

Ecophysiological responses of invasive and indigenous mytilids in the Ría de Vigo (NW Spain)

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Abstract – The impact of an alien species is correlated with its abundance and potential to colonise new environments. Consequently, the crucial aspects that give a mytilid species invasive potential include its strength and capacity for adhesion to a wide variety of substrates and its ecological tolerance to environmental conditions. The alien black pygmy mussel *Xenostrobus securis* settles together with the indigenous and raft cultured mussel *Mytilus galloprovincialis* on hard substrates of the inner coastline of the Ría de Vigo (Atlantic Coast NW Spain). Key parameters for the ecological success of these species could include their byssus structure and ecological plasticity. In this study, we assessed their comparative ability to attach to different substrates under different environmental conditions, using both in situ and laboratory approaches. Byssus threads secreted by *X. securis* were found to be extremely thin and their thickness did not vary with mussel size. This thinness of byssus filaments in *X. securis* was compensated by the secretion of a huge number (up to thousand of byssus filaments). Although no increase in attachment strength was found with increasing mussel size in this species, multiple byssus thread secretion might be a key factor in the successful invasion of inner areas of the estuary. Furthermore, *X. securis* exposes a much lower shell area to lift and drag forces in mussel conglomerates. By contrast, the indigenous species *M. galloprovincialis* secretes thicker byssus threads, which provide stronger in situ attachment displaying a linear increase in strength relative to the size of mussels. The exposure of both mytilid species to different abiotic factors in the laboratory confirmed the weaker byssus secretion and limited variation in attachment strength in the alien species. On one hand, *X. securis* seemed to be better adapted to life on soft bottoms and not capable of much plasticity when attached to different substrates. However, *X. securis* might be also well adapted to variable environmental conditions, e.g., salinity fluctuations, with no need to modify its byssus properties to ensure secure attachment. Attachment strength of *M. galloprovincialis* was higher than *X. securis* in all abiotic conditions tested. Results are discussed in relation to the ecological tolerance and impact of these mytilid species.

Key words: Ecophysiology / Invasion / Attachment strength / Byssus secretion / Bivalves / *Xenostrobus securis* / *Mytilus galloprovincialis*

1 Introduction

A potentially invasive species can enhance its invasive capacity through a number of advantageous physiological characteristics (Sarà et al. 2008). For sessile species like mytilids, one such characteristic may be the potential for adhesion to different substrates. Recently, the presence of the alien mytilid *X. securis* (black pygmy mussel) was reported in the inner part of Ría de Vigo (Galicia, NW Spain) by Garci et al. (2007) (Fig. 1). This species is endemic to the brackish waters of New Zealand and Australia and was introduced to Europe (Spain and Italy; Garci et al. 2007; Lazzari and Rinaldi 1994; Sabelli and Speranza 1994) and Japan (Kimura et al. 1999; Kohama et al. 2001). *X. securis* is considered to be a serious threat

as a bioinvader with a negative impact on indigenous ecosystems (Darrigran 2002). Today, the indigenous *Mytilus galloprovincialis* and the alien *X. securis* share the same habitat in Ensenada San Simón (Fig. 1) in the inner part of Ría de Vigo (NW Spain). *M. galloprovincialis* settles on hard substrates, whereas *X. securis* is able to live not only on hard rocks but also on muddy and soft bottoms in the inner Ría (Garci et al. 2007). Both these mytilids are filter feeders but in contrast to *M. galloprovincialis*, *X. securis* is usually found on muddy sediments in eutrophic bays and estuaries (Kimura 1994). Hard substrates in the inner Ría de Vigo are not fully occupied by *M. galloprovincialis*, thus offering space for other species, including *X. securis*, to settle on the granite rocks. Competition for space and food are central issues for ongoing research on the cohabitation of these two species.

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Coexistence mechanisms of native and exotic bivalves with similar niches have been reported in several studies based on comparison of ecological traits (Harger 1972; Griffiths and Hockey 1987; Safrieli and Sasson-Frostig 1988; Van Erkom Schurink and Griffiths 1990; Zardi et al. 2006, 2007, 2008; Rius and McQuaid 2006; Nicastro et al. 2008, 2010; Dutton and Hofmann 2008; Bownes and McQuaid 2010 among others). Together with its potential to settle on different substrates, like hard rocks and soft and muddy bottoms (personal field observations), *X. securis* also shows high tolerance to variations in salinity between 5 and 37 psu (Wilson 1968; Kimura et al. 1995). Physical stress plays a significant role in the dynamics of intertidal communities, has an impact on the coexistence of competing species (Connell 1961; Dayton 1971; Suchanek 1978), and may also influence the success of biological invasions (Bownes and McQuaid 2010).

According to Garcí et al. (2007), more research is needed to understand dynamics of *X. securis* in Galician waters. In addition, the edible mussel *M. galloprovincialis*, cultivated in the outer areas of Ría de Vigo, has a great economic importance in the region (Labarta et al. 2004). Mussel cultivation in the Rías Baixas (Galicia, NW Spain) represents a total production of about 250 000 t of mussels per year, which is about 40% of the total European output and 15% of world mussel production (Labarta et al. 2004). If *X. securis* were to colonise outer areas of the Rías, larvae of this species might settle on ropes where *M. galloprovincialis* is being cultivated, resulting in high economic losses for the mussel industry.

The dynamics of invasion and coexistence between species depend on environmental heterogeneity and its interaction with animal biological responses. Important abiotic factors for population dynamics in the littoral zone include temperature, salinity and water flow/turbulence, as well as the sedimentary effect of the natural substrate and transport of mud or sand (Zardi et al. 2008).

Furthermore, the attachment capacity of a mytilid species is an important factor determining their potential to live in different habitats. Mussels secrete numerous byssus filaments through the foot (Yonge 1962; Price 1983), with each thread connecting proximally to a common stem rooted within the byssus gland of the foot and ultimately to the byssus retractor muscles (Brown 1952; Price 1983; Waite et al. 2002). The distal section of the byssus threads, together with the adhesive plaque establishes the anchorage point (Brown 1952; Waite et al. 2002). Byssogenesis of different species of the *Mytilus* genus was found to vary according to many abiotic factors, such as temperature (Allen et al. 1976), salinity and water velocity (Van Winkle 1970; Babarro and Carrington 2011), agitation or vigorous shaking (Young 1985), oxygen tension (Widdows and Bayne 1971), wave action associated with wind (Price 1982), and circadian and tidal rhythms (Martella 1974). The potential to secrete byssus threads is considered the main source of variability in the force necessary to dislodge mussels in nature, together with other parameters like byssus diameter and the strength of the thread material (Bell and Gosline 1996).

A number of studies have explored the attachment strength of mussels on artificial substrates (Waite 2002; Walter and Liebezeit 2003; Lekang et al. 2003; Filgueira et al. 2007;

Burkett et al. 2009; Brenner and Buck 2010). In general, attachment strength can be seen to be influenced by the composition of the substrate material, surface area available, exposure to stress and type of spat collector. Specifically, Brenner and Buck tested a number of spat collector types for the analysis of the attachment properties of the blue mussel *Mytilus edulis* and observed that nylon (nylon-polyethylene and nylon-polypropylene) and natural fibres offered a better surface for the adhesion of mussel spat than other materials such as polyester. Another interesting aspect is that mussels prefer high-energy surfaces (Yamamoto 1995; Waite 2002; Burkett et al. 2009). High-energy substrates like aluminium and glass (Burkett et al. 2009) or polar surfaces like glass and slate (Waite 2002) may help the individuals to maximise adhesion-enhancing adhesive bonding between the mussel plaques and surfaces. By contrast, surfaces like acrylic, PVC and T-2 (Burkett et al. 2009), or teflon, paraffin and acetal (Waite 2002) represent low energy surface values that minimize an animal's ability to establish adhesion. Nevertheless, the trend correlating surface energy and adhesion is not perfect and variations could have resulted from differences in surface chemistry and moduli changes (Burkett et al. 2009).

An alien species may displace indigenous species due to superior competitive abilities and physiological tolerances (Holway 1999; Byers 2000; Steffani and Branch 2005), but interactions with the environment and native species make the invasiveness of an alien unpredictable, and competitive ability can be equalled by the resistance of a community to invasions (Paini et al. 2008).

In this study, we aimed to evaluate several ecophysiological responses (byssus attachment strength, morphometry, structure and filament number) of the mytilid *X. securis* under field and laboratory conditions, where mussels were subjected to natural and controlled variation in a number of different abiotic parameters (salinity, flow speed and substrate). The performance of this alien species was compared with the native mytilid *M. galloprovincialis* to explore the degree of adaptation of the alien when facing more saline and turbulent waters than those found in its native environment. We determined the attachment strength and associated byssus apparatus to evaluate the potential of *X. securis* to colonise the rougher and full marine environments in the outer Ría de Vigo where mussel raft culture of the indigenous species *M. galloprovincialis* is carried out.

2 Materials and methods

2.1 Field experiment

Field experiments were conducted at two coastal sites in the inner Ría de Vigo (NW Spain), selected according to their environmental conditions. Both sampling sites were located near the city of Vigo (Fig. 1): one in the inner estuary (San Simón: SS) and the other at the mouth of the river Verdugo (Sampaio: SP). Field data was collected during February 2010. In San Simón, both the indigenous mussel *M. galloprovincialis* and the alien *X. securis* are present in the intertidal zone. In Sampaio (SP) there is only *X. securis*. In situ dislodgement force was assessed for both mytilid species relative to the size range encountered in the field.

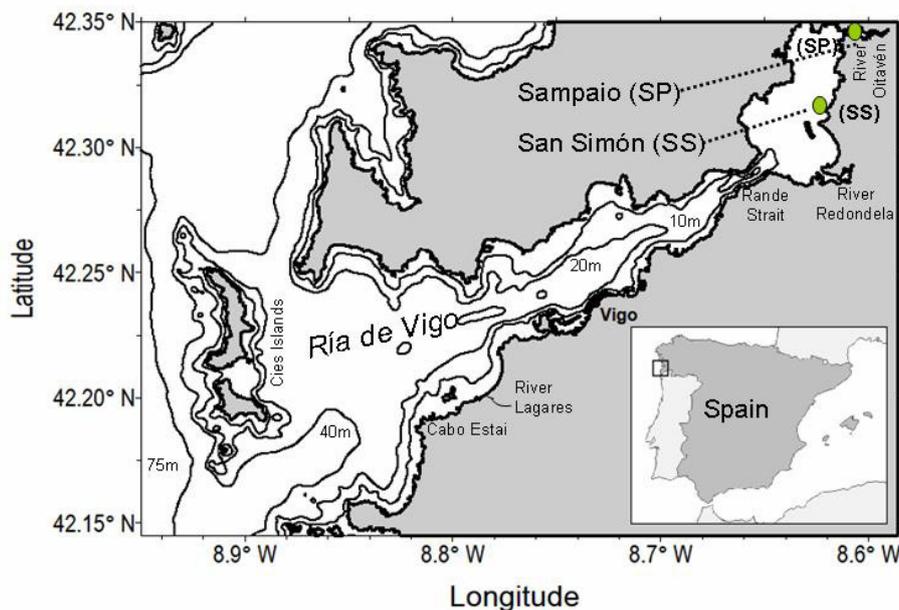


Fig. 1. Experimental sites located in Ría de Vigo (NW Spain). San Simón (SS) and Sampaio (SP) indicate the inner sheltered site and the site at the mouth of river Verdugo flowing into the Ría, respectively.

2.1.1 Abiotic parameters

Temperature and salinity at the sampling sites were measured using a multi-parameter sensor (XS PC300, Eutech Inst.). Flow speed was measured in situ using a 2D-ACM flow meter (Falmouth Scientific, Inc. Cataumet, MA 02534 USA).

2.1.2 Attachment strength

Attachment strengths of eighty mussels, covering the mussel size range encountered in the field (20–60 mm and 15–45 mm shell length for *M. galloprovincialis* and *X. securis*, respectively), were measured by connecting the mussels to a spring scale (Kern MH, resolution of 0.01 N) with a thin monofilament fishing line through a 2-mm diameter hole drilled through the shell valves close to the posterior margin. Only animals directly attached to the hard substrate were considered. The spring scale was pulled perpendicular (90° pull angle) to the substrate until dislodgement occurred (Bell and Gosline 1997; Babarro and Fernández Reiriz 2010). After dislodgment, a number of 5 to 15 individuals (depending on the size class, see below) were measured to the nearest millimetre along the antero-posterior (shell length, L), dorso-ventral (shell height, H) and lateral axes (shell width, W) using vernier callipers. The shell surface area of the sampled mussels was measured by image analysis (IA), with shell height and width as the main axes defining the area over which the lift force acts (Bell and Gosline 1996). IA measurements were performed using the software QWin (© Leica Imaging Systems) on a computer (PC AMD Athlon XP 3000+) connected to a video camera (Leica IC A) on a stereo microscope (Leica MZ6). Camera and light settings were established at the beginning of the analysis and kept constant throughout.

2.1.3 Byssus secretion in situ: thickness

The thickness (diameter) of the byssus threads secreted per size class of the mussels was measured using computer-assisted image analysis (IA). IA was performed using 15 byssus threads per mussel size class. Considering the mussel size range encountered in situ for attachment strength measurements (see Sect. 2.1.2), we sampled fresh individuals (different ones from those used for the attachment measurements) to obtain the entire byssus and tried to cover a significant range of mussel size. These individuals were grouped within the following size classes: 20, 30, 40, 50 and 60 ± 2 mm (*M. galloprovincialis*) and 22 mm, 27, 31, 34, and 38 ± 2 mm (*X. securis*). Proximal and distal portions of the filaments were subdivided into sections along the thread in order to obtain an integrated thickness measurement and were easily distinguishable in the stereo microscope. For the software used and camera conditions, please refer to Section 2.1.2 (shell surface area measurements).

2.2 Laboratory experiments

2.2.1 Maintenance of animals

Individuals of *M. galloprovincialis* and *X. securis* were collected from San Simón (SS) in the inner area of Ría de Vigo (Fig. 1) and transported to the laboratory (within 30 min approx.). Collection was made carefully by scraping the rocks to avoid damaging the byssus gland or byssus stem. Only mussels of 33 ± 2 mm shell length were collected for the laboratory experiment, as these represented the most frequent size class in the field. Mussels were acclimated for a week in a

flow-through device (Babarro and Fernández Reiriz 2010), using seawater with the following characteristics: 10 μm -filtered seawater, salinity 35 psu and temperature 15 °C, at a calm flow speed of 0.1 cm s^{-1} . Daily pulses of phytoplankton (Tahitian *Isochrysis* aff. *galbana*, T-ISO) mixed with sediment were added to feed the animals (approx. 1.0 mg L^{-1}), simulating mean values of food availability in the natural environment of Galician Rías (Babarro et al. 2000). At the end of the acclimatisation, byssus threads secreted by the individuals were removed carefully with a razor blade.

2.2.2 Factors tested

Attachment strength of the two mytilid species was investigated and compared according to the following factors: (1) species, *M. galloprovincialis* or *X. securis*, (2) flow speed, 5 or 25 cm s^{-1} , and (3) substrate, rectangular slates (28 \times 18 cm, length \times width) or nylon ropes (2 cm diameter).

A set of two slates simulating the “mussel bed set up” (one slate per species) was placed on the bottom of a flume tank (see Sect. 2.2.4) with twenty individuals on each slate. Initially, the slates with the mussels were placed in the maintenance tank (flow = 0.1 cm s^{-1}) for 24 h to allow individuals can attach to the substrate. Moreover, before the mussels were transferred to the flume tank, they were fixed to the slate plate using a nylon mesh (1 cm pore size) to prevent them from falling off if water circulation pressure was excessive. The nylon mesh was removed after 24 h and as long as no dislodgements of individuals were observed with the experimental flow speed selected in the flume tank, the experiment was started. Animals were exposed to the chosen flow speeds for one week and attachment strength of the individuals to the slates was then measured as reported above (see Sect. 2.1.2).

Simultaneously, a second substrate, “rope set up” was also run in the flume tank. Six ropes were installed along the width of the flume tank, all facing the tested flow. Each rope was suspended from a grid at the top of the flume tank, and maintained vertically using weights at the bottoms of the ropes. The simultaneous testing of both substrates (slates and nylon ropes) receiving seawater at different depths in the water column of the flume tank (40 cm, see below) did not influence the experimental flow speed. Flow speed values were measured at different depths of the water column in the vicinity of the mussels by using a 2D-ACM flow meter (Falmouth Scientific, Inc. Cataumet, MA 02534 USA). Twenty animals per rope and three ropes per species were socked, allowing the animals to form conglomerates in a multilayer disposition. Initially, we used an elastic cotton net to keep animals attached to the ropes. This net progressively degraded and was completely cut off as soon as the animals attached themselves to the substrate. Once the cotton mesh was completely removed and mussels did not become dislodged from the ropes, the experiment was started. Attachment strength of the mussels to the nylon ropes was measured after one week of exposure to each flow speed (see Sect. 2.1.2). Two sets of individuals from the maintenance tanks were used for the two experimental flow speeds tested in the flume tank.

Next, a separate experiment was done to estimate the byssus production per individual for each species. A new set

of slates with vertical posts was set up (Fig. 2) and new individuals of both species were transferred from the maintenance tanks to the flume tank. Two slates (one per species) were placed on the bottom of the flume tank with twelve mussels of each species individually glued to vertical posts using “5 min” epoxy (Imedio S.A. Madrid, Spain). Individuals were suspended 6 mm above the substrate with their posterior ends facing upstream (Fig. 2), separated by a sufficient distance (one shell length) to avoid any overlap of the byssus threads secreted. For this experiment, we followed the scheme described by Carrington et al. (2008) for an optimal counting of the byssus threads in different bivalve species. The number of byssus threads secreted per individual after one week of exposure to each flow speed was counted and used for the morphometric analysis.

2.2.3 Substrates

Slate and nylon ropes were selected as substrates to simulate natural (mostly monolayer on the granitic rocks) and culture (multilayer on culture ropes) dispositions in the laboratory. For this, slate and nylon ropes were chosen as appropriate substrates for mussel attachment processes. Slate represents a high-energy surface (Waite 2002) and a good substrate for mussel attachment after minimal exploration of the surface (Young 1985). Moreover, secreted byssus is easy to collect from this relatively smooth surface by scraping off the adhesive plaques. By contrast, traditional nylon ropes commonly used for mussel aquaculture purposes (Filgueira et al. 2007) were selected as a substrate for the animals in the water column of the flume tank. Nylon also represents a good surface for adhesive purposes of the mussels compared with other materials (Brenner and Buck 2010).

2.2.4 Flow speed

A circulating flume tank was used to expose mussels to a constant water velocity. The working section of the flume tank corresponded to a water column of 80 cm length \times 60 cm width \times 40 cm height. The seawater flowed through a system of collimators (PVC pipes of 2 cm diameter \times 100 cm length) to avoid an excess of turbulence before entering the working section containing the mussels. The flow in the chamber was generated by an axial flow pump and operated at different hertz values, which were converted to flow measurements (cm s^{-1}) using a 2D-ACM flow meter (see Sect. 2.1.1). Considering the fluid dynamic conditions and shape of the flume tank, the flow generated was turbulent even at relatively low velocities (3–5 cm s^{-1}) according to Reynolds number (>10000) (Vogel 1994; Ackerman 1999). Velocity measurements in the vicinity of the experimental mussels (ropes in the water column and slates on the bottom of the flume tank) were measured during the experiment to record the actual current flow.

The experiments in the flume tank were started when the animals were attached to the substrates and their valves were clearly open. The temperature of the seawater in the flume tank remained constant at 15 ± 0.5 °C and individuals were fed following the conditions described in Section 2.2.1. The

water of the flume tank was replaced every three days to remove faeces and other mussel excretions. The experimental flow speeds tested here were chosen to represent calmer and rougher regimes representative of hydrodynamics in the Ría de Vigo, including the influence of the cyclic semidiurnal tides on the water flow speed of the Ría and values obtained for the raft culture disposition (Míguez et al. 2001; Petersen et al. 2008).

2.2.5 Byssus secretion in the laboratory: quantitative values, thickness, length and surface texture

The number of byssus threads secreted by a single individual and thickness and length values of the filaments were recorded at the end of each flow trial. The number of byssus threads could only be estimated for *M. galloprovincialis*. *X. securis* showed a massive secretion of byssus threads, which made the counting imprecise. When viewed in detail, each single filament of *X. securis* is composed of multiple threads with diminutive adhesive plaques (Fig. 2). Threads secreted by *M. galloprovincialis* were easily counted using a binocular dissection microscope (Nikon SMZ-10 at 4-fold magnification). For complete byssus collection, filaments were carefully cut from the proximal section, close to the stem, and the plaque was scraped off at the end of the distal part attached to the slate. Only new threads secreted during the experiment and attached to the slate were considered. The remaining old threads secreted before the experiment were easily recognizable by their equal and shorter size, dark brown colour (Bell and Gosline 1996) and lack of plaque at the end of the distal part.

For thickness measurements of the byssus secreted in the laboratory, see Section 2.1.3. Approximately hundred threads from 10 mussels (10 threads per mussel) were analysed for each mytilid species. The length of the entire byssus and its proximal and distal portions were also measured for both species by Image analysis (IA).

Scanning electron microscopy (SEM) was used to examine the surface texture of the proximal and distal sections of the byssus secreted by the two species.

2.3 Statistical analysis

In situ attachment strength of the mussels in relation to individual size was fitted to linear functions. Projected shell area of mussels as a function of individual size was fitted to potential allometric equations ($Y = aX^b$). Three-way ANOVA was used to estimate the influence of species, flow speed and substrate on attachment strength variability (logarithmic transformed data) of the 33 mm (shell length) mussels selected for the laboratory experiment. One-way ANOVA was used to test the effect of flow speed on the amount of byssus threads secreted by *M. galloprovincialis* and the percentage of the thread length that each section of the byssus (proximal and distal) represented. The effect of species and flow speed on the proximal and distal thickness values of the byssus threads was tested by a two-way ANOVA. Independence of cases was assumed and normality checked with the Shapiro-Wilk

Table 1. Field: Abiotic parameters of seawater at San Simón (SS) and Sampaio (SP) sites during sampling (February 2010). Ranges of values include both low and high tides.

Site	Temperature (°C)	Flow speed (cm s ⁻¹)	Salinity
San Simón (SS)	11.3–13.2	0–2.3	32.0–32.4
Sampaio (SP)	9.8–10.8	8.0–123.1	6.2–32.0

test. Homoscedasticity between experimental groups of mussels was established using Levene's test. When data did not fit normality tests or variances were not homogenous, the non-parametric Kolmogorov-Smirnov test was used. All analyses performed with STATISTICA 6.0 except the normality test, for which R software version 2.10.1 was used.

3 Results

3.1 Field experiment

3.1.1 Abiotic parameters

Flow speed, temperature and salinity data collected during sampling of mussels at the field stations are presented in Table 1. Flow speed varied widely at Sampaio (SP) depending on the tidal regime and the impact of freshwater flow, with values ranging between 8.0 and 123.1 cm s⁻¹. Similarly, salinity fluctuated abruptly at the Sampaio site (SP) within the range of 6.2 and 32.0 psu, including low and high tide cycles. Temperature values of seawater during sampling of individuals at Sampaio (SP) varied between 9.8 and 10.8 °C.

Mean values of flow speed in the inner Ría de Vigo site (San Simón, SS) varied over the range 0 and 2.3 cm s⁻¹. Temperature and salinity values fluctuated over the range 11.3 and 13.2 °C and 32.0 and 32.4 psu, respectively, during the period of mytilid sampling, which included both low and high tides.

3.1.2 Attachment strength and animal's shape

The shapes of the two mytilid species are illustrated in Figure 3a. According to the size range observed in the field (Fig. 3b), the black pygmy mussel *X. securis* is a mytilid usually smaller than 40 mm shell length. For a given shell length (Fig. 3a), the main difference between the two species corresponded to the shell height value, which was significantly higher for indigenous *M. galloprovincialis*. This morphological difference between the two species means that the shell area exposed to lifting forces in a mussel bed is also higher for *M. galloprovincialis* over the whole of its size distribution (Fig. 3b). There was a significant increase in attachment strength of *M. galloprovincialis* with mussel size, from values of 8 N (individuals of 20 mm shell length) to 31 N (individuals of 60 mm shell length) (Fig. 4a). The relationship between attachment strength and mussel size was not significant in the alien *X. securis*, regardless of experimental site. Attachment strength values of the alien *X. securis* varied between 6 and 12 N for the whole mussel size range (Fig. 4b).

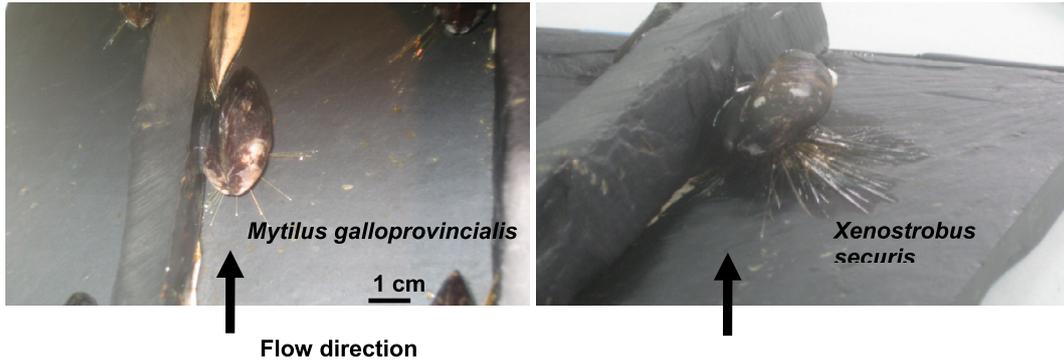
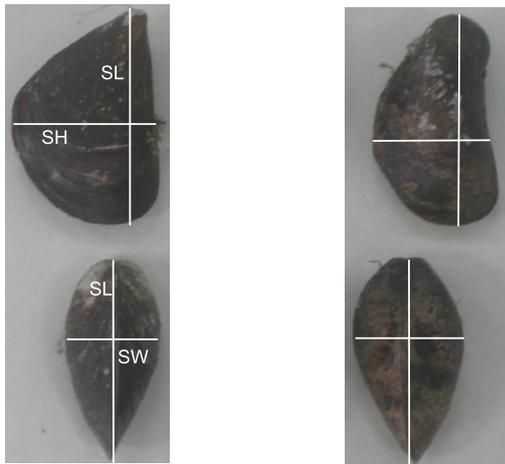
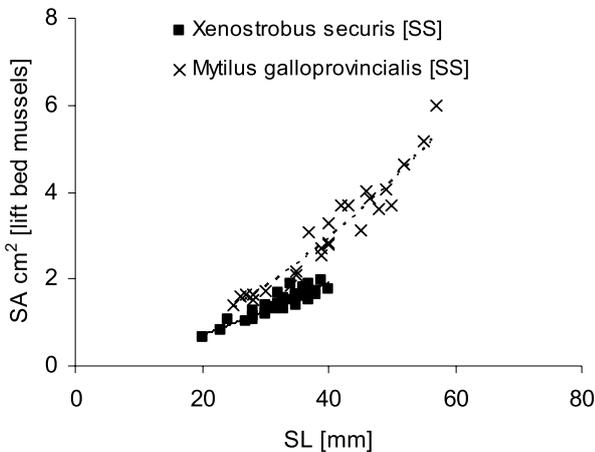


Fig. 2. Individual layout of the mussels on slates for byssus counting.

Mytilus galloprovincialis *Xenostrobus securis*



(a)



(b)

Fig. 3. Field results: (a) Shape and morphology of the mytilids *Mytilus galloprovincialis* and *Xenostrobus securis*; (b) shell area (SA) values as a function of mussel size (shell length, SL) for both mytilids. *M. galloprovincialis*: $SA = 0.0062 SL^{1.67}$, $r^2 = 0.94$, $p < 0.001$, $n = 35$; *X. securis*: $SA = 0.0098 SL^{1.43}$, $r^2 = 0.87$, $p < 0.001$ $n = 35$.

During dislodgement measurements, the way the byssus broke was observed in both mytilid species. Filaments of *M. galloprovincialis* occasionally broke at the thread level (proximal section), although both substrate and adhesive plaque were very common failure points. Byssus of *X. securis* frayed inconsistently along the thread like chewing gum.

3.1.3 Byssus thickness

Thickness of the byssus secreted by the mytilids followed a similar pattern to that of mussel attachment strength (Figs. 4c–4d). Linear increase in the attachment strength of *M. galloprovincialis* with increasing size was correlated with linear increase of thread diameter in both proximal and distal sections (Fig. 4c). Byssus thickness in threads secreted by *M. galloprovincialis* varied between 92 μm (proximal) and 52 μm (distal), for the smallest mussel size class of 20 mm shell length, and 134 μm (proximal) and 120 μm (distal) for the 60 mm (largest) mussel size class (Fig. 4c).

As with the attachment strength variability, proximal and distal thickness of the byssus threads of the black pigmy mussel *X. securis* did not follow any significant pattern with increasing size (Fig. 4b). Thickness of byssus varied over the range of 26 and 41 μm in the proximal section and 13 and 19 μm in the distal section for the mussel size range analysed (Fig. 4d).

3.2 Laboratory experiment

3.2.1 Attachment strength as a function of species, flow speed and substrate

Three-way ANOVA on the attachment strength variability of the mussels showed that all factors tested (species, flow and substrate) caused highly significant effects. The strongest effect was species ($p < 0.001$). Indeed, attachment strengths of *M. galloprovincialis* were two (slate) or three (ropes) times higher than those of *X. securis* ($p < 0.001$) (Figs. 5a–5b). Otherwise, there was a positive effect of increasing flow speed on attachment strength of the mussels ($p < 0.01$), although this effect was dependent on the substrate and occurred only when the animals were on slates (Fig. 5a). Both mytilids increased attachment strength by approximately two-fold on slates when

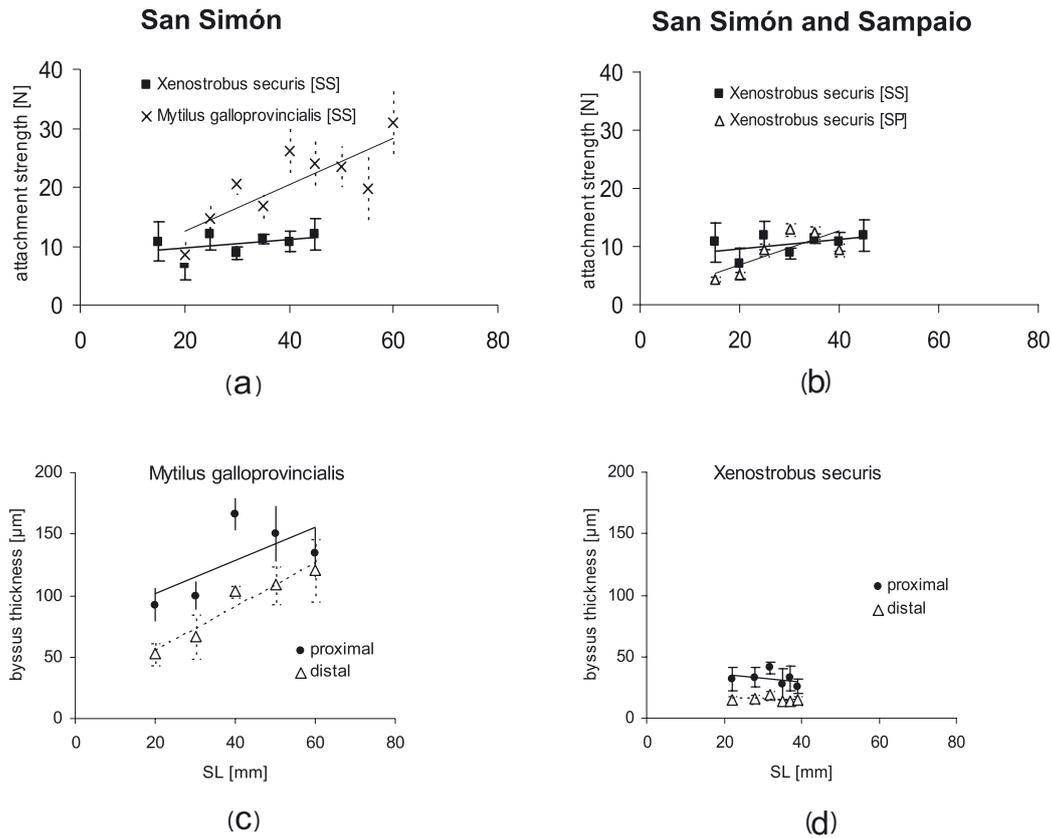


Fig. 4. Field results: (a) attachment strength (AS) values as a function of mussel size (shell length, *SL*) for *M. galloprovincialis* and *X. securis* in San Simón (SS). *M. galloprovincialis*: $AS = 0.39 SL + 4.77$, $r^2 = 0.65$, $p < 0.01$, $n = 9$; *X. securis*: not significant; (b) attachment strength values as a function of mussel size (shell length) for *X. securis* in both experimental sites: San Simón (SS) and Sampaio (SP), relationships are not significant; (c) byssus thickness (BT) values as a function of mussel size (shell length, *SL*) for *M. galloprovincialis* for both proximal and distal sections of the filaments. proximal: not significant; distal: $BT = 1.78 SL + 19.17$, $r^2 = 0.93$, $p < 0.001$, $n = 5$; (d) byssus thickness values as a function of mussel size (shell length) for *X. securis* for both proximal and distal sections of the filaments, relationships are not significant.

facing a flow of 25 cm s^{-1} compared with one of 5 cm s^{-1} (Fig. 5a). No effect of flow speed on attachment strength of either mytilid was recorded when animals were attached to nylon ropes, values ranging from 3.5 N for *X. securis* to 9.5 N for *M. galloprovincialis* regardless of flow speed (Fig. 5b). In the substrate comparison, a significant effect was found on the attachment strength of mussels ($p < 0.001$) but this was dependent on species. Interestingly, *M. galloprovincialis* attached more strongly to nylon ropes (up to 9.5 N; $p < 0.0001$) than to slates (4–7 N for both experimental flows) (Figs. 5a–5b). Attachment strength of *X. securis* individuals was not significantly different between slates and nylon ropes, with values ranging between 2.7 and 4.3 N (Figs. 5a–5b).

3.2.2 Byssus secretion: quantitative values, thickness, length and surface texture

The amount of byssus threads secreted by *M. galloprovincialis* was significantly higher (40%) when individuals were exposed to higher flow speed (Fig. 6). Such increase in byssus secretion might also be correlated with the higher attachment

strength observed for *M. galloprovincialis* exposed to higher flow speed on slates (Fig. 5a). No recordings were made of the number of filaments secreted by *X. securis* (see Materials and Methods). With regard to byssus thickness values, filaments secreted by *X. securis* were 3–5 times thinner than byssus secreted by *M. galloprovincialis* depending on the byssus section (Table 2). Thickness of the distal section of the byssus only differed according to species, with mean values of $70 \mu\text{m}$ and $15 \mu\text{m}$ for *M. galloprovincialis* and *X. securis*, respectively. Both species and flow speed had significant effects on the thickness of proximal byssus (Table 2). Indeed, proximal byssus was much thicker for filaments secreted by *M. galloprovincialis* individuals with values of $100 \mu\text{m}$, although there were no differences with flow speed within this species (Table 2). There was a 22% increase in the thickness of proximal filaments secreted by *X. securis* (from 28 to $35 \mu\text{m}$; $p < 0.001$) with the tested increase in flow speed. Interestingly, the length of this proximal section of the byssus secreted by *X. securis* was also modified when individuals were faced with higher flow speed. The proximal section of byssus represented 6.9% of the thread length in *X. securis* at 5 cm s^{-1} , and increased up to 11% of the whole thread at a flow speed

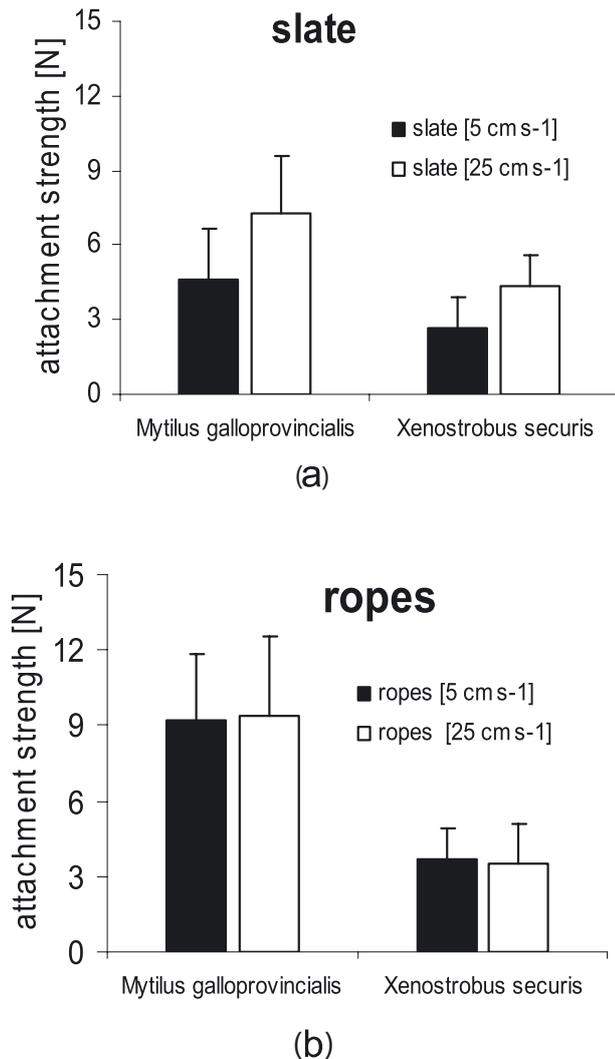


Fig. 5. Laboratory results: (a) attachment strength values of *M. galloprovincialis* and *X. securis* on slate as a function of flow speed on the slate disposition ($n = 15$ for each species); (b) attachment strength values of *M. galloprovincialis* and *X. securis* on the nylon ropes as a function of flow speed ($n = 15$ for each species).

of 25 cm s⁻¹ ($p < 0.001$). Proximal length of the byssus secreted by *M. galloprovincialis* remained unchanged (22 and 24%) when subjected to different flow speeds (Table 2).

Length of entire byssus filaments in *X. securis* did not differ between the flow rates tested in the laboratory (average 10.6 mm). Similarly to *X. securis*, no differences in thread length were found for *M. galloprovincialis* exposed to different flow speeds (mean value of 13.2 mm; Table 2). A species comparison of byssus length, however, showed that filaments were significantly longer in *M. galloprovincialis* than in *X. securis* ($p < 0.05$).

Scanning electron microscopy of byssus threads secreted by the two mytilids revealed differences in surface texture of the threads (Fig. 7). Distal sections in both mytilids were generally smooth with no differences between species. Proximal sections, however, were clearly corrugated in both species which highlighted interspecific differences in structure along

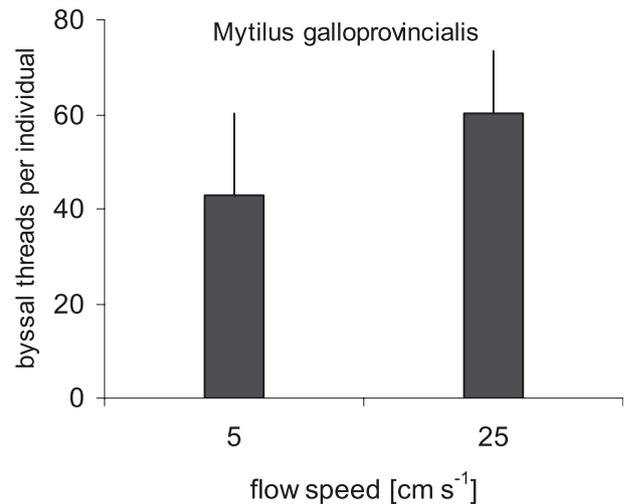


Fig. 6. Laboratory results: number of byssus threads secreted by *M. galloprovincialis* on slate exposed to different flow speed values.

the thread. More interestingly, proximal section of the byssus in threads secreted by *M. galloprovincialis* showed more crimped and corrugated fibres. The proximal section of the byssus secreted by *X. securis* showed not only transverse grooves, like *M. galloprovincialis*, but also longitudinal ones that alternated along the thread forming a completely different structure (Fig. 7).

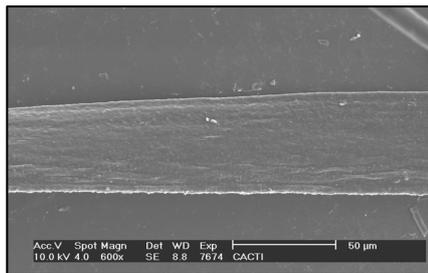
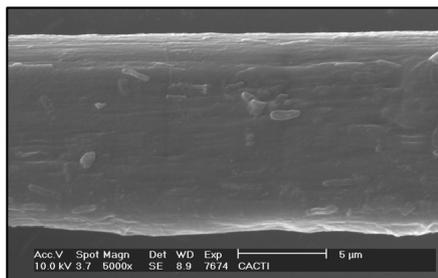
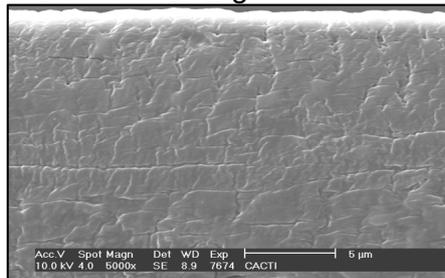
4 Discussion

The alien species *X. securis* is able to colonise a wide variety of substrates from muddy/soft bottoms to hard rocks. *X. securis* can also inhabit very different estuarine bottoms with broadly differing granulometry values in terms of proportions of gravel, sand and mud in the inner areas of Ría de Vigo (Pascual et al. 2010). Moreover, *X. securis* has also been reported as a wide tolerant species with regard to environmental salinity, between 5 and 37 psu (Kimura et al. 1995), which corresponds to the environmental range in our studied area. Our field observations showed that both species live together on natural hard substrates on the inner coastline of Ría de Vigo (San Simón SS). However, the indigenous mussel *M. galloprovincialis* is practically absent from soft bottoms in the inner Ría de Vigo San Simón (SS) and Sampaio sites (SP) where *X. securis* grows at high density (Garci et al. 2007).

Xenostrobus securis attached to natural rocks in the field with similar strength regardless of the environment, even though the abiotic parameters differed sharply. At first view, *X. securis* might appear to have a broad ecological tolerance here, establishing a rather constant attachment strength regardless of habitat and mussel size. However, our laboratory experiments demonstrated a certain ability of the alien species to modify attachment strength through its byssus properties when subjected to differences in flow speed, though at a significantly lower magnitude (5 to 25 cm s⁻¹) than reported at the field station in SP where the conditions were roughest (up to 123 cm s⁻¹). Thus, attachment strength of the alien species

Table 2. Laboratory results: byssus thread measurements for the studied mytilids (shell length: 33 ± 2 mm). Values are means \pm SD. Statistics presented in the Table were performed for flow speed comparisons in each species independently. ns: not significant.

	<i>Mytilus galloprovincialis</i>			<i>Xenostrobus securis</i>		
	5 cm s ⁻¹	25 cm s ⁻¹	<i>p</i>	5 cm s ⁻¹	25 cm s ⁻¹	<i>p</i>
Thread length (mm)	12.6 \pm 4.2	13.9 \pm 3.6	ns	10.4 \pm 2.2	10.8 \pm 2.0	ns
Proximal section (% whole thread)	24.2 \pm 6.2	22.6 \pm 6.9	ns	6.9 \pm 2.4	11.0 \pm 2.6	<i>p</i> < 0.000
Proximal thickness (μ m)	100.4 \pm 23.4	99.9 \pm 20.0	ns	28.5 \pm 6.0	34.9 \pm 5.8	<i>p</i> < 0.000
Distal thickness (μ m)	71.5 \pm 20.9	68.7 \pm 16.6	ns	14.2 \pm 1.6	15.4 \pm 2.7	ns

Mytilus galloprovincialis**Distal smooth****Proximal corrugated*****Xenostrobus securis*****Distal smooth****Proximal corrugated****Fig. 7.** Laboratory results: scanning electron micrographs of the proximal and distal surfaces of the byssus secreted by *M. galloprovincialis* and *X. securis*.

might be a more dynamic ecophysiological parameter than expected from the field data.

Attachment strength of *X. securis* to hard rocks was significantly lower than *M. galloprovincialis* for most of the size range. Despite the lower attachment strength in the field, *X. securis* survived both in the relatively calm and brackish waters of San Simón and in the most exposed site at Sampaio (mouth of the River Verdugo), where flow speeds are greater. It is well known that the forces exerted on organisms in clusters are smaller than the forces the same individuals would experience if alone (Johnson 2001; Vogel 1984), which means that forces will decrease with increasing cluster density (Carrington et al. 2008; Brenner and Buck 2010). This could be one of the reasons why individuals are able to grow and form dense conglomerates in the highly exposed mouth of the River Verdugo (Sampaio SP). On the other hand, the alien

X. securis might be able to colonise the inner areas of the Ría de Vigo at lower density values because of its great tolerance to salinity fluctuation in the estuary, combined with its byssus characteristics.

In San Simón, *X. securis* colonised hard substrates not fully occupied by the indigenous mussel *M. galloprovincialis*. Clearly, the higher attachment strength of *M. galloprovincialis* would be a dominant characteristic for this species in terms of space competition, i.e., for natural rocks. Differences in thickness of byssus secreted by the two mytilids might help to explain this. A single thread of *M. galloprovincialis* was much thicker in both proximal (3 \times) and distal (5 \times) parts of the filaments than a thread secreted by *X. securis*, and a thicker filament would be able to sustain a higher tension load. The extremely thin byssus secreted by *X. securis* was counterbalanced by its production of a huge amount of byssus threads.

This might be considered as an important species-specific feature that could help this alien mytilid to become an invader. To our knowledge, only *X. securis* (present study) and the dreissenid mussel *Mytilopsis sallei* (the black striped mussel from Indian tropical waters; Udhayakumar and Karande 1989) present such an extraordinary capacity to produce up to 1 000 filaments per byssus gland.

Apart from the amount of byssus, *X. securis* secreted the thinnest byssus threads recorded for any of the Mytilidae and even some species of Dreissenidae (see Braze and Carrington 2006; Kennedy 2011, for the exact name of the species), although such comparisons may be influenced by mussel size if this has a relationship with byssus morphometry as seen in our study. Moreover, the area of the plaques attached to the substrate was also 8 times larger in *M. galloprovincialis* (0.8 mm²) than in *X. securis* (0.1 mm²) (preliminary observations). *X. securis* has, together with *Modiolus modiolus*, the smallest plaque size of several, previously studied, bivalve molluscs (Udhayakumar and Karande 1989; Braze and Carrington 2006). Threads and plaques are moulded by the mussel foot, which may vary in size (Braze and Carrington 2006). For a given size of animal, the foot organ of the black pygmy mussel *X. securis* is much smaller than that of *M. galloprovincialis* (Fig. 8) and although this also represents a species characteristic, it might be also correlated with secretion of the much thinner byssus and smaller adhesive plaques.

As a consequence of the tidal cycle movements of seawater, and assuming that larvae of both species appear in the seawater samples at inner Ría de Vigo (Santaclara et al. 2007), both mytilids would have an equal potential to colonise different substrates on the coastline, i.e., soft bottoms and hard substrates. The fact that the alien species colonises both soft bottoms and hard rocks could indicate a wider range of tolerance that might be correlated with its byssus. Meadows and Shand (1989) observed that semi-infaunal species produce a very large number of thin threads, whereas epifaunal species produce a smaller number of thicker threads. Most likely, by the secretion of great number of thin threads, this alien bivalve would be more effective at inhabiting semi-infaunal conditions, as the threads can create an extensive network of individual attachments to small particles (Pearce and LaBarbera 2009). *Mytilus* species attach themselves to rocks and other hard substrates with a smaller number of thick threads that provide a more reliable tether against wave action or predation (Bell and Gosline 1996, 1997; Carrington 2002).

Another interesting aspect is related to the byssus surface texture observations in the two mytilids. In a comparison of mytilid species (Braze and Carrington 2006), the surface texture of the proximal byssus of *X. securis* resembled that of *M. modiolus* (Fig. 6 in Braze and Carrington 2006). *Mytilus galloprovincialis* can be grouped together with the blue mussel *M. edulis* according to the scheme presented by Braze and Carrington. Both *Mytilus* species have a corrugated and crimped proximal byssus section, related to large extensibility values typically observed in epibyssate individuals (Bell and Gosline 1996). By contrast, proximal byssus in *X. securis* presented longitudinal and transverse grooves alternating along this byssus section. Hypothetically, a less extensible proximal byssus in *X. securis* would reflect a similar ecological

transitional mode to that reported for *M. modiolus*, adapted to live commonly on muddy/soft bottoms but also on hard substrates (Stanley 1972; Holt et al. 1998; present study) through changes in the byssus properties (number of threads, morphometry and, most likely, mechanical properties).

As previously mentioned, *X. securis* may live across a wide salinity range (Kimura et al. 1995) and is more tolerant to this abiotic factor than *M. galloprovincialis* (Braby and Somero 2006). Consequently, salinity variation could also play a key role regulating the field distribution of both mytilids, though wide differences in environmental salinity did not affect attachment strength of the alien mussel. For *Mytilus* sp., salinity represents a more significant factor than temperature in regulating species distribution of *M. galloprovincialis*, *M. trossulus* and *M. edulis* (Braby and Somero 2006). In our survey, such lower tolerance to salinity variation in *M. galloprovincialis* was obvious, since this mytilid was not present near the mouth of river Verdugo (Sampaio SP) where the alien *X. securis* is the highly dominant species. The precise contribution of these two factors (species-specific byssus properties and salinity adaptation patterns) in explaining the distribution and survival of the alien species cannot be properly differentiated using our experimental design.

The laboratory experiment demonstrated that *M. galloprovincialis* had the strongest attachment under the abiotic conditions tested (different flow speeds and substrates). The attachment strength of both mytilids was significantly lower in the laboratory than in the field experiment for a given mussel size, i.e., 33 mm shell length. These differences could be due to the relatively shorter and longer periods of exposure that individuals had to the natural (field, life cycle) and artificial substrates (lab, 1–2 weeks), respectively, and the heterogeneity of the natural environment compared with the fixed abiotic environment selected for the laboratory experiment. Moreover, different types of aggregation established by the mussels in field and laboratory conditions (cluster density, byssus network, multilayer disposition, etc.), and other biotic factors like predation pressure might have contributed to these differences. Again, differences in attachment strength between species might be partially accounted for by the secretion of thicker and longer byssus threads in *M. galloprovincialis*. Nevertheless, several aspects can be remarked in the species comparison. Animals may adjust their dislodgement force by varying the number of byssus threads, their thickness and the byssus material strength (Bell and Gosline 1997; Carrington 2002). Specifically for Ría de Vigo, differences in the abiotic environment between outer and inner areas of the Ría are responsible for changes in the byssus morphology and its mechanical functioning of single threads of *M. galloprovincialis* (Babarro and Carrington 2011). In our survey, despite the lack of byssus thread number data for the alien *X. securis*, we observed that these two mytilids followed different strategies to increase attachment strength when necessary. *M. galloprovincialis* attached to slates secreted a higher number of filaments (40%) when exposed to a higher flow speed, though byssus thickness remained unchanged. By contrast, *X. securis* attached to slates secreted proximally thicker and longer byssus when exposed to higher flow speed, which might be correlated with the observed increase in attachment

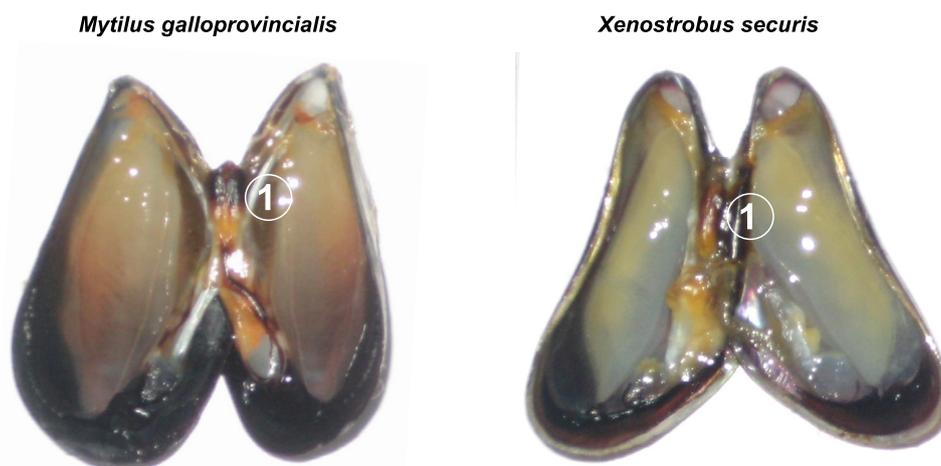


Fig. 8. Photographs of the internal anatomy of *M. galloprovincialis* and *X. securis* showing the differences in foot organ size (1 within a circle).

strength. This morphometric plasticity of the proximal byssus in *X. securis*, together with the resemblance of its surface texture to another transitional species, *M. modiolus*, and its extraordinary potential to secrete byssus filaments combine to make *X. securis* a species with an extremely wide tolerance to abiotic changes, i.e., flow speed and substrates, which in turn might be the basis for its great invasive potential.

Attachment strength of *M. galloprovincialis* on nylon ropes was twice that of the same species on slates at a flow speed of 5 cm s^{-1} , but such a difference in attachment strength between substrates was not observed for *X. securis*, leading to even greater differences between the species when they were put on ropes. Interestingly, flow speed increase did not cause any change in the attachment strength of either mytilid when they were attached to nylon ropes. As was noted above, hydrodynamic forces that individuals face in conglomerates decrease with the increasing cluster density (Carrington et al. 2008; Brenner and Buck 2010) as a consequence of the neighbourhood protection against the impact of the current (Buck and Buchholz 2005). This protective effect of mussel clumps needs to be quantified more precisely in future surveys, but it is plausible to hypothesise that *M. galloprovincialis* might have experienced higher load values when attached in a vertical position on ropes, due to its higher shell surface (and weight) exposed to the flow. *X. securis* might be more “hydrodynamic” in terms of body shape to be firmly attached on ropes, with a hypothetically lower weight factor, and this may have helped it to establish more compact and uniform clusters on the ropes in the flume tank (personal observations). This hypothesis needs to be confirmed with new research considering drag and lift reduction analysis, using individuals of both species forming conglomerates.

The alien species demonstrated great adaptability to different ecosystems by growing in heterogeneous environments with a wide fluctuation in salinity, flow speed and substrate type. The ecological tolerance reported here, together with its growth rates and density of individuals in inner Ría de Vigo (Garci et al. 2007) imply that it could threaten the indigenous mussel *M. galloprovincialis* cultivated in Galicia Rías. Cultivation of *M. galloprovincialis* takes place mainly in the outer

areas of the Ría de Vigo. *M. galloprovincialis* is an important cultivated marine resource in Galician (NW Spain) providing jobs for 11 500 people and generating 420 millions euros annually (Santaclara et al. 2007). More than 3000 rafts are used to cultivate this indigenous mussel along the coastline, with an extraordinary socio-economic impact in the area (see Introduction). Moreover, the alien *X. securis* could affect other species by altering marine and brackish water ecosystems at different levels.

In summary, in situ attachment strength of *M. galloprovincialis* was significantly higher than that of *X. securis*, and a laboratory experiment confirmed this pattern on both slate and rope substrates. Byssus thickness secreted by each of these mytilid species might represent the basis of these attachment strength differences. Secretion of an extremely thin but vast number of byssus filaments by the alien *X. securis* is extraordinary (not previously recorded in other Mytilidae) and highlights a great advantage that, coupled with its capacity to inhabit heterogeneous environments, could allow this species to become invasive. Surprisingly, *X. securis* showed a certain plasticity enabling it to modify morphological characteristics of the (proximal) byssus to potentially increase attachment strength on hard substrates under rougher conditions. Moreover, analysis of the surface texture of the byssus suggested that *X. securis* can be grouped together with the well-known transitional species *M. modiolus* (from partially buried to epibyssate). These byssus features, together with the species-specific euryhaline response of the alien mytilid, could mean it represents a threat for the ecosystem of the Ría de Vigo as a whole.

Nevertheless, the overall results presented here can be interpreted in two different but compatible ways. First, the alien species secreted weaker byssus and presented a limited ability to modify byssus characteristics and attachment strength on nylon ropes. This would make *X. securis* better adapted to inhabit soft bottoms (with a profuse network established with its abundant byssus threads) and less capable of much plasticity on other substrates. At the same time, *X. securis* might also be well adapted to a multitude of environments (i.e., wide salinity fluctuation) by establishing a secure attachment with no need

to modify byssus secretion properties. Attachment strength of *M. galloprovincialis* was higher than *X. securis* in all abiotic conditions tested. Consequently, this indigenous species would not suffer extreme competition by the alien on natural rocks unless salinity values were to vary abruptly. In the latter case, *X. securis* would be the better performer. Further research is necessary to understand adult competition patterns for food and space as well as larval dispersal and recruitment patterns in the inner and outer Ría. Such investigation would confirm the true potential of this alien species to hypothetically colonise areas where raft mussel culture is carried out.

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