

Eco-physiological responses of invasive and indigenous mytilids in the Ría de Vigo**(NW Spain)**Jose M.F. Babarro^{1,*} and Malwenn Lassudrie²¹Instituto de Investigaciones Marinas (IIM-CSIC), Eduardo Cabello 6,
36208 Vigo, Spain²Université de Bretagne Occidentale – Institut Universitaire Européen de la Mer (IUEM),
3 Rue des Archives, CS 93837, 29238 Brest Cedex 3, France*Corresponding author: Jose M.F. Babarro Email: jbabarro@iim.csic.es
Tel.: +34 986 231930 Ext. 207; Fax: +34 986 292762**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 (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). 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. In 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

38 *X. securis* in all abiotic conditions tested. Results are discussed in relation to the ecological
tolerance and impact of these mytilid species.

40

Keywords: eco-physiology, invasion, attachment strength, bivalves, *Xenostrobus securis*, *Mytilus*
42 *galloprovincialis*

44

1 Introduction

46 A potentially invasive species can enhance its invasive capacity through a number of advantageous
physiological characteristics (Sara et al. 2008). For sessile species like mytilids, one such
48 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 Rıa de Vigo
50 (Galicia, NW Spain) by Garcı 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; Garcı et al. 2007;
52 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
54 indigenous ecosystems (Darrigran 2002). Today, the indigenous *Mytilus galloprovincialis* and the
alien *X. securis* share the same habitat in Ensenada San Simon (Fig. 1) in the inner part of Rıa de
56 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 Rıa (Garcı et al. 2007). Both
58 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 Rıa de
60 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
62 ongoing research on the cohabitation of these two species.

Coexistence mechanisms of native and exotic bivalves with similar niches have been reported in
64 several studies based on comparison of ecological traits (Harger 1972; Griffiths and Hockey 1987;
Safriel and Sasson-Frostig 1988; Van Erkom Schurink and Griffiths 1990; Zardi et al. 2006, 2007,
66 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
68 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
70 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
72 influence the success of biological invasions (Bownes and McQuaid 2010).

According to Garci et al. (2007), more research is needed to understand dynamics of *X. securis* in
74 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
76 in the Rías Baixas (Galicia, NW Spain) represents a total production of about 250 000 tons of
mussels per year, which is about 40 % of the total European output and 15 % of world mussel
78 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
80 economic losses for the mussel industry.

The dynamics of invasion and coexistence between species depend on environmental heterogeneity
82 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
84 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
86 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
88 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
90 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
92 (Allen et al., 1976), salinity and water velocity (Van Winkle 1970), agitation or vigorous shaking
(Young 1985), oxygen tension (Widdows and Bayne 1971), wave action associated with wind
94 (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
96 nature, together with other parameters like byssus diameter and the strength of the thread material
(Bell and Gosline 1996).

98 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;
100 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
102 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-
104 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
106 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
108 (Waite 2002) may help the individuals to maximise adhesion-enhancing adhesive bonding between
the mussel plaques and surfaces. In contrast, surfaces like acrylic, PVC and T-2 (Burkett et al.
110 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
112 energy and adhesion is not perfect and variations could have resulted from differences in surface
chemistry and moduli changes (Burkett et al. 2009).

114 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
116 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.
118 2008).

In this study, we aimed to evaluate several eco-physiological responses (byssus attachment strength,
120 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
122 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
124 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
126 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.

128

130 **2 Materials and Methods**

2.1 Field experiment

132 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
134 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
136 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
138 species relative to the size range encountered in the field.

140 **2.1.1 Abiotic parameters**

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

144

2.1.2 Attachment strength

146 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
148 measured by connecting the individuals 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
150 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
152 (Bell and Gosline 1997; Babarro and Fernández Reiriz 2010). After dislodgment, a number of 5 -
15 individuals (depending on the size class, see below) were measured to the nearest millimetre
154 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
156 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
158 (© 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
160 established at the beginning of the analysis and kept constant throughout.

162 **2.1.3 Byssus secretion *in situ*: thickness**

The thickness (diameter) of the byssus threads secreted per size class of the mussels was measured
164 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
166 measurements (see section 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
168 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
170 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

172 software used and camera conditions, please refer to section 2.1.2 (shell surface area
measurements).

174

2.2 Laboratory experiments

176 2.2.1 Maintenance of animals

Individuals of *M. galloprovincialis* and *X. securis* were collected from San Simón (SS) in the inner
178 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
180 mussels of 33 ± 2.0 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-
182 through device (Babarro and Fernández Reiriz 2010), using seawater with the following
characteristics: 10 μm -filtered seawater, salinity 35 ppt and temperature 15 °C, at a calm flow speed
184 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
186 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
188 blade.

190 2.2.2 Factors tested

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

194 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 section 2.2.4) with twenty individuals on each slate. Initially, the slates
196 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
198 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
200 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
202 one week and attachment strength of the individuals to the slates was then measured as reported
above (see 2.1.2).

204 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

206 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
208 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
210 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 soaked, allowing the
212 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
214 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.
216 Attachment strength of the mussels to the nylon ropes was measured after one week of exposure to
each flow speed (see 2.1.2). Two sets of individuals from the maintenance tanks were used for the
218 two experimental flow speeds tested in the flume tank.

220 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 for detail) and new individuals of
222 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
224 individually glued to vertical posts using “5 minute” epoxy (Imedio S.A. Madrid, Spain).
Individuals were suspended 6 mm above the substrate with their posterior ends facing upstream
226 (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)
228 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
230 for the morphometric analysis.

232 **2.2.3 Substrates**

Slate and nylon ropes were selected as substrates to simulate natural (mostly monolayer on the
234 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 (see
236 Introduction). 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
238 easy to collect from this relatively smooth surface by scraping off the adhesive plaques. In contrast,
traditional nylon ropes commonly used for mussel aquaculture purposes (Filgueira et al. 2007) were

240 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
242 Buck 2010; see Introduction).

244 **2.2.4 Flow speed**

A circulating flume tank was used to expose mussels to a constant water velocity. The working
246 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
248 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
250 hertz values, which were converted to flow measurements (cm s^{-1}) using a 2D-ACM flow meter
(see section 2.1.1). Considering the fluid dynamic conditions and shape of the flume tank, the flow
252 generated was turbulent even at relatively low velocities ($3\text{-}5 \text{ cm s}^{-1}$) according to Reynolds number
($> 10\,000$) (Vogel 1994; Ackerman 1999). Velocity measurements in the vicinity of the
254 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.

256 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
258 constant at $15 \pm 0.5 \text{ }^\circ\text{C}$ and individuals were fed following the conditions described in the section
2.2.1. The water of the flume tank was replaced every three days to remove faeces and other mussel
260 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
262 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).

264

2.2.5 Byssus secretion in the laboratory: quantitative values, thickness, length and surface 266 texture

The number of byssus threads secreted by a single individual and thickness and length values of the
268 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
270 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.*
272 *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

274 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.
276 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
278 distal part.

For thickness measurements of the byssus secreted in the laboratory, see section 2.1.3.
280 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
282 measured for both species by Image analysis (IA).

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

286 **2.3 Statistical analysis**

In situ attachment strength of the mussels in relation to individual size was fitted to linear functions.
288 Projected shell area of mussels as a function of individual size was fitted to potential allometric
equations ($Y=a.X^b$). Three-way ANOVA was used to estimate the influence of species, flow speed
290 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
292 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.
294 The effect of species and flow speed on the proximal and distal thickness values of the byssus
threads was tested by two-way ANOVA. Independence of cases was assumed and normality
296 checked with the Shapiro-Wilk test. Homoscedasticity between experimental groups of mussels was
established using Levene's test. When data did not fit normality tests or variances were not
298 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.

300

302 **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
306 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,

308 salinity fluctuated abruptly at the Sampaio site (SP) within the range of 6.2 – 32.0 ppt, including
low and high tide cycles. Temperature values of seawater during sampling of individuals at
310 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 –
312 2.3 cm s⁻¹. Temperature and salinity values fluctuated over the range 11.3 – 13.2 °C and 32.0 –
32.4 ppt, respectively, during the period of mytilid sampling, which included both low and high
314 tides.

316 **3.1.2 Attachment strength and animal's shape**

The shapes of the two mytilid species are illustrated in Fig. 3a. According to the size range
318 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
320 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
322 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.*
324 *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
326 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
328 (Fig. 4b).

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

334 **3.1.3 Byssus thickness**

Thickness of the byssus secreted by the mytilids followed a similar pattern to that of mussel
336 attachment strength (Fig. 4c-d). Linear increase in the attachment strength of *M. galloprovincialis*
with increasing size was correlated with linear increase of thread diameter in both proximal and
338 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
340 length, and 134 µm (proximal) and 120 µm (distal) for the 60 mm (largest) mussel size class (Fig.
4c).

342 As with the attachment strength variability, proximal and distal thickness of the byssus threads of
the black pygmy mussel *X. securis* did not follow any significant pattern with increasing size (Fig.
344 4b). Thickness of byssus varied over the range of 26 – 41 μm in the proximal section and 13 – 19
 μm in the distal section for the mussel size range analysed (Fig. 4d).

346

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
350 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
352 (ropes) times higher than those of *X. securis* ($p < 0.001$) (Fig. 5a-b). Otherwise, there was a positive
effect of increasing flow speed on attachment strength of the mussels ($p < 0.01$), although this effect
354 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 facing a flow of
356 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
358 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
360 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) (Fig. 5a-b).
362 Attachment strength of *X. securis* individuals was not significantly different between slates and
nylon ropes, with values ranging between 2.7 – 4.3 N (Fig. 5a-b).

364

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

366 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
368 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
370 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.*
372 *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 μm and 15 μm for *M.*
374 *galloprovincialis* and *X. securis*, respectively (Table 2). In contrast, both species and flow speed had
significant effects on the thickness of proximal byssus (Table 2). Indeed, proximal byssus was much

376 thicker for filaments secreted by *M. galloprovincialis* individuals (Table 2) with values of 100 μm ,
although there were no differences with flow speed within this species (Table 2). In contrast, there
378 was a 22 % increase in the thickness of proximal filaments secreted by *X. securis* (from 28 to 35
 μm ; $p < 0.001$; Table 2) with the tested increase in flow speed. Interestingly, the length of this
380 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
382 in *X. securis* at 5 cm s^{-1} , and increased up to 11 % of the whole thread at a flow speed of 25 cm s^{-1}
($p < 0.001$; Table 2). Proximal length of the byssus secreted by *M. galloprovincialis* remained
384 unchanged (22 – 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
386 laboratory (mean value of 10.6 mm; Table 2). Similarly to *X. securis*, no differences in thread
length were found for *M. galloprovincialis* exposed to different flow speeds (mean value of 13.2
388 mm; Table 2). A species comparison of byssus length, however, showed that filaments were
significantly longer in *M. galloprovincialis* than in *X. securis* ($p < 0.05$).

390
Scanning electron microscopy of byssus threads secreted by the two mytilids revealed differences in
392 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
394 which highlighted interspecific differences in structure along the thread. More interestingly,
proximal section of the byssus in threads secreted by *M. galloprovincialis* showed more crimped
396 and corrugated fibres (Fig. 7). 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
398 the thread forming a completely different structure (Fig. 7).

400

4 Discussion

402 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
404 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
406 species with regard to environmental salinity, between 5 and 37 ppt (Kimura et al. 1995), which
corresponds to the environmental range in our studied area. Our field observations showed that
408 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

410 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).

412 *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 ecologically 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 ($5 - 25 \text{ cm s}^{-1}$) 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 might be a more dynamic eco-physiological parameter than expected from the field data.

422 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×) and distal (5×) 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
444 produce up to 1000 filaments per byssus gland.

Apart from the amount of byssus, *X. securis* secreted the thinnest byssus threads recorded for any of
446 the Mytilidae and even some species of the family Dreissenidae (see Brazee and Carrington 2006
and Kennedy 2011 for the exact name of the species), although such comparisons may be
448 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 9 times larger in *M.*
450 *galloprovincialis* (0.81 mm^2) than in *X. securis* (0.09 mm^2) (preliminary observations). *X. securis*
has, together with *Modiolus modiolus*, the smallest plaque size of several, previously studied,
452 bivalve molluscs (Udhayakumar and Karande 1989; Brazee and Carrington 2006). Threads and
plaques are moulded by the mussel foot, which may vary in size (Brazee and Carrington 2006). For
454 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
456 might be also correlated with secretion of the much thinner byssus and smaller adhesive plaques.

458 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
460 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
462 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
464 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
466 conditions, as the threads can create an extensive network of individual attachments to small
particles (Pearce and LaBarbera 2009). In contrast, *Mytilus* species attach themselves to rocks and
468 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; Bell and Gosline, 1997; Carrington,
470 2002).

Another interesting aspect is related to the byssus surface texture observations in the two mytilids.
472 In a comparison of mytilid species (Brazee and Carrington 2006), the surface texture of the
proximal byssus of *X. securis* resembled that of *M. modiolus* (Fig. 6 in Brazee and Carrington
474 2006). *Mytilus galloprovincialis* can be grouped together with the blue mussel *M. edulis* according
to the scheme presented by Brazee and Carrington. Both *Mytilus* species have a corrugated and
476 crimped proximal byssus section, related to large extensibility values typically observed in

epibyssate individuals (Bell and Gosline 1996). In contrast, proximal byssus in *X. securis* presented
478 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
480 reported for *M. modiolus*, adapted to living commonly on muddy/soft bottoms but also on hard
substrates (Stanley 1972; Holt et al. 1998; present study) through changes in the byssus properties
482 (number of threads, morphometry and, most likely, mechanical properties).

484 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).
486 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
488 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
490 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
492 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
494 and survival of the alien species cannot be properly differentiated using our experimental design.

496 The laboratory experiment demonstrated that *M. galloprovincialis* had the strongest attachment
under the abiotic conditions tested (different flow speeds and substrates). It was noted that
498 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
500 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
502 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
504 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
506 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
508 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). In our
510 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. In 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 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* (see above), 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. In contrast, *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. However, as was shown in the present survey, *X. securis* is

able to attach and perform in rougher and full marine environments. *M. galloprovincialis* is an
546 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
548 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
550 marine and brackish water ecosystems at different levels.

552 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.
554 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
556 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
558 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
560 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
562 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
564 ecosystem of the Ría de Vigo as a whole.

Nevertheless, the overall results presented here can be interpreted in two different but compatible
566 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
568 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
570 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.*
572 *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
574 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
576 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
578 culture is carried out.

580

582 **Acknowledgements.** We would like to thank E. Silva Caride for technical assistance in the field and laboratory. We
 584 also thank Jesús Méndez (CACTI, University of Vigo) for SEM analysis of the byssus texture. This study was funded
 by the project AGL2010-16464 Dirección General de Investigación y Gestión del Plan Nacional de I+D+i (Ministerio
 de Ciencia e Innovación 2011-2013, Spanish Government).

586

References

- 588 Ackerman J.D., 1999, Effect of velocity on the filter feeding of dreissenid mussels (*Dreissena polymorpha*
 and *Dreissena bugensis*): implications for trophic dynamics. *Can. J. Fish. Aquat. Sci.* 56, 1551-1561.
- 590 Allen J.A., Cook M., Jackson D.J., Preston S., Worth E.M., 1976, Observations on the rate of production and
 mechanical properties of the byssus threads of *Mytilus edulis* L. *J. Mollus. Stud.* 42, 279-289.
- 592 Babarro J.M.F., Fernández-Reiriz M.J., Labarta U., 2000, Feeding behaviour of seed mussel *Mytilus*
galloprovincialis: environmental parameters and seed origin. *J. Shellfish Res.* 145 (2), 204-213.
- 594 Babarro J.M.F., Fernández-Reiriz M.J., 2010, Secretion of byssal threads in *Mytilus galloprovincialis* after
 spawning stress. *J. Comp. Physiol. B* 180, 95-104.
- 596 Bell E.C., Gosline J.M., 1996, Mechanical design of mussel byssus: material yield enhances attachment
 strength. *J. Exp. Biol.* 199, 1005-1017.
- 598 Bell E.C., Gosline J.M., 1997, Strategies for life in flow: tenacity, morphometry, and probability of
 dislodgement of two *Mytilus* species. *Mar. Ecol. Prog. Ser.* 159, 197-208.
- 600 Bownes S.J., McQuaid D., 2010, Mechanisms of habitat segregation between an invasive (*Mytilus*
galloprovincialis) and an indigenous (*Perna perna*) mussel: adult growth and mortality. *Mar. Biol.* 157,
 602 1799-1810.
- Braze S.L., Carrington E., 2006, Interspecific comparison of the mechanical properties of mussel byssus.
 604 *Biol. Bull.* 211, 263-274.
- Brenner M., Buck B.H., 2010, Attachment properties of blue mussel (*Mytilus edulis* L.) byssus threads on
 606 culture-based artificial collector substrates. *Aquacult. Eng.* 42, 128-139.
- Braby C.E., Somero G.N., 2006, Following the heart: temperature and salinity effects on heart rate in native
 608 and invasive species of blue mussels (genus *Mytilus*). *J. Exp. Biol.* 209, 2554-2566.
- Brown C.H., 1952, Some structural proteins of *Mytilus edulis* L. *Quart. J. Micros. Sci.* 93, 487-502.
- 610 Buck B.H., Buchholz C.M., 2005, Response of offshore cultivated *Laminaria saccharina* to hydrodynamic
 forcing in the North Sea. *Aquaculture* 250, 674-691.
- 612 Burkett J.R., Wojtas, J.L., Cloud, J.L., Wilker, J.J., 2009, A method for measuring the adhesion strength of
 marine mussels, *J. Adhesion*, 85 (9), 601-615.
- 614 Byers J.E., 2000, Differential susceptibility to hypoxia aids estuarine invasion. *Mar. Ecol. Prog. Ser.* 203,
 123-132.
- 616 Carrington E., 2002, Seasonal variation in the attachment strength of the blue mussel: causes and
 consequences. *Limnol. Oceanogr.* 47, 1723-1733.
- 618 Carrington, E., Moeser G.M., Thompson S.B., Coutts L.C., Craig C.A., 2008, Mussel attachment on rocky
 shores: the effect of flow on byssus production. *Int. Comp. Biol.* 48, 801-807.
- 620 Connell J.H., 1961, The influence of interspecific competition and other factors on the distribution of the
 barnacle *Chthamalus stellatus*. *Ecology* 42, 710-723.
- 622 Darrigran G, 2002, Potential impact of filter-feeding invaders on temperate inland freshwater environments.
Biol. Invasions 4, 145-156.
- 624 Dayton P.K., 1971, Competition, disturbance and community organization: the provision and subsequent
 utilization of space in a rocky shore intertidal community. *Ecol. Monogr.* 41, 351-389.
- 626 Dutton J.M., Hofmann G.E., 2008, Spatial and temporal variation in distribution and protein ubiquitination
 for *Mytilus* congeners in the California hybrid zone. *Mar. Biol.* 154, 1067-1075.
- 628 Filgueira R., Peteiro L.G., Labarta U. Fernández-Reiriz M.J., 2007, Assessment of spat collector ropes in
 Galician mussel farming. *Aquacult. Eng.* 37, 195-201.

- 630 Garci M.E., Trigo J.E., Pascual S., Gonzalez A.F., Rocha F., Guerra A., 2007, *Xenostrobus securis*
 (Lamarck, 1819) (Mollusca: Bivalvia): first report of an introduced species in Galician waters. *Aquacult.*
 632 *Int.* 15, 19-24.
- Griffiths C.L., Hockey P.A.R., 1987, A model describing the interactive roles of predation, competition and
 634 tidal elevation in structuring mussel population. *S. Afr. J. Marine Sci.* 5, 547-556.
- Harger J.R., 1972, Competitive co-existence: maintenance of interacting associations of the sea mussel
 636 *Mytilus edulis* and *Mytilus californianus*. *Veliger* 14, 387-410.
- Holt T.J., Rees E.I., Hawkins S.J., Seed R., 1998, Biogenic Reefs, Vol. 9: An Overview of Dynamic and
 638 Sensitivity Characteristics for Conservation Management of Marine SACs. Scottish Association of Marine
 Sciences/UK Marine SACs Project, Oban, Scotland.
- Holway D.A., 1999, Competitive mechanisms underlying the displacement of native ants by the invasive
 640 Argentine ant. *Ecology* 80, 238-251.
- Johnson A.S., 2001, Drag, drafting, and mechanical interactions in canopies of the red alga *Chondrus*
 642 *crispus*. *Biol. Bull. Mar. Biol. Lab.* 201 (2), 126-135.
- Kennedy V.S., 2011, Biology of the uncommon dreissenid bivalve *Mytilopsis leucophaeata* (Conrad, 1831)
 644 in central Chesapeake Bay. *J. Mollus. Stud.* 77 (2), 154-164.
- Kohama T., Montani S., Kajiwaru Y., Yamada M., 2001, Population dynamics of sessile bivalves *Mytilus*
 646 *galloprovincialis* and *Xenostrobus securis* in hyper eutrophicated bay, Japan. *Bull. Jpn Soc. Fish Sci.* 67,
 648 664-671.
- Kimura T., 1994, The population dynamics of *Musculista senhousia* (Benson) and *Limnoperna fortunei*
 650 *kikuchii* Habe in Lake Hamana, especially the ecological study of larval recruitment. PhD thesis, Mie
 University, Mie, Japan.
- Kimura T., Kakuta T., Kurokura H., 1995, Salinity tolerance and osmoregulation in freshwater and brackish
 652 water mytilids (Mytilidae: Genus *Limnoperna*). *Bull. Soc. Sea Water Scientist. Jpn.* 49,148-152.
- Kimura T., Masaaki T., Yasuhiro S., 1999, *Limnoperna fortunei kikuchii* Habe, 1981 (Bivalvia : Mytilidae) is
 654 a synonym of *Xenostrobus securis* (Lamarck, 1918) : introduction into Japan from Australia and/or New
 656 Zealand. *Japanese Jpn J. Malacol.* 58, 101-117.
- Labarta, U., Fernández-Reiriz, M.J., Pérez-Camacho, A., Pérez Corbacho, E., 2004, Bateeiros, mar, mejillón.
 658 Una perspectiva bioeconómica. CIEF. Fundación Caixagalicia, Santiago de Compostela, 262 pp., ISBN
 84-95491-69-9.
- Lazzari G., Rinaldi E., 1994, Alcune considerazioni sulla presenza di specie extra Mediterranee nelle lagune
 660 salmastre di Ravenna. *B. Malacol.* 30, 195-202.
- Lekang O.-I., Stevik T.K., Borno A.M., 2003, Evaluation of different combined collectors used in longlines
 662 for blue mussel farming. *Aquacult. Eng.* 27, 89-104.
- Martella T., 1974, Some factors influencing byssus thread production in *Mytilus edulis* (Mollusca: Bivalvia)
 664 Linnaeus, 1758. *Water Air Soil Poll.* 3, 171-177.
- Meadows P.S., Shand P., 1989, Experimental analysis of byssus thread production by *Mytilus edulis* and
 666 *Modiolus modiolus* in sediments. *Mar. Biol.* 101, 219-226.
- Míguez B.M., Farina-Busto L., Figueiras F.G., Pérez F.F., 2001, Succession of phytoplankton assemblages
 668 in relation to estuarine hydrodynamics in the Ría de Vigo: A box model approach. *Sci. Mar.* 65 (1), 65-76
- Nicastro K.R., Zardi G.I., Mcquaid C.D., 2008, Movement behaviour and mortality in invasive and
 670 indigenous mussels: resilience and resistance strategies at different spatial scales. *Mar. Ecol. Prog. Ser.*
 672 372, 119-126.
- Nicastro K.R., Zardi G.I., Mcquaid C.D., 2010, Differential reproductive investment, attachment strength
 674 and mortality of invasive and indigenous mussels across heterogeneous environments. *Biol. Invasions*, 12,
 2165-2177.
- Paini D.R., Funderburk J.E., Reitz S.R., 2008, Competitive exclusion of a worldwide invasive pest by a
 676 native. Quantifying competition between two phytophagous insects on two host plant species. *J. Anim.*
 678 *Ecol.* 77, 184-190.
- Pascual S., Villalba A., Abollo E., Garci M., González A.F., Nombela M., Posada D., Guerra A., 2010, The
 680 mussel *Xenostrobus securis*: a well-established alien invader in the Ria de Vigo (Spain, NE Atlantic). *Biol.*
Invasions 12, 2091-2103.
- Pearce T., LaBarbera M., 2009, Biomechanics of byssal threads outside the Mytilidae: *Atrina rigida* and
 682 *Ctenoides mitis*. *J. Exp. Biol.* 212, 1449-1454.

- 684 Petersen J.K., Nielsen T.G., van Duren L., Maar M., 2008, Depletion of plankton in a raft culture of *Mytilus galloprovincialis* in Ría de Vigo, NW Spain. I. Phytoplankton. *Aquat. Biol.* 4, 113-125.
- 686 Price H.A., 1982, An analysis of factors determining seasonal variation in the byssal attachment strength of *Mytilus edulis* L. *J. Mar. Biol. Ass. U.K.* 62, 147-155.
- 688 Price H.A., 1983, Structure and formation of the byssus complex in *Mytilus* (Mollusca, Bivalvia). *J. Mollus. Stud.* 49, 9-17.
- 690 Rius M., McQuaid C.D., 2006, Wave action and competitive interaction between the invasive mussel *Mytilus galloprovincialis* and the indigenous *Perna perna* in South Africa. *Mar. Biol.* 150 (1), 69-78.
- 692 Sabelli B., Speranza S., 1994, Rinvenimento di *Xenostrobus* sp. (Bivalvia, Mytilidae) nella laguna di Venecia. *B. Malacol.* 29, 311-318.
- 694 Safriel U.N., Sasson-Frostig Z., 1988, Can colonizing mussel outcompete indigenous mussel? *J. Exp. Mar. Biol. Ecol.* 117, 211-226.
- 696 Santaclara F.J., Espiñeira M., Vieites J.M., 2007, Molecular Detection of *Xenostrobus securis* and *Mytilus galloprovincialis* Larvae in Galician Coast (Spain). *Mar. Biotechnol.* 9, 722-732.
- 698 Sará G., Romano C., Widdows J., Staff F.J., 2008, Effect of salinity and temperature on feeding physiology and scope for growth of an invasive species (*Brachidontes pharaonis* - MOLLUSCA: BIVALVIA) within the Mediterranean sea. *J. Exp. Mar. Biol. Ecol.* 363 (1-2), 130-136.
- 700 Stanley S.M., 1972, Functional morphology and evolution of byssally attached bivalve molluscs. *J. Paleontol.* 46, 165-212.
- 702 Steffani C.N., Branch G.M., 2005, Mechanisms and consequences of competition between an alien mussel, *Mytilus galloprovincialis*, and an indigenous limpet, *Scutellastra argenvillei*. *J. Exp. Mar. Biol. Ecol.* 317, 127-142.
- 704 Suchanek T.H., 1978, The ecology of *Mytilus edulis* L. in exposed rocky inertial communities. *J. Exp. Mar. Biol. Ecol.* 31, 105-120.
- 708 Udhayakumar, M., Karande, A.A., 1989, Byssal threads of *Mytilopsis sallei* (Recluz) and their adhesive strength. *Proc. Indian Acad. Sci. (Anim. Sci.)* 98 (1), 65-76.
- 710 Van Erkom Schurink C., Griffiths C.L., 1990, Marine mussels of Southern Africa-their distribution patterns, standing stocks, exploitation and culture. *J. Shellfish Res.* 9, 75-85.
- 712 Van Winkle W., 1970, Effect of environmental factors on byssal thread formation. *Mar. Biol. (Berlin)* 7, 143-148.
- 714 Vogel S., 1984, Drag and flexibility in sessile organisms. *Am. Zool.* 24 (1), 37-44.
- Vogel S., 1994, *Life in moving fluids*, 2nd ed. Princeton University Press, Princeton, NJ.
- 716 Waite J.H., 2002, Adhesion á la moule. *Integr. Comp. Biol.*, 42, 1172-1180.
- 718 Waite J.H., Vaccaro E., Sun C., Lucas J.M., 2002, Elastomeric gradients: a hedge against stress concentration in marine holdfasts? *Phil. Trans. R. Soc. Lond. B.* 357, 143-153.
- 720 Walter U., Liebezeit G., 2003, Efficiency of blue mussel (*Mytilus edulis*) spat collectors in highly dynamic tidal environments of the Lower Saxonian coast (southern North Sea). *Biomol. Eng.* 20, 407-411.
- 722 Widdows J., Bayne B.L., 1971, Temperature acclimation of *Mytilus edulis* with reference to its energy budget. *J. Mar. Biol. Ass. U.K.* 51, 827-843.
- 724 Wilson B., 1968, Survival and reproduction of the mussel *Xenostrobus securis* (Lam.) (Mollusca-Bivalvia-Mytilidae) in a Western Australian estuary. I. Salinity tolerance. *J. Nat. Hist.* 2, 307-328.
- 726 Yamamoto, H., 1995. Marine adhesive proteins and some biotechnological applications. *Biotechnol. Genet. Eng.* 13, 133-165.
- 728 Yonge C.M., 1962, On the primitive significance of the byssus in the Bivalvia and its effects in evolution. *J. Mar. Biol. Assoc. U.K.* 42, 113-125.
- 730 Young G.A., 1985, Byssus-thread formation by the mussel *Mytilus edulis*: effects of environmental factors. *Mar. Ecol. Prog. Ser.* 24, 261-271.
- 732 Zardi G.I., Nicastro K.R., Mc Quaid C.D., Rius M., Porri F., 2006, Hydrodynamic stress and habitat partitioning between indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) mussels: constraints of an evolutionary strategy. *Mar. Biol.* 150, 79-88.
- 734 Zardi G.I., McQuaid C.D., Nicastro K.R., 2007, Balancing survival and reproduction: seasonality of wave action, attachment strength and reproductive output in indigenous *Perna perna* and invasive *Mytilus galloprovincialis* mussels. *Mar. Ecol. Prog. Ser.* 334, 155-163.
- 736

738 Zardi G.I., Nicastro K.R., Mc Quaid C.D., Erlandsson J., 2008, Sand and wave induced mortality in invasive
740 (*Mytilus galloprovincialis*) and indigenous (*Perna perna*) mussels. Mar. Biol. 153, 853-858.

742

Figure Legends

744

746

Figure 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.

750

752

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

754

Figure 3. Field results: (a) Shape and morphology of the mytilids *M. galloprovincialis* and *X. 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} r² = 0.94 p < 0.001 n = 35] *X. securis*: [SA = 0.0098 SL^{1.43} r² = 0.87 p < 0.001 n = 35].

756

758

Figure 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 r² = 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 r² = 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.

760

762

764

766

768

770

772

Figure 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 on the nylon ropes disposition [n = 15 for each species].

774

776

778

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

780

782

784

786

788

790

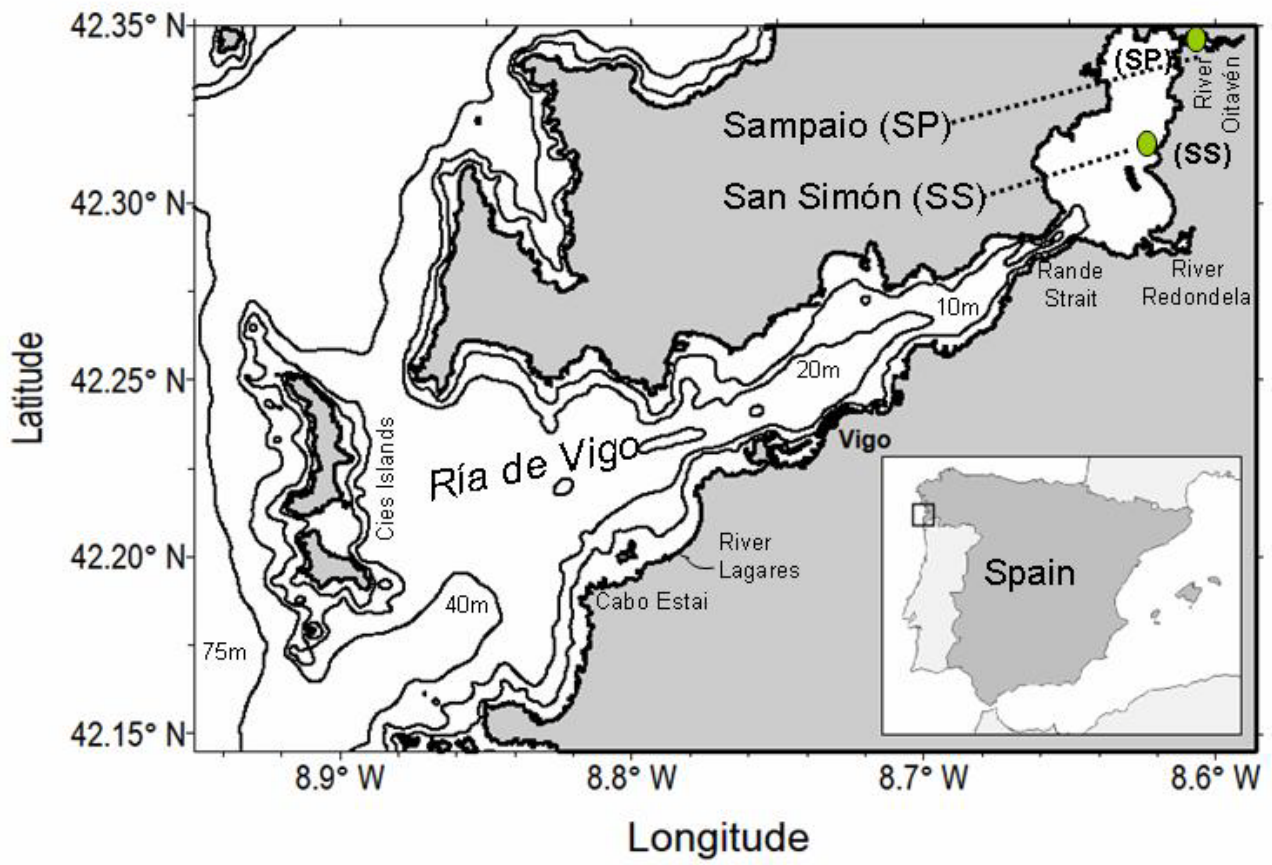
792

Figure 7. Laboratory results: Scanning electron micrographs of the proximal and distal surfaces of the byssus secreted by *M. galloprovincialis* and *X. securis*.

Figure 8. Photographs of the internal anatomy of *M. galloprovincialis* and *X. securis* showing the differences in foot organ size [1 within a circle].

794

796



798

800

802

804

806

808

810

812

814

816

818

Figure 1

820



822

flow direction

824

826

828

830

832

834

836

838

840

842

844

846

848

Figure 2

850

852

854

a

856

Mytilus galloprovincialis

Xenostrobus securis

858

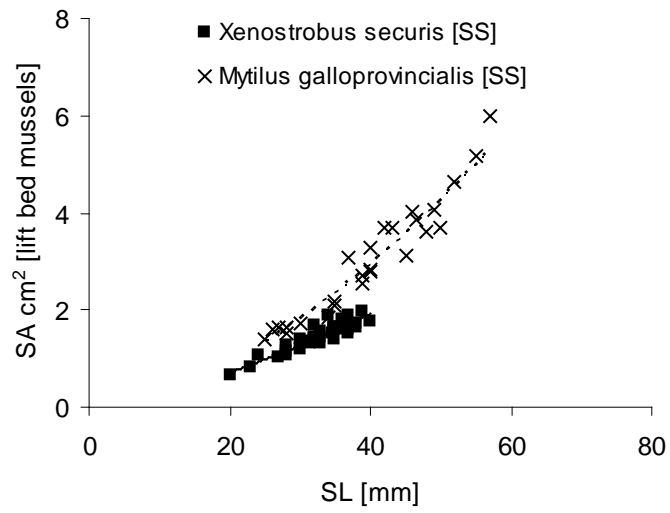


860

862

864

b



866

868

870

872

Figure 3

874

876

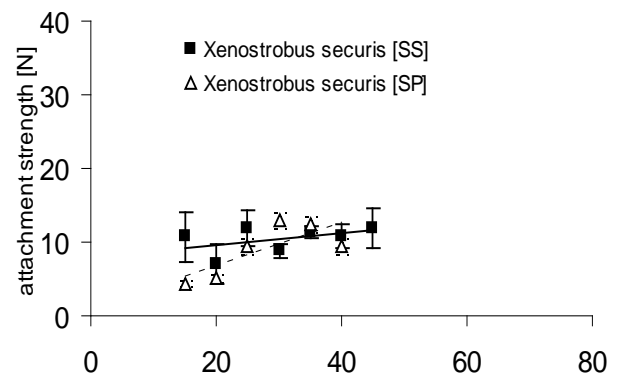
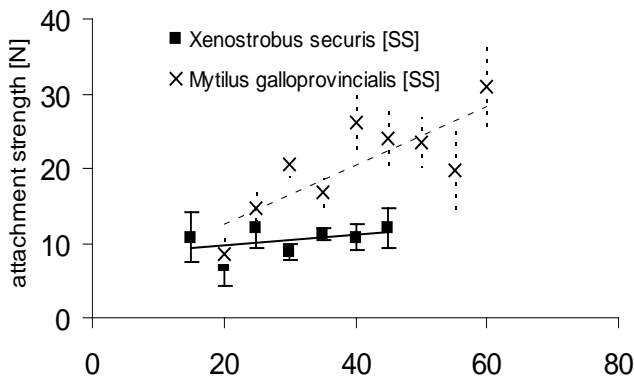
a

b

878

San Simón

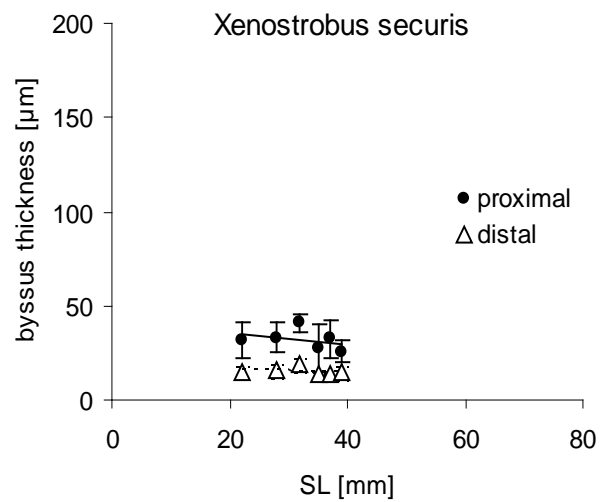
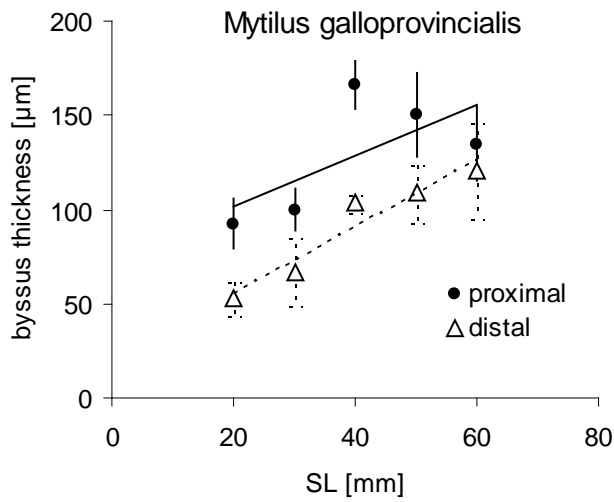
San Simón and Sampaio



880

c

d



882

884

886

888

890

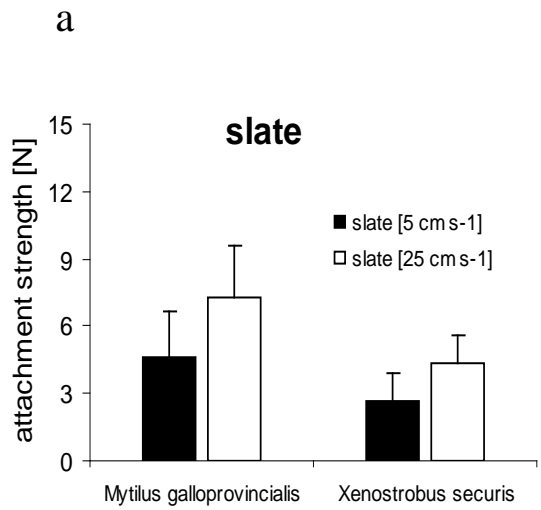
892

894

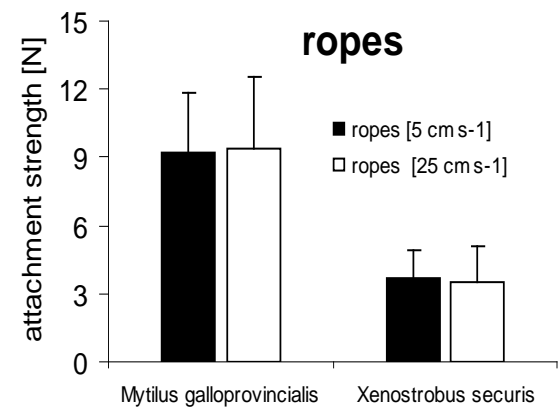
896

Figure 4

898



b



900

902

904

906

908

910

912

914

916

918

920

922

924

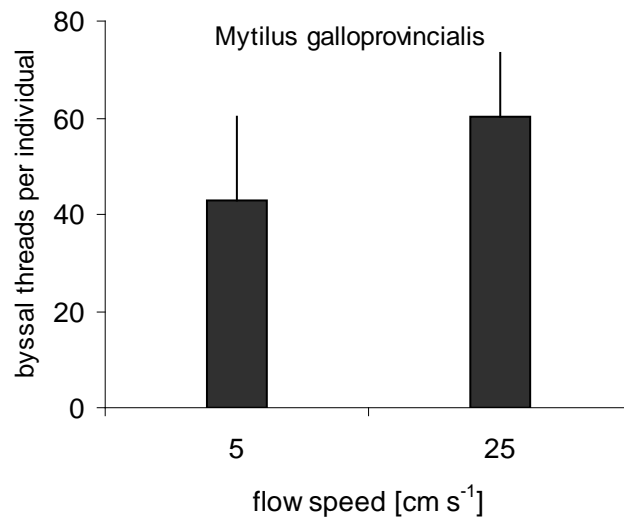
926

928

930

Figure 5

932



934

936

938

940

942

944

946

948

950

952

954

956

958

960

Figure 6

962

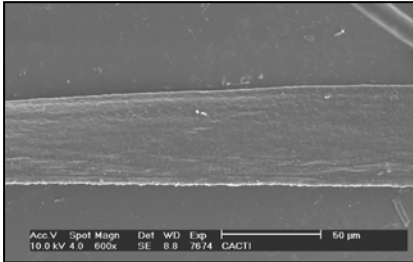
964

966

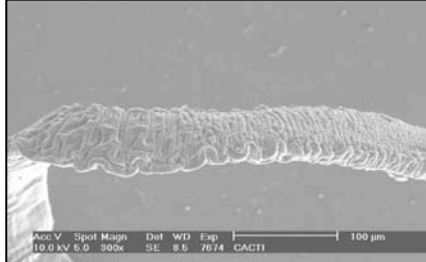
Mytilus galloprovincialis

968

distal smooth



proximal corrugated



970

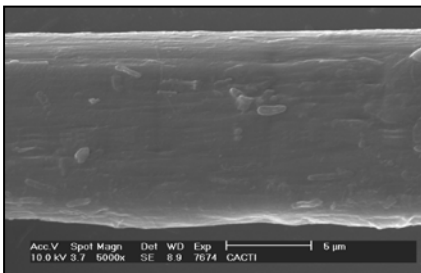
972

974

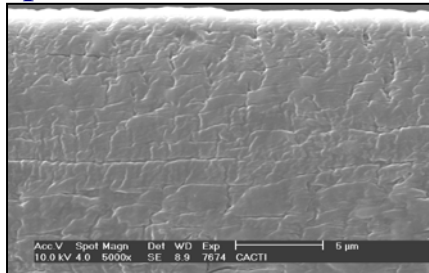
Xenostrobus securis

976

distal smooth



proximal corrugated



978

980

982

984

986

988

990

992

994

996

Figure 7

998

1000

Mytilus galloprovincialis

1002

1004



1006

1008

1010

1012

1014

1016

1018

1020

1022

1024

1026

1028

1030

1032

1034

1036

1038

Xenostrobus securis



Figure 8

1040

1042 **Table 1.** Field: Abiotic parameters of seawater at San Simón (SS) and Sampaio (SP) sites during sampling
1044 (February 2010). Ranges of values include both low and high tides.

Sites	temperature (°C)	flow speed (cm s ⁻¹)	salinity (ppt)
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

1046

1048

1050

1052

1054

1056

1058

1060

1062

1064

1066

1068

1070

1072

1074

1076

1 **Table 2.** Laboratory results: Byssus thread measurements for the studied mytilids (shell length: 33.0 ± 2.0 mm).
 2 Values are means \pm SD. Statistics presented in the Table were performed for flow speed comparisons in each species independently.
 3 ns: not significant
 4

	<i>Mytilus galloprovincialis</i>			<i>Xenostrobus securis</i>	
	5 cm s ⁻¹	25 cm s ⁻¹		5 cm s ⁻¹	25 cm s ⁻¹
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 p < 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 p < 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

5
 6
 7
 8
 9
 10
 11
 12
 13
 14
 15
 16

