of *X. securis* was mainly concentrated in the innermost location (L1), with much lower recruitment at any other location landward of the Rande Strait and only anecdotal records seaward of that point. A similar, but inverse pattern of recruitment was observed for *M. galloprovincialis*. Such drastic changes in recruitment patterns may be only possible if caused by some type of hydrographic discontinuity, which could create a refuge for dispersion and limits larval exchange (Roegner 2000; Banas and Hickey 2005). Dispersion along the main axis of an estuary is largely impacted by lateral processes generated by shoreline irregularities, such as bends in the main channel, inlets, marshes and straits (MacVean and Stacey 2011; Giddings et al. 2012). These barriers to dispersal are particularly evident during periods of low river discharge (Giddings et al. 2012) and are thus usually associated with convergent fronts (i.e. tidal traps, straits, flow curvatures). They may also be seasonal and cause alterations in longitudinal dispersion (Okubo 1973; MacVean and Stacey 2011; Giddings et al. 2012). In the Ría de Vigo, the Rande Strait is a topographical feature that modifies along-channel flow and can also act as a barrier to dispersal (Gómez-Gesteira et al. 1999). The Rande Strait also encloses the very shallow San Simón basin, where *X. securis* settlement is concentrated and where Gómez-Gesteira et al. (1999) estimated a high probability of particle retention (~ 80%) during the summer, even 28 days after release. The estimated retention period within the San Simón basin may enable the complete development of *X. securis* larvae (i.e. one month: Wilson 1968), preventing flushing to unsuitable habitats. The formation of a seasonal dispersal barrier during summer in the Rande Strait may explain why, despite an almost continuous breeding season with planktotrophic larvae potentially present in the water column during almost

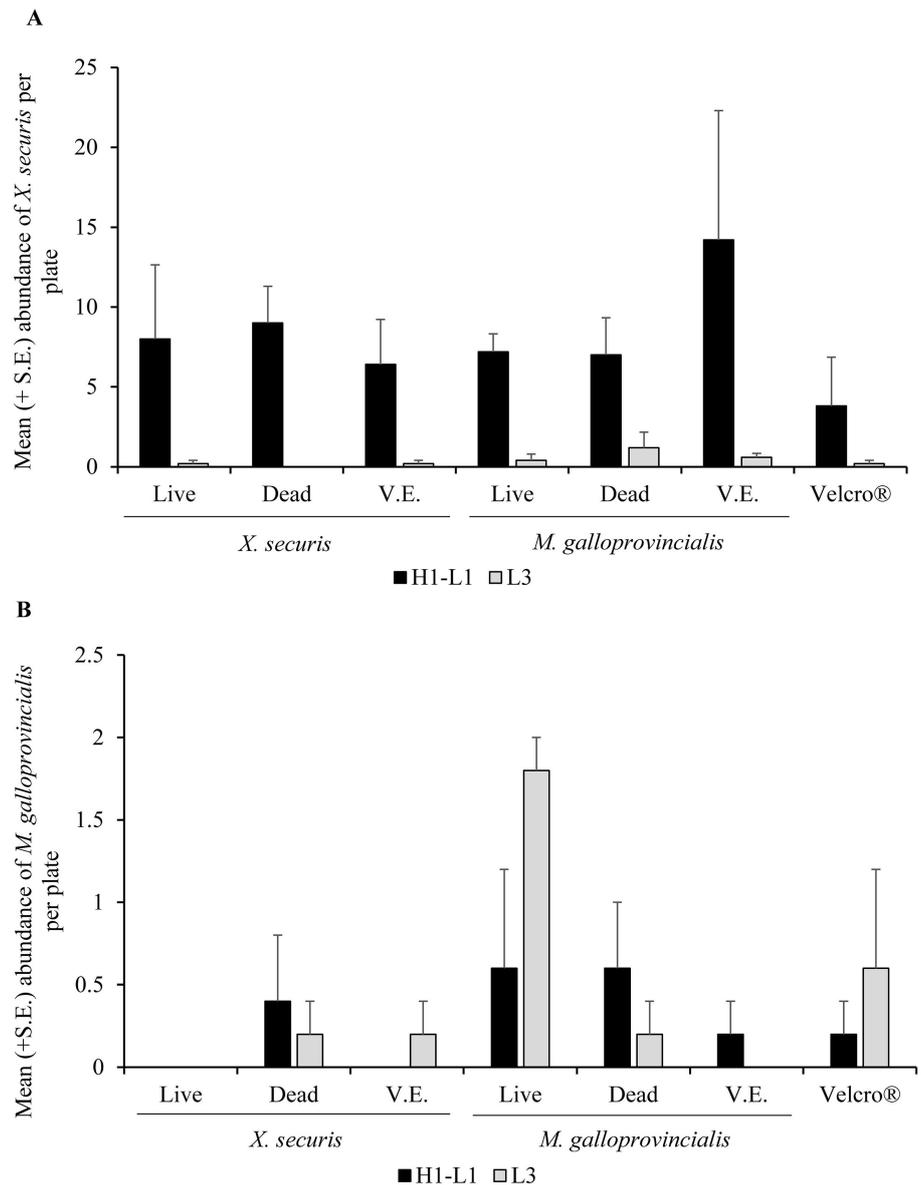


Figure 6. Mean (+SE) abundance of recruits (individuals/plate; $n = 5$) between June and August 2015. (A) *X. securis* and (B) *M. galloprovincialis* on different treatments at H1–L1 and L3. Treatments: plates with live *X. securis*; plates with dead *X. securis*; (V.E.) plates with Velcro® previously embedded in a liquid composed of water and crushed *X. securis* residues; plates with live *M. galloprovincialis*; plates with dead *M. galloprovincialis*; (V.E.): plates with Velcro® previously embedded in a liquid composed of water and crushed *M. galloprovincialis* residues; plates with Velcro®.

10 months (Montes et al. 2020), the *X. securis* recruitment period occurred between July and September. By contrast, in its native range the recruitment period lasted longer, i.e. 7 months, almost matching the breeding season, which lasts 7–8 months (Wilson 1969). Gametogenesis of *X. securis* may be adapted to retention patterns in their native region, but successful settlement in the invaded regions may be limited to the period during which retention on suitable habitats is possible. These results are reinforced by the positive correlation between *X. securis* recruitment and temperature, which is also related to increased retention of water within the ria (Barton et al. 2015).

Wind forcing may also play a role in *X. securis* recruitment, as suggested by the negative correlation with UI observed at the innermost location H1–L1 (Figure 3C). Although not significant, this negative relationship might reflect the increment of settlement during downwelling events. Downwelling events act counter to the estuarine circulation by trapping water masses in the ria and increasing the residence time to 9.5 days, while upwelling events enhance estuarine circulation accelerating the flushing of the Ría de Vigo and decreasing the residence time to 4.5 days (Torres López et al. 2001; Barton et al. 2015). Relaxation of downwelling or upwelling events could therefore potentially contribute to greater larval retention and recruitment of *X. securis*, while upwelling events could allow larval flushing beyond the distribution of adults (Montes et al. 2018), which would explain the sporadic settlement episodes at the outermost location of Ría de Vigo (H3; Figure 2D) and even invasion in adjacent estuaries (i.e. Ría de Pontevedra; Gestoso et al. 2012).

Recruitment of *M. galloprovincialis* was enhanced by upwelling, as shown by the positive correlation observed at the outermost location between settlement and UI (Figure 3F). This positive relationship, although not significant, might reflect the delivery of larvae during upwelling episodes, which allows oceanic water charged with larvae from the outer coast to enter the ria. This relationship may also explain the positive correlation between *M. galloprovincialis* recruitment and salinity, as the intrusion of upwelling water is characterized by higher salinity and lower temperature (Barton et al. 2015), with a time lag of 2 to 4 days from wind forcing in the middle part of the ria (Montero et al. 1999; Barton et al. 2015). This delay may also explain the delayed settlement of *M. galloprovincialis* in the middle of the San Simón basin (H2–L4) relative to settlement in the outermost location sampled (H3).

Low-frequency time-series also enabled identification of spatio-temporal variability in both *M. galloprovincialis* and *X. securis* recruits, even between very close locations. For both species, recruitment was higher and first occurred at the location with the largest adult population (Gestoso et al. 2012; Montes et al. 2018), which was the outermost location for *M. galloprovincialis* (H2–L4) and the innermost location for *X. securis* (H1–L1), indicating coupled abundance between recruits and adults. Nonetheless, there was also a high level of variation in recruitment at the lowest spatial scale examined, with recruitment varying greatly between experimental plates separated by 1 m intervals. The variability on rocky shores at small, within-habitat scales is consistently observed in recruitment studies of benthic invertebrates (Underwood and Chapman 1996; O’Leary and Potts 2011; Smale 2013). Patchiness reflects the large number of factors influencing recruitment, including larval production, survival and transport to suitable habitats and also selection of optimum substrates to enhance post-settlement survival. The high heterogeneity and complexity of the substrate

may account for patchiness in recruitment of mussels and other invertebrates (Palomo et al. 2007). Substrate selection by larvae in response to biological cues of conspecifics or heterospecifics, or to cues associated with biofilms can be an important driver of variation (Chiba and Noda 2000; Tamburri et al. 2008; Gribben et al. 2009; Whalan et al. 2012; Morello and Yund 2016). *Mytilus galloprovincialis* recruits were indeed more abundant on plates with live conspecifics, as observed for other mussel species (Chiba and Noda 2000; Morello and Yund 2016). By contrast, the treatment did not significantly affect recruitment of *X. securis*, indicating that synthetic surfaces (plates covered with Velcro®), with less structurally complex habitats, were not less attractive as settlement surfaces than the shells of live or dead mussels. Diverse invasive invertebrates show a high capacity to settle and grow on a wide range of natural and artificial substrates, which appears to be a common life-history trait of invasive species (e.g. Dafforn et al. 2009; Pinochet et al. 2020). Further experiments in the laboratory are necessary to elucidate the role of physical and biological cues on recruitment of both species.

Biotic interactions such as predation, competition and facilitation are important drivers of post-settlement mortality, especially in mussel beds (McQuaid and Lindsay 2005; Rius and McQuaid 2009). The predation exclusion experiment did not detect any significant effect of predation on the recruitment density of *X. securis* (Table 1; Figure 4). Conversely, Gestoso et al. (2014) detected a significant effect of predation on *X. securis* in the Ría de Vigo, although only in one location where predators were particularly abundant, mainly the crab *Carcinus maenas* (Linnaeus, 1758) and the gastropod *Nucella lapillus* (Linnaeus, 1758). As predation abundance also depends on many factors, we suggest that predator pressure on *X. securis* is limited to some outbreaks. On the other hand, *M. galloprovincialis* showed a consistent increment in recruitment success in the exclusion treatment (Table 1; Figure 4), suggesting greater predation pressure on this species, as previously reported (Veiga et al. 2011). In addition, the predation pressure on *M. galloprovincialis* was spatio-temporally very variable, which is possibly related to the seasonal dynamics of different predators. These findings may also reflect the greater adaptation of local predators to local prey, reducing the predator pressure on the invader.

Predation affected the size of mussels. Thus, larger individuals of both *M. galloprovincialis* and *X. securis* were found in the control treatment than in the exclusion treatment, suggesting that smaller mussels were more vulnerable to predation in the control treatments. A size-dependent effect of predation on mussels is a common characteristic of predator-prey interactions (Paine 1976; Bertolini et al. 2018). The fact that smaller mussels were found in the exclusion treatment (Table 2; Figure 5), even when there were no differences in density between treatments (Table 1; Figure 4), also suggests that self-thinning processes may play a major role in post-settlement mortality for *X. securis* (see Alunno-Bruscia et al. 2000).

Post-settlement mortality associated with intra-specific competition for food or space may have been enhanced in the exclusion treatment because of the greater survival of small individuals. Our findings suggest that density-dependent mortality self-regulates population size when predation pressure is low, as in the case of *X. securis* (Tables 1, 2; Figure 5A), while predation may play a more important role in regulating the population size in *M. galloprovincialis* (Tables 1, 2; Figure 5B).

Although environmental restrictions may play a role in regulating the abundance of *X. securis*, the wide tolerance of adults and juveniles to environmental conditions such as temperature and salinity (Wilson 1969; Garci et al. 2007; Gestoso et al. 2012; Iwasaki and Yamamoto 2014), together with the lack of a large predation pressure, suggests that the main limiting factor for the spread of the invasive mussel is the mismatch between the larval dispersal strategy and the oceanographic conditions in the study area.

The reproductive characteristics of *X. securis* are typical of successful invaders: high reproductive flexibility (Wilson 1968, 1969; Montes et al. 2020), precocious sexual maturity (15.6 mm), rapid gametogenesis, accumulation of mature gametes and an extended spawning period of 10 months with no resting period (Montes et al. 2020). *Xenostrobus securis* has been recorded as an invading species that usually lives in high-density populations in different coastal areas from the Mediterranean, including Italian and French lagoons, southern Korea, China, Japan or Atlantic coasts of Spain (Barbieri et al. 2011; Gestoso et al. 2012; Iwasaki and Yamamoto 2014, and references therein). Although in certain areas its rate of expansion can be of up to 23.9 km year⁻¹ (Iwasaki et al. 2004), the distribution of adults and juveniles in the study area has remained unchanged since 2012 (Montes et al. 2018). Spread of the invasive mussel is limited to the Rande Strait in the Ría de Vigo (~ 6 km from the location initially invaded in the first 5 years after its first record; Gestoso et al. 2012), which supports the hypothesis that a seasonal dispersal barrier associated with topographic features limits larval dispersal. It is possible that the large dispersal potential of the species could lead to invasion of adjacent estuaries under sporadic favourable hydrodynamic conditions. For example, episodes of continued upwelling might allow larval flushing outside of the ria, and under favourable oceanographic conditions, to the posterior invasion of adjacent estuaries. Such a mechanism could explain the spread of *X. securis* to the adjacent Ría de Pontevedra (Gestoso et al. 2012) and emphasizes the need for a better understanding of dispersal patterns of invasive species for effective expansion control. However, we cannot ignore the fact that human activities *via* recreational and commercial shipping or aquaculture activities may interfere with natural dispersal mechanisms and, subsequently, favour secondary spread (Ruiz et al. 2006; Acosta and Forrest 2009). This is relevant in the study area where inshore fisheries (including aquaculture vessels) account for most of the recreational

boating and, therefore, should be considered as a putative important pathway for secondary spread. Our study findings also highlight the need for dynamic risk-assessment tools to enable evaluation of the invasion risk associated with temporal and spatial variability in the biological characteristics of invasive species and in local hydrodynamics.

Acknowledgements

We thank Esther Pérez and Jone Molina for assistance with field sampling and sample processing. We greatly appreciate the constructive criticism provided by three anonymous reviewers.

Funding Declaration

This study was funded by the Consellería de Educación e Ordenación Universitaria Xunta de Galicia, specifically by the European Regional Development Funds (ERDF GRC2013-004, ED431D 2017/20 and ED431C 2017/46) within the framework of the ERDF project for Galicia 2014-2020 “Unha maneira de facer Europa”. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- Acosta H, Forrest BM (2009) The spread of marine non-indigenous species via recreational boating: a conceptual model for risk assessment based on fault tree analysis. *Ecological Modelling* 220: 1586–1598, <https://doi.org/10.1016/j.ecolmodel.2009.03.026>
- Alunno-Bruscia M, Petraitis PS, Bourget E, Fréchette M (2000) Body size-density relationship for *Mytilus edulis* in an experimental food-regulated situation. *Oikos* 90: 28–42, <https://doi.org/10.1034/j.1600-0706.2000.900104.x>
- Alvarez I, Prego R, Gómez-Gesteira M (2003) Hydrographic characterization of a winter-upwelling event in the Ria of Pontevedra (NW Spain). *Estuarine Coastal Shelf Science* 56: 869–876, [https://doi.org/10.1016/S0272-7714\(02\)00309-8](https://doi.org/10.1016/S0272-7714(02)00309-8)
- Alvarez I, Gómez-Gesteira M, deCastro M, Dias JM (2008) Spatiotemporal evolution of upwelling regime along the western coast of the Iberian Peninsula. *Journal of Geophysical Research Oceans* 113: C07020, <https://doi.org/10.1029/2008JC004744>
- Babarro JMF, Abad MJ (2013) Co-existence of two mytilids in a heterogeneous environment: mortality, growth, and strength of shell and byssus attachment. *Marine Ecology Progress Series* 476: 115–128, <https://doi.org/10.3354/meps10122>
- Banas NS, Hickey BM (2005) Mapping exchange and residence time in a model of Willapa Bay, Washington, a branching, macrotidal estuary. *Journal of Geophysical Research: Oceans* 110: C11, <https://doi.org/10.1029/2005JC002950>
- Barbieri M, Maltagliati F, Di Giuseppe G, Cossu P, Lardicci C, Castelli A (2011) New records of the pygmy mussel *Xenostrobus securis* (Bivalvia: Mytilidae) in brackish-water biotopes of the western Mediterranean provide evidence of its invasive potential. *Marine Biodiversity Records* 4: 1–4, <https://doi.org/10.1017/S175526721100042X>
- Barton ED, Largier JL, Torres R, Sheridan M, Trasviña A, Souza A, Pazos Y, Valle-Levinson A (2015) Coastal upwelling and downwelling forcing of circulation in a semi-enclosed bay: Ria de Vigo. *Progress in Oceanography* 134: 173–189, <https://doi.org/10.1016/j.pocean.2015.01.014>
- Bertolini C, Montgomery WI, O'Connor NE (2018) Habitat with small inter-structural spaces promotes mussel survival and reed generation. *Marine Biology* 165: 163, <https://doi.org/10.1007/s00227-018-3426-8>
- Bilton DT, Paula J, Bishop JDD (2002) Dispersal, genetic differentiation and speciation in estuarine organisms. *Estuarine, Coastal and Shelf Science* 55: 937–952, <https://doi.org/10.1006/ecss.2002.1037>
- Cáceres-Martínez J, Robledo JAF, Figueras A (1993) Settlement of mussels *Mytilus galloprovincialis* on an exposed rocky shore in Ria de Vigo, NW Spain. *Marine Ecology Progress Series* 93: 195–198, <https://doi.org/10.3354/meps093195>
- Cáceres-Martínez J, Robledo JAF, Figueras A (1994) Settlement and post-larvae behaviour of *Mytilus galloprovincialis*: field and laboratory experiments. *Marine Ecology Progress Series* 112: 107–117, <https://doi.org/10.3354/meps112107>
- Carl C, Poole AJ, Williams MR, de Nys R (2012) Where to Settle-Settlement Preferences of *Mytilus galloprovincialis* and Choice of Habitat at a Micro Spatial Scale. *PLoS ONE* 7: e52358, <https://doi.org/10.1371/journal.pone.0052358>
- Cheng BS, Hovel KA (2010) Biotic resistance to invasion along an estuarine gradient. *Oecologia* 164: 1049–1059, <https://doi.org/10.1007/s00442-010-1700-7>

- Chiba S, Noda T (2000) Factors maintaining topography-related mosaic of barnacle and mussel on a rocky shore. *Journal of the Marine Biological Association of the United Kingdom* 80: 617–622, <https://doi.org/10.1017/S0025315400002435>
- Colgan DJ, da Costa P (2013) Invasive and non-invasive lineages in *Xenostrobus* (Bivalvia: Mytilidae). *Molluscan Research* 33: 272–280, <https://doi.org/10.1080/13235818.2013.826574>
- Connell JH (1985) The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. *Journal of Experimental Marine Biology and Ecology* 93: 11–45, [https://doi.org/10.1016/0022-0981\(85\)90146-7](https://doi.org/10.1016/0022-0981(85)90146-7)
- Dafforn KA, Glasby TM, Johnston EL (2009) Links between estuarine condition and spatial distributions of marine invaders. *Diversity and Distributions* 15: 807–821, <https://doi.org/10.1111/j.1472-4642.2009.00587.x>
- Denny MW, Wethey DS (2001) Physical processes that generate patterns in marine communities. In: Bertness MD, Gaines SD, Hay ME (eds), *Marine community ecology*. Sinauer Associates, Sunderland, pp 3–37
- García ME, Trigo JE, Pascual S, González AF, Rocha F, Guerra A (2007) *Xenostrobus securis* (Lamarck, 1819) (Mollusca: Bivalvia): first report of an introduced species in Galician Waters. *Aquaculture International* 15: 19–24, <https://doi.org/10.1007/s10499-006-9062-1>
- Gestoso I, Olabarria C, Arenas F (2012) The invasive mussel *Xenostrobus securis* along the Galician Rías Baixas (NW of Spain): status of invasion. *Cahiers de Biologie Marine* 53: 391–396
- Gestoso I, Arenas F, Olabarria C (2014) Biotic resistance and facilitation of a nonindigenous mussel vary with environmental context. *Marine Ecology Progress Series* 506: 163–173, <https://doi.org/10.3354/meps10828>
- Giddings SN, Fong DA, Monismith SG, Chickadel CC, Edwards KA, Plant WJ, Wang B, Fringer OB, Horner-Devine AR, Jessup AT (2012) Frontogenesis and Frontal Progression of a Trapping-Generated Estuarine Convergence Front and Its Influence on Mixing and Stratification. *Estuaries and Coasts* 35: 665–681, <https://doi.org/10.1007/s12237-011-9453-z>
- Gómez-Gesteira M, Montero P, Leitao P, Ruiz-Villarreal M, Neves R, Pérez-Villar V (1999) A two-dimensional particle tracking model for pollution dispersion in A Coruña and Vigo Rias (NW Spain). *Oceanologica Acta* 22: 167–177, [https://doi.org/10.1016/S0399-1784\(99\)80043-7](https://doi.org/10.1016/S0399-1784(99)80043-7)
- Gosselin L, Qian P (1997) Juvenile mortality in benthic marine invertebrates. *Marine Ecology Progress Series* 146: 265–282, <https://doi.org/10.3354/meps146265>
- Gribben PE, Wright JT, O'Connor WA, Steinberg P (2009) Larval settlement preference of a native bivalve: The influence of an invasive alga versus native substrata. *Aquatic Biology* 7: 217–227, <https://doi.org/10.3354/ab00196>
- Grosholz ED (1996) Contrasting rates of spread for introduced species in terrestrial and marine systems. *Ecology* 77: 1680–1686, <https://doi.org/10.2307/2265773>
- Hunt HL, Scheiblin RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Marine Ecology Progress Series* 155: 269–301, <https://doi.org/10.3354/meps155269>
- Iwasaki K, Yamamoto H (2014) Recruitment and population structure of the non-indigenous brackish-water mytilid *Xenostrobus securis* (Lamarck, 1819) in the Kino River, Japan. *Aquatic Invasions* 4: 479–487, <https://doi.org/10.3391/ai.2014.9.4.06>
- Iwasaki K, Kinoshita K, Okoshi K, Kimura T, Kosuge T, Suzuki T, Nishi E, Nishikawa T, Hayashi I, Henmi Y, Furota T, Mukai H, Yamaguchi T, Yamanishi R (2004) Range expansion of non-indigenous marine benthos introduced into Japan through human activities. *Bulletin of Plankton Society Japan* 51: 132–144
- Karlson R, Hurd L (1999) Disturbance, coral reef communities and changing ecological paradigms. *Coral Reefs* 12: 117–125, <https://doi.org/10.1007/BF00334469>
- Lavín A, Díaz del Río G, Cabanas JM, Casas G (1991) Afloramiento en el noroeste de la Península Ibérica. Índices de afloramiento para el punto 43 °N 11 °W: período 1966-1989. *Informes Técnicos Instituto Español de Oceanografía* 91: 1–40
- Leonard GH, Ewanchuk PJ, Bertness MD (1999) How recruitment, intraspecific interactions, and predation control species borders in a tidal estuary. *Oecologia* 118: 492–502, <https://doi.org/10.1007/s004420050752>
- MacVean LJ, Stacey MT (2011) Estuarine Dispersion from Tidal Trapping: A New Analytical Framework. *Estuaries and Coasts* 34: 45–59, <https://doi.org/10.1007/s12237-010-9298-x>
- McKindsey CW, Bourget E (2000) Explaining mesoscale variation in intertidal mussel community structure. *Marine Ecology Progress Series* 205: 155–170, <https://doi.org/10.3354/meps205155>
- McQuaid CD, Lindsay JR (2005) Interacting effects of wave exposure, tidal height and substratum on spatial variation in densities of mussel *Perna perna* plantigrades. *Marine Ecology Progress Series* 301: 173–184, <https://doi.org/10.3354/meps301173>
- Menge BA (1991) Relative importance of recruitment and other causes of variation in rocky intertidal community structure. *Journal of Experimental Marine Biology and Ecology* 146: 69–100, [https://doi.org/10.1016/0022-0981\(91\)90255-U](https://doi.org/10.1016/0022-0981(91)90255-U)
- Montero P, Gómez-Gesteira M, Taboada JJ, Ruiz-Villarreal M, Santos AP, Neves RR, Prego R, Pérez-Villar V (1999) On residual circulation of the Ria of Vigo, using a 3-D baroclinic model. *Boletín del Instituto Español de Oceanografía* 15: 31–38

- Montes A, Lorenzo-Abalde S, González-Fernández Á, Vázquez, Olabarria C (2018) Use of monoclonal antibody-based assay for the early detection of an invasive bivalve in plankton samples. *Marine Pollution Bulletin* 133: 320–327, <https://doi.org/10.1016/j.marpolbul.2018.05.004>
- Montes A, Olabarria C, Vázquez E (2020) Reproductive plasticity in the invasive - *Xenostrobus securis* (Bivalvia, Mytiloidea) in northwestern Spain. *Journal of Sea Research* 159: 101893, <https://doi.org/10.1016/j.seares.2020.101893>
- Morello S, Yund P (2016) Response of competent blue mussel (*Mytilus edulis*) larvae to positive and negative settlement cues. *Journal of Experiment Marine Biology and Ecology* 480: 8–16, <https://doi.org/10.1016/j.jembe.2016.03.019>
- Nielsen KJ, Franz DR (1995) The influence of adult conspecifics and shore level on recruitment of the ribbed mussel *Geukensia demissa* (Dillwyn). *Journal of Experiment Marine Biology and Ecology* 188: 89–98, [https://doi.org/10.1016/0022-0981\(94\)00190-0](https://doi.org/10.1016/0022-0981(94)00190-0)
- Nombela M, Vilas F, Evans G (1995) Sedimentation in the mesotidal rias of Galicia (northwestern Spain): Ensenada de San Simón, inner part of the Ria de Vigo. *Special Publication International Association of Sedimentology* 24: 133–149, <https://doi.org/10.1002/9781444304138.ch9>
- O’Leary JK, Potts DC (2011) Using hierarchical sampling to understand scales of spatial variation in early coral recruitment. *Coral Reefs* 30: 1013–1023, <https://doi.org/10.1007/s00338-011-0789-4>
- Okubo A (1973) Effect of shoreline irregularities on streamwise dispersion in estuaries and other embayments. *Netherlands Journal of Sea Research* 6: 213–224, [https://doi.org/10.1016/0077-7579\(73\)90014-8](https://doi.org/10.1016/0077-7579(73)90014-8)
- Paine RT (1976) Size-Limited Predation: An Observational and Experimental Approach with the *Mytilus-Pisaster* Interaction. *Ecology* 57: 858–873, <https://doi.org/10.2307/1941053>
- Palomo M, People J, Chapman MG, Underwood AJ (2007) Separating the effects of physical and biological aspects of mussel beds on their associated assemblages. *Marine Ecology Progress Series* 344: 131–142, <https://doi.org/10.3354/meps07002>
- Perez-Arlucea M, Mendez G, Clemente F, Nombela M, Rubio B, Filgueira M (2005) Hydrology, sediment yield, erosion and sedimentation rates in the estuarine environment of the Ria de Vigo, Galicia, Spain. *Journal of Marine Systems* 54: 209–226, <https://doi.org/10.1016/j.jmarsys.2004.07.013>
- Pernet F, Tremblay R, Bourget E (2003) Settlement success, spatial pattern and behavior of mussel larvae *Mytilus* spp. in experimental ‘down-welling’ systems of varying velocity and turbulence. *Marine Ecology Progress Series* 260: 125–140, <https://doi.org/10.3354/meps260125>
- Pineda J, Porri F, Starczak V, Blythe J (2010) Causes of decoupling between larval supply and settlement and consequences for understanding recruitment and population connectivity. *Journal of Experiment Marine Biology and Ecology* 392: 9–21, <https://doi.org/10.1016/j.jembe.2010.04.008>
- Pinochet J, Urbina MA, Lagos ME (2020) Marine invertebrate larvae love plastics: Habitat selection and settlement on artificial substrates. *Environmental Pollution* 257: 113571, <https://doi.org/10.1016/j.envpol.2019.113571>
- Queiroga H, Blanton J (2005) Interactions between behaviour and physical forcing in the control of horizontal transport of decapod crustacean larvae. *Advances in Marine Biology* 47: 107–214, [https://doi.org/10.1016/S0065-2881\(04\)47002-3](https://doi.org/10.1016/S0065-2881(04)47002-3)
- Queiroga H, Cruz T, dos Santos A, Dubert J, González-Gordillo JI, Paula J, Peliz A, Santos AMP (2007) Oceanographic and behavioural processes affecting invertebrate larval dispersal and supply in the western Iberia upwelling ecosystem. *Progress in Oceanography* 74: 174–191, <https://doi.org/10.1016/j.pocean.2007.04.007>
- R Core Team (2014) R: a Language and Environment for Statistical Computing. The R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org/>
- Rius M, McQuaid CD (2009) Facilitation and competition between invasive and indigenous mussels over a gradient of physical stress. *Basic and Applied Ecology* 10: 607–613, <https://doi.org/10.1016/j.baae.2009.03.008>
- Rodríguez SR, Ojeda FP, Inestrosa NC (1993) Settlement of benthic marine invertebrates. *Marine Ecology Progress Series* 97: 193–207, <https://doi.org/10.3354/meps097193>
- Roegner GC (2000) Transport of molluscan larvae through a shallow estuary. *Journal of Plankton Research* 22: 1779–1800, <https://doi.org/10.1093/plankt/22.9.1779>
- Roughgarden J, Iwasa Y, Baxter C (1985) Demographic theory for an open marine population with space-limited recruitment. *Ecology* 66: 54–67, <https://doi.org/10.2307/1941306>
- Ruiz GM, Fegley L, Fofonoff P, Yongxu C, Lamaitre R (2006) First records of *Eriocheir sinensis* H. Milne Edwards, 1853 (Crustacea: Brachyura: Varunidae) for Chesapeake Bay and the mid-Atlantic coast of North America. *Aquatic Invasions* 1: 137–142, <https://doi.org/10.3391/ai.2006.1.3.7>
- Scrosati RA, Ellrich JA (2016) A 12-year record of intertidal barnacle recruitment in Atlantic Canada (2005–2016): relationships with sea surface temperature and phytoplankton abundance. *PeerJ: Life and Environment* 4: e2623, <https://doi.org/10.7717/peerj.2623>
- Shanks AL, Brink L (2005) Upwelling, downwelling, and cross-shelf transport of bivalve larvae: test of a hypothesis. *Marine Ecology Progress Series* 302: 1–12, <https://doi.org/10.3354/meps302001>

- Shanks AL, Shearman RK (2009) Paradigm lost? Cross-shelf distributions of intertidal invertebrate larvae are un-affected by upwelling or downwelling. *Marine Ecology Progress Series* 385: 189–204, <https://doi.org/10.3354/meps08043>
- Smale DA (2013) Multi-scale patterns of spatial variability in sessile assemblage structure do not alter predictably with development time. *Marine Ecology Progress Series* 482: 29–41, <https://doi.org/10.3354/meps10273>
- Smith HK (2010) Factors affecting the abundance and size of *Pisaster ocharceus ochraceus* in the rocky intertidal zone of southern British Columbia. *Bioscience Horizons* 3: 179–187, <https://doi.org/10.1093/biohorizons/hzq023>
- Tamburri MN, Luckenbach MW, Breitburg DL, Bonniwell SM (2008) Settlement of *Crassostrea ariakensis* larvae: effects of substrate, biofilms, sediment and adults chemical cues. *Journal of Shellfish Research* 27: 601–608, [https://doi.org/10.2983/0730-8000\(2008\)27\[601:SOCALE\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2008)27[601:SOCALE]2.0.CO;2)
- Torres López S, Varela RA, Delhez E (2001) Residual circulation and thermohaline distribution of the Ria de Vigo: A 3-D hydrodynamical model. *Scientia Marina* 65: 277–289, <https://doi.org/10.3989/scimar.2001.65s1277>
- Underwood AJ, Chapman MG (1996) Scales of spatial patterns of distribution of intertidal invertebrates. *Oecologia* 107: 212–224, <https://doi.org/10.1007/BF00327905>
- Vázquez E, Young CM (1998) Ontogenetic changes in phototaxis during larval life of the Ascidian *Polyandrocarpa zorritensis* (Van Name, 1931). *Journal of Experimental Marine Biology and Ecology* 231: 267–277, [https://doi.org/10.1016/S0022-0981\(98\)00094-X](https://doi.org/10.1016/S0022-0981(98)00094-X)
- Vázquez E, Young CM (2000) Effects of low salinity on metamorphosis in estuarine colonial ascidians. *Invertebrate Biology* 119: 433–444, <https://doi.org/10.1111/j.1744-7410.2000.tb00113.x>
- Veiga P, Rubal, M, Arenas F, Incera M, Olabarria C, Sousa-Pinto I (2011) Does *Carcinus maenas* facilitate the invasion of *Xenostrobus securis*? *Journal of Experimental Marine Biology and Ecology* 406: 14–20, <https://doi.org/10.1016/j.jembe.2011.05.035>
- Vitousek PM, D'Antonio CM, Loope LL, Westbrooks R (1996) Biological invasions as global environmental change. *American Scientist* 84: 468–478
- Whalan S, Webster N, Negri A (2012) Crustose coralline algae and a cnidarian neuropeptide trigger larval settlement in two coral reef sponges. *PLoS ONE* 7: 1–8, <https://doi.org/10.1371/journal.pone.0030386>
- White JW, Morgan SG, Fisher JL (2014) Planktonic larval mortality rates are lower than widely expected. *Ecology* 95: 3344–3353, <https://doi.org/10.1890/13-2248.1>
- Wilson BR (1968) Survival and reproduction of the mussel *Xenostrobus securis* (Lam.) (Mollusca: Bivalvia: Mytilidae) in a Western Australian estuary. *Journal of Natural History* 2: 307–328, <https://doi.org/10.1080/00222936800770341>
- Wilson BR (1969) Survival and reproduction of the mussel *Xenostrobus securis* (Lamarck) (Mollusca; Bivalvia; Mytilidae) in a Western Australian estuary. Part II: Reproduction, growth and longevity. *Journal of Natural History* 3: 93–120, <https://doi.org/10.1080/00222936800770341>
- Young CM, Chia FS (1987) Abundance and distribution of pelagic larvae as influenced by predation, behavior, and hydrographic factors. In: Giese AC, Pearse JS, Pearse VB (eds), *Reproduction of marine invertebrates*. Blackwell Scientific Publications. Palo Alto, California, pp 385–464

Supplementary material

The following supplementary material is available for this article:

Figure S1. Weekly temperature and salinity values at the studied locations.

Figure S2. Detailed description of field experiments.

This material is available as part of online article from:

http://www.reabic.net/aquaticinvasions/2021/Supplements/AI_2021_Montes_et_al_SupplementaryMaterial.pdf