Byssus secretion of *Mytilus galloprovincialis*: Effect of site at macro and micro-geographical scales within Ría of Vigo (NW Spain)

Jose M.F. Babarro[1,*] Emily Carrington[2]

[1*]Instituto de Investigaciones Marinas CSIC, Eduardo Cabello 6, 36208 Vigo, Spain

*Corresponding author. Email: jbabarro@iim.csic.es

Tel.: +34 986 231930 Ext. 207; Fax: +34 986 292762

[2]Friday Harbor Laboratories, Department of Biology, University of Washington, Friday Harbor, WA 98250, USA

Email: ecarring@u.washington.edu

ABSTRACT

The effect of abiotic environment on mussel tenacity and associated parameters was investigated for *Mytilus galloprovincialis* in the Ría of Vigo (NW Spain). Site was examined at macro-geographical (outer exposed CE vs. inner sheltered SS sites) and micro-geographical (intertidal vs. subtidal locations) scales. Site significantly influenced mussel tenacity, shape and byssus thread diameter, while location did not. Qualitative analysis of the byssus corroborated the importance of site; animals inhabiting the rougher outer Ría secreted stronger and stiffer threads regardless of location and had a higher potential to form cross-links or metal chelation in the byssal collagen to gain structural integrity when needed.

When animals were transplanted between exposed and sheltered sites, asymmetrical changes were observed in tenacity, endogenous indices, byssus morphology and mechanical properties after three months. Individuals moved from the sheltered to the exposed sites shifted all parameters, suggesting mussels have a plastic response to rougher environments by increasing byssus size and mechanical integrity. By contrast, animals transplanted from the exposed to the sheltered sites shifted tenacity, endogenous indices and thread length but not thread diameter nor mechanical properties. In

summary, we report the highly dynamic nature of the mussel ability to modify byssus tenacity when subjected to abrupt environmental changes. Animals have the potential to change byssus diameter and mechanical properties to increase strength in stressful abiotic conditions, and can re-allocate energy for vital structures like gonadal and soft tissue growth in more benign environments.

Key words: *Mytilus galloprovincialis*; tenacity; byssus secretion; environment; plasticity

INTRODUCTION

In the tidal zone of estuaries, environmental factors like temperature, salinity, aerial exposure and hydrodynamics represent key factors to determine population dynamics. Mussels are sessile and gregarious organisms capable of withstanding hydrodynamic challenges of the sea due to their ability to secrete an extracellular structure named byssus, an array of collagenous threads secreted in the ventral groove of the foot (Waite 1992). Each thread is proximally attached to a common stem that connects via the root to the byssus retractor muscle (Brown 1952) and distally to the substratum through the adhesive plaque. The structure of this byssus apparatus has to be replaced continuously because of thread decay over time (about 4 to 6 weeks; Carrington 2002; Moeser and Carrington 2006). The process of thread replacement can represent up to 8-15% of total energy expenditure of the mussel (Hawkins and Bayne 1985). Moreover, a morphological feature like shell mass and/or thickness, which affects the survival of intertidal animals facing aerial exposure, wave action and predation, may represent a high metabolic cost (up to 25-50% of the total energy that can be allocated into the shell formation; Gardner & Thomas 1987). Different environments, within the same estuary (i.e. sheltered vs. exposed areas) may cause several morphological changes in corporal parameters of the mussels, such as shell thickness, height and width (Raubenheimer and Cook 1990; Akester and Martel 2000; Steffani and Branch 2003; Beadman et al. 2003) and shift energy allocation patterns to other vital structures. Unbalanced patterns in energy allocation between shell

53 and soft tissue growth, reproductive tissues and byssal attachment may be more common in littoral
54 zones with limited food availability than in subtidal environments where food resources are less
55 scarce (Lachance et al. 2008).
56 Many factors (biotic and abiotic) influence attachment strength of mussels, such as temperature,
57 salinity, wave action, food availability and predators, which may be due to temporal and spatial
58 variability in byssus secretion. Variability in attachment strength of individuals has been proposed
59 to be not only based on the number of byssal threads secreted by the animals, but also on thread
60 thickness (Bell and Gosline 1997; Zardi et al. 2007; Babarro et al. 2008; Babarro and Fernández
61 Reiriz 2010). Moeser et al. (2006) reported that seasonal variations in attachment strength do not
62 always reflect variability in thread number, which suggests other factors might play a significant
63 role, such as thread mechanical properties, decay rates and other endogenous parameters such as
64 reproductive condition. Tensile mechanical properties of the byssus were quantified in several
65 surveys (Smeathers and Vincent 1979; Bell and Gosline 1996; Carrington and Gosline 2004; Brazee
66 and Carrington 2006). For that, the breaking force was estimated as the maximum force supported
67 by an individual thread and the breaking strain corresponded to the total distance a thread can
68 extend before failure divided by the initial thread length (Moeser and Carrington 2006). In general
69 terms, strength of the entire byssal structure should increase with an increase of breaking force and
70 strain; higher extensibility allows the individual thread to stretch and realign within the byssal
71 complex in order to realign and recruit more threads facing load (Bell and Gosline 1996). The
72 extensibility of the byssus in *Mytilus* sp. is due to the proximal section and the yield behaviour of
73 the distal section (becoming less stiff) before thread failure which establishes a triphasic pattern for
74 the whole-thread tensile behaviour for many *Mytilus* species (Bell and Gosline 1996): threads are
75 initially stiff, then yield, and finally stiffen again before structural failure.
76 When plasticity patterns, in qualitative terms, are considered for the byssus structure of the mussels
77 under stressful conditions, there is evidence for the formation of quinone-derived cross-links in
78 mussel byssal plaques with enhanced levels of 5,5´-dihydroxyphenyl-alanine cross-links when

individuals are exposed to increasing flow regimes (McDowell et al. 1999). The whole thread structure is mainly collagenous (Pujol et al. 1970; 1976; Sun and Waite 2005) but the distal part has a supplementary composition in alanine and glycine that make it similar to silk fibroin (Qin and Waite 1998) whereas proximal section has additional components similar to those encountered in elastin (Coyne et al. 1997; Waite et al. 2002). Both proximal and distal sections have common histidine-rich residues at their terminal flanking domains with important implications for the intra- and inter-molecular stabilization of assembled preCols in the byssus (Qin and Waite 1998). Specifically for the case of the byssal collagens, metal chelate complexes joining Zn^{2+}, Cu^{2+} and Fe^{2+} represent a significant cross-link alternative involving histidine, dopa (3,4-dihydroxyphenylalanine) or even cysteine residues (Lucas et al. 2002; Harrington and Waite 2007) that gives integrity and structural strength to the byssus apparatus. Recently, we have reported how the amount of basic residues, i.e. histidine and lysine, in the byssal collagen represent a significant contribution to the byssal strength of mussels under endogenous stress such as post-spawning events (Babarro and Fernández Reiriz 2010) although results of this work referred only to the distal section of the byssal filaments.

Mytilus galloprovincialis represents a widely distributed and cultured bivalve along the coastline of Rías Gallegas (NW Spain) and individuals may tolerate occasionally abrupt fluctuations of the abiotic factors between outer and inner sites of the Rías. Here, we examine the influence of the spatial gradient along the Ría of Vigo between the outer exposed vs. inner sheltered littoral sites as well as the vertical tidal zone gradient (intertidal vs. subtidal locations) on several phenotypic characteristics of rocky shore mussels, including endogenous parameters and byssus morphology, mechanics and composition. Reciprocal transplant experiments were designed to test the hypotheses that (1) both horizontal (outer vs. inner Ría) and vertical (intertidal vs. subtidal) spatial gradients along the Ría of Vigo affect byssal attachment strength, (2) differences in mussel tenacity between experimental locations reflect differences in byssus morphometry and material properties.

105

106 **MATERIALS AND METHODS**

107 **Environment**

108 Field studies were conducted in two littoral sites of Ría of Vigo (NW Spain) with strong differences
109 in environmental conditions. Both experimental sites are located near the city of Vigo and separated
110 by 30 kms (Figure 1): one location at the exposed outer Ría Cabo Estay (CE) and the other at the
111 inner sheltered zone in the Ensenada of San Simón (SS). Experiments were performed during fall
112 2007 and spring-summer 2008. For the first outplant experiment (fall 2007), two spatial gradients
113 were considered: one along horizontal line from the outer exposed (CE) to inner sheltered (SS) Ría
114 sites and, the other a vertical gradient within each site from intertidal to subtidal (20-0% air
115 exposure, respectively). The main goal was to investigate the significance of both macro- and
116 micro-geographical environmental levels on mussel tenacity and associated parameters. This
117 experiment lasted for 3 months between October-December 2007. A second reciprocal transplant
118 experiment was performed between exposed and sheltered intertidal sites with the main goal of
119 investigating the plasticity patterns in the mussel's performance under changing environments. This
120 survey was carried out between May-July 2008. Environment in both experiments was characterised
121 by analyzing seawater (weekly) from experimental sites for total particulate matter (TPM) and
122 chlorophyll-a values. Temperature was measured by using data loggers (Vemco Division, Nova
123 Scotia, Canada). Salinity, current speed and wave height of seawater were obtained from Reports of
124 MeteoGalicia for both experimental sites (Xunta de Galicia, Autonomic Government).

125

126 **Outplant and transplant design**

127 Subtidal individuals of *M. galloprovincialis* were collected from a raft system in the Ría of Vigo
128 (~4.5 cm shell length) and used for the outplant experiment during fall 2007 placing the mussels in
129 subtidal and intertidal locations at both outer exposed and inner sheltered sites. Two sets of slates
130 were placed at each site (Figure 2), one set for the intertidal and the other set for the subtidal

131 locations. Each set consisted in 4 slates per population (A-D) with 20 individuals per slate (N=80 for
132 each site). We have selected such number of individuals initially for the field experiment because
133 testing the different eco-physiological parameters required a number of individuals that cannot be
134 used for other purposes i.e. after measuring mussel tenacity, individuals are not suitable for other
135 byssus test because of its breakage. Beside, as consequence of aggregations formed by the mussels
136 in the field, very often was not that easy to collect entire byssus for a single mussel to characterise
137 the three main components of the study: amino acids analysis, mechanical properties and
138 morphometrics. Moreover, interconnected threads between individuals made occasionally mussels
139 unavailable for obtaining entire byssus. With such initial number of animals, we ensured reliable
140 samples sizes for the different tests proposed. Animals were allowed to establish primary attachment
141 in the laboratory for 2 days before transportation to the field conditions to avoid difficulties in
142 establishing byssus under natural tidal conditions. Nylon net was used to cover mussel populations,
143 allowing the seawater to flow in but preventing predation by macro-invertebrates initially when
144 attachment was weaker during first days after its transportation from the laboratory. After this initial
145 period in the field, the net was progressively cut and degraded; therefore, individuals were allowed
146 to freely attach to slates and naturally exposed to each environment.
147
148 For the reciprocal transplant experiment (spring-summer 2008), mussels from the exposed CE and
149 sheltered SS intertidal sites were carefully collected (4.5-4.9 cm shell length) and transported to the
150 laboratory for few days. Byssal threads were removed by snipping them with scissors to avoid
151 pulling them by hand and disturb the byssus structure of the mussels and/or foot organ. Individuals
152 from each intertidal site were divided into two sub-groups, native (returned to original sites; SS→SS
153 and CE→CE) and transplanted (moved to new site; SS→CE: CE→SS). A similar slate assemblage
154 to that used in the outplant experiment was designed.
155
156 **Tenacity**

157 Attachment force of individuals from each experimental site was measured by connecting the
158 mussel to a spring scale (Kern MH, resolution of 0.01N) with a thin monofilament fishing line
159 through a 2-mm diameter hole drilled through the shell valves close to the posterior margin. Then,
160 dislodgment force of the mussels from the substratum was recorded. The spring scale was pulled
161 perpendicular (normal) to the substrate until dislodgement occurred (Bell and Gosline 1997;
162 Babarro and Fernández Reiriz 2010). Attachment force (F) was normalized by mussel size in order
163 to obtain tenacity in N m^{-2} according to the formula Tenacity = F/PA, where PA is the projected area
164 of the individuals (m^{-2}; see next paragraph). Only animals directly attached to the slate surface were
165 considered.
166
167 **Shape and gonadal/condition index**
168 After dislodgment, mussels were measured according to the antero-posterior (shell length, L),
169 dorso-ventral (height, H) and lateral (width, W) axis to the nearest millimetre with vernier callipers.
170 The shell area of the experimental mussels was measured by image analysis (IA) of an ellipse
171 approximately with shell height and width as major and minor axes, respectively. IA measurements
172 were performed using the software QWin (© Leica Imaging Systems) on a PC (AMD Athlon XP
173 3000+) connected to a video camera (Leica IC A) on a stereo microscope (Leica MZ6). Camera and
174 light settings were established at the beginning of the analysis and kept constant throughout the
175 whole analysis. Shell thickness was determined as shell mass/surface area ratio (Beadman et al.
176 2003). The mass of the shell was determined as described below (see condition index) and the
177 surface area of the shell was estimated following the formula $A=l\ (h^2 + w^2)^{0.5}\ 0.5\pi$, where A is the
178 surface area (cm^2), l is length (cm), w is width (cm) and h is height (cm).
179 Condition index was obtained according to the formula: $CI=(DW_{tissue}/DW_{shell})x100$, where DW_{tissue}
180 corresponds to dry weight of soft tissues and DW_{shell} to dry weight of the shell (Freeman 1974).
181 Gonadal index was obtained as the proportion of mussel biomass composed of mantle tissue (site of
182 gametogenesis in *Mytilus*). Wet mantle was dissected from the wet body and together with the rest

of organs were freeze-dried for 48 hours. Samples of the mantle and the rest of tissues were weighed to the nearest 0.001 g and gonadal index was calculated as the dry weight of the mantle divided by the whole soft body (sum of the dry weight of the mantle and remaining tissues) (Carrington 2002; Babarro and Fernández Reiriz 2010).

Byssus size, mechanical properties and amino acid composition

Thickness and length of the threads secreted by the mussels *in situ* were measured by Image Analysis (IA; see previous section for shell area measurements) performed on a number of 30-40 threads for each experimental population. Byssal threads of *Mytilus* sp. are ovate in cross-section (Smeathers and Vincent 1979; Brazee and Carrington 2006) which creates elliptical profiles with a major and minor axis. Here, IA performed for thread's diameter measurements refer to apical photographs of the thread disposed on the plane. In order to collect the entire byssus, adjacent mussels (not manipulated) to those used for dislodgement measures were used. Proximal and distal portions of the thread were subdivided into sections along the thread in order to get an integrated measure of both portions.

The tensile properties of byssal threads from the different experimental mussel populations were tested according to Bell and Gosline (1996), using an Instron-5565 tensometer. Maximum load (N), strain at maximum load (mm/mm), initial modulus (MPa), yield force (N) as well as scaled force to break (N) were measured for whole threads, and material strength values were measured separately for proximal and distal thread regions. All mechanical tests were conducted in sea water at 15 ±1ºC at an extension rate of 10 mm/min.

The percentage of collagen content in the byssal threads can be estimated by quantifying the amount of hydroxyproline. Collagen quantity as well as quality (amino acid composition) was measured for both proximal and distal sections of the byssal threads of the experimental mussels. Hydrolysis of the byssal collagen was performed following Lucas et al. (2002). Briefly, proximal/distal segments

209 of the threads were hydrolysed in 6 mol^{-1} HCl with 0.01 ml of redistilled phenol. Three replicates of
210 five mussels each were used for HPLC analysis of each mussel population, pooling 3-5
211 proximal/distal segments from each animal. Threads were hydrolysed in vacuo for 24h at 110ºC and
212 samples were then flash-evaporated at 60ºC. A volume of PCA (perchloric acid) was added to the
213 dry hydrolysed thread material and amino acids were quantified following Babarro and Fernández
214 Reiriz (2010). Determination of amino acids was performed by reverse-phase high-performance
215 liquid chromatography of the dabsyl derivatives. All amino acids standards and dabsyl chloride
216 were purchased from Sigma. Amino acid separation method consisted in a slight modification of
217 that reported by Krause et al. (1995) changing the dilution buffer by a mixture of both mobile
218 phases A and B (see below) in the same proportion than that used at starting point of the gradient
219 profile. The chromatograph was a Waters Alliance HPLC System with a 2690 separations module
220 and a Waters 996 photodiode array detector (440-480 nm). The stationary phase was a C_{18} column
221 (Waters Symmetry, 150 x 4.6 mm, 3.5 µm particle size, 100 Å pore size) thermostated at 50ºC
222 either by an Alliance System column oven. Twenty µL of the derivatized samples were injected.
223 Dabsylated amino acids were eluted at a flow-rate of 1 mL/min using a gradient made with phase A
224 (9 mM sodium dihydrogenphosphate, 4% dimethylformamide and 0.1-0.2% triethylamine titrated to
225 pH 6.55 with phosphoric acid) and B (80% aqueous acetonitrile) with a gradient profile that
226 corresponds to that used by Pinho et al. (2001). For quantification, nor-leucine was used as internal
227 standard.
228
229 **Statistical analysis**
230 Two-way ANOVA was used to estimate the effects of both site (outer exposed CE and inner
231 sheltered SS) and tidal exposure (intertidal and subtidal) on the endogenous parameters (shape,
232 byssus morphology and soft tissues state), mussel tenacity, byssal mechanical properties and amino
233 acid composition (all dependent variables log-transformed) in the outplant experiment. Two-way
234 ANOVA was also used to estimate the effects of both site and origin of native and transplanted

mussels on the same log-transformed parameters described before and obtained in the second transplant experiment (spring-summer 2008). Homogeneous groups were established *a posteriori* by using Tukey test. When variances were not homogeneous (Levene test), non-parametric test Kolmogorov-Smirnov and Mann-Whitney were used. T-test was used to establish differences by shape and morphological values of mussels from the beginning (CE t=0 and SS t=0) to the end of the experiment as well as those transplanted individuals with regard to their original sites. T-test was also used for specific comparisons between amino acid residues of the two distinct regions of the byssus i.e. proximal and distal. All analyses were performed using STATISTICA 6.0 software.

RESULTS

Outplant Experiment: effect of both horizontal and vertical spatial gradients on byssal attachment strength, body size parameters and byssus properties

Environment

Abiotic conditions for the outer exposed CE and inner sheltered SS experimental sites at Ría of Vigo are presented in Figure 3. Air/seawater daily maximum temperature values fluctuated widely for both intertidal (11.5-29.4ºC) and subtidal (11.5-21.2ºC) locations at both inner (Figure 3A) and outer (Figure 3C) sites. Mean air/seawater temperature values were 13.4-14.1ºC (outer CE) and 12.3-12.5ºC (inner SS) including both intertidal and subtidal locations, respectively. Daily minimum and maximum salinity values varied between 34.5-35.9 psu and 22.3-31.4 psu for both the exposed CE (mean 35.5 psu) and the sheltered SS (mean 25.9 psu) sites, respectively (Figure 3B). Mean values of current speed and wave height were approx. 2 fold and 15-fold higher at the exposed outer site CE (0.08 ±0.04 m/s and 1.20 ±0.61 m, respectively) as compared to the inner sheltered SS (0.04 ±0.03 m/s and 0.08 ±0.05 m, respectively) (Figure 3D-E). Chlorophyll-a (Chl-a µg/l) and total particulate matter (TPM mg/l) values in seawater at the experimental locations are presented in Figure 3F. Chl-a values were rather similar between both sites (1.6-1.9 µg/l) with the exception of

261 the highest value reported for the exposed CE in October 2007 (5.3 µg/l) (Figure 3F). TPM showed
262 constant values for the exposed CE site (approx. 0.8 mg/l) and much higher variability for the
263 sheltered SS between minimum values of 0.4 mg/l and maximum of 5.8 mg/l (Figure 3F).
264
265 **Corporal parameters**
266 Animals living at the exposed CE had significantly higher shell width and lower shell height than
267 those from the sheltered SS site ($P<0.001$; Table 1). No significant differences were encountered for
268 the vertical zonation. Due to inverse pattern between differences in height and width values of
269 mussels, shell area differences of the individuals were balanced and not significantly different
270 between sites CE and SS (approx. 4.9 cm^2 mean values for both populations) but significantly
271 higher values were reported for subtidal (5.3 cm^2) than intertidal (4.6 cm^2) locations ($P<0.05$) (Table
272 1). Shell thickness (ST) showed differences by site but not by vertical distribution of the animals
273 (Table 1), values being 14-24% higher in the exposed CE population ($P<0.001$) as compared to the
274 sheltered SS. By contrast, gonadal (GI) and condition (CI) index values were significantly higher in
275 the sheltered SS also regardless of vertical zonation and compared to the exposed CE (Table 1),
276 values ranging from the highest 25% (GI) and 18% (CI) in the sheltered SS to the range of 13-18%
277 (GI and CI) in the exposed CE (Table 1).
278
279 **Tenacity**
280 Variability of mussel tenacity according to site and vertical zonation is presented in Table 2.
281 ANOVA showed that mussel tenacity varied significantly with site ($P<0.01$) but not with the
282 vertical zonation in the littoral coastline. Tenacity was significantly higher in the exposed CE
283 mussels regardless of intertidal or subtidal disposition (approx. 4.5 10^{-4} N m^{-2}) as compared to the
284 sheltered SS (range of 2.9-3.6 10^{-4} N m^{-2} for both littoral dispositions) (Table 2).
285
286 **Byssus size, mechanical properties and amino acid composition**

287 Thickness and length values of the byssus secreted according to the site and vertical zonation of the
288 mussels are presented in Table 2. ANOVA showed that only distal sections of the byssus varied
289 significant by site ($P<0.01$) but not by vertical zonation of the animals. Distal thickness was
290 significantly higher in the exposed CE (range of 97-106 μm for both intertidal and subtidal
291 populations) as compared to the sheltered SS (range of 90-95 μm) (Table 2). No significant
292 differences were observed for the proximal sections of the byssus secreted by mussels with respect
293 to sites and vertical zonation with a range of values of 159-170 μm (Table 2). Similarly, no
294 significant effect was reported for site and vertical zonation factors on the length of the byssus
295 secreted by experimental mussels, values ranging between 16-20 mm for all experimental
296 conditions (Table 2).
297 A number of mechanical properties of the threads secreted by the experimental mussels in the field
298 are presented in Table 3. Site factor caused a significant effect on maximum load, modulus, yield,
299 proximal strength and scaled force values of the byssal threads although vertical zonation did not
300 produce any effect (Table 3). Strain and distal strength did not change significantly with either site
301 or vertical zonation, values ranged between 0.615-0.801 mm/mm and 54-87 MPa for all
302 experimental populations, respectively (Table 3). Thread maximum load, yield and modulus values
303 were significantly higher for the exposed CE mussels as compared to the sheltered SS, regardless of
304 the vertical zonation (Table 3). Scaled force values of the threads were also significantly higher for
305 the exposed CE mussels as compared to the sheltered SS individuals with no effect of the vertical
306 zonation (Table 3). Threads secreted by CE exposed mussels were, therefore, significantly stronger
307 and stiffer due to differences in both proximal (strength) and distal (yield) sections (Table 3).
308 Specific amino acid compositional analyses of the acid-hydrolysed distal and proximal regions of
309 the threads secreted by the experimental mussels are illustrated in Table 1. Byssal collagen quantity,
310 according to amino acid hydroxyproline (and hydroxylysine) content was not significantly affected
311 by site or tidal exposure (ANOVA; data not shown) and this result was reported regardless of
312 byssus section proximal or distal (see mean values in Table 1). Nevertheless, mean values of both

hydroxyproline and hydroxylysine were clearly higher in distal section (10-12%) than in proximal segments of the byssus (8.0-9.1%) (Table 1) ($P<0.05$). A significant effect of the site factor ($P<0.01$) was observed on the histidine plus lysine residues at both proximal and distal regions of the byssus (Table 1; ANOVA; data not shown). By contrast, no effect was detected for the tidal exposure. The sum of histidine and lysine residues was higher in the threads secreted by the exposed CE mussels regardless of proximal (mean value of 4.5%) or distal (mean value of 3%) sections of the byssus as compared to the sheltered SS mussels (mean of 2.8% and 1.8% for both proximal and distal sections, respectively; Table 1).

Transplant Experiment: effect of origin and site on byssal attachment strength, body size parameters and byssus properties between the exposed CE and sheltered SS intertidal sites

Environment

Air/seawater temperature (mean) values were 17.5ºC ±1.0 and 17.9ºC ±1.9 for the exposed CE and sheltered SS, respectively (Figure 4A) with minimum of 14.3ºC (sheltered SS) and 16.1ºC (exposed CE) and, maximum of 21.9ºC (sheltered SS) and 19.2ºC (exposed CE) (Figure 4A). Salinity mean values were 35.6 ±0.2 and 34.2 ±0.4 psu for both outer CE and inner SS sites, respectively (Figure 4B) with no significant fluctuations. Mean values of current flow and wave height were approx. 2 fold and 11-fold higher at the exposed outer CE site (0.12 ±0.06 m/s and 1.2 ±0.4 m, respectively) as compared to the sheltered inner SS site (0.06 ±0.03 m/s and 0.11 ±0.03 m, respectively) (Figure 4C-D). Chlorophyll-a (chl-a µg/l) and total particulate matter (TPM mg/l) values in seawater at the experimental sites are presented in Figure 4E-F. Mean chl-a values were clearly higher in the sheltered SS (mean values of 3.1 ±1.9 µg/l) as compared to the exposed CE (mean of 0.68 ±0.40 µg/l) (Figure 4E) whereas TPM was also higher in the sheltered SS as consequence of peak values at the beginning of the sampling period (mean of 3.52 mg/l) and compared to the exposed CE (mean of 0.46 mg/l) (Figure 4F).

339 **Corporal parameters**

340 Shape of native (CE→CE and SS→SS) and transplanted mussels (CE→SS and SS→CE) from both
341 exposed CE and sheltered SS sites are presented in Table 4. Growth was practically zero or residual
342 in native (CE→CE; 4.49 cm) and transplanted (SS→CE; 4.51-4.57 cm) mussels located in the
343 exposed CE site when compared to CE t=0 and SS t=0 (Table 4). Similarly, shell height remained
344 unchanged between native CE→CE (2.26-2.30 cm) and transplanted SS→CE (2.71-2.74 cm)
345 mussels during the sampling period (Table 4). Shell width, however, showed a significant increase
346 for the sheltered SS (1.74 cm, SS t=0) transplanted mussels to the exposed site (SS→CE, 1.83 cm)
347 ($P<0.001$; Table 4) whereas no significant change was observed for the exposed CE→CE native
348 mussels (1.88-1.90 cm; Table 4). This caused that shell area increased also significantly ($P<0.01$)
349 for the former population (5.03 cm^2 for SS→CE mussels from 4.71 cm^2 at SS t=0) as compared to
350 CE→CE native (NS; Table 4).

351 When animals are located in the sheltered SS site, growth was significant for several morphological
352 values (Table 4). Shell length values of both native (4.51-4.89 cm) and transplanted (4.49-4.92 cm)
353 mussels increased at similar rate ($P<0.001$; Table 4). Shell width was also reported to increase at
354 similar rate between SS→SS native (1.74-1.93 cm) and CE→SS (1.88-2.06 cm) transplanted
355 mussels (Table 4). However, a much higher increment was reported in shell height of the exposed
356 mussels transplanted to sheltered site (2.26-2.46 cm for CE→SS; $P<0.001$) as compared to
357 unchanged values of SS→SS native (2.71-2.80 cm; NS) (Table 4). Shell area values presented,
358 therefore, a significant increase in SS→SS native (4.71-5.41 cm^2; $P<0.001$) but also for the
359 transplanted CE→SS mussels (4.25-5.05 cm^2; $P<0.001$) (Table 4).

360 Shell thickness was significantly higher in the exposed CE→CE native animals (296 mg cm^{-2}) as
361 compared to the sheltered SS→SS native mussels (222 mg cm^{-2}) ($P<0.001$; Figure 5A-B).
362 Transplanted animals modified shell thickness increasing (248 mg cm^{-2}; SS→CE) and decreasing
363 (275 mg cm^{-2}; CE→SS) values as compared to their original sites but values did not reach the native
364 non-transplanted populations in any circumstances (Figure 5A-B). Gonadal (GI) and condition (CI)

365 index values showed no differences between natives and transplanted individuals. Native mussels of
366 the exposed CE site (CE→CE) showed lower gonadal (13%) and condition (6%) indices ($P<0.001$)
367 as compared to native mussels of the sheltered SS site (SS→SS) (25% and 11% for both gonadal
368 and condition indices, respectively; Figure 5C-D). After transplantation, in both directions,
369 transplanted mussels reached similar values that characterised native populations i.e. SS→CE and
370 CE→SS mussels decreased and increased significantly both GI and CI values, respectively (Figure
371 5C-D).
372
373 **Tenacity**
374 Tenacity of mussels in the transplant experiment is presented in Figure 6. ANOVA showed that
375 origin has no effect on mussel tenacity but the site did ($P<0.01$). Tenacity of native CE→CE
376 mussels was two-fold higher than that of SS→SS native mussels, however after 3 months of
377 transplant from exposed CE to sheltered SS conditions, mussels decreased significantly its tenacity
378 down to values not significantly different than SS→SS natives (Figure 6). Mussels from the
379 sheltered SS site transplanted to the exposed CE site (SS→CE) showed a significant increase in
380 tenacity (35%) up to values not significantly different than CE→CE native (Figure 6). It is
381 important to note that mortality was not reported for all mussel populations with the exception of
382 the sheltered SS mussels transplanted to the exposed site CE (SS→CE) in which a 33% of the
383 population was lost. Clearly animals were weak to make a secure byssus under the latter
384 experimental condition.
385
386 **Byssus size, mechanical properties and amino acid composition**
387 Thickness of the byssus secreted by mussels in the transplant experiment is illustrated in Table 5.
388 Variability of the proximal thickness of the byssus remained unaffected by site and origin factors,
389 neither the interaction term was significant (Table 5). Values varied within the range 120-135 μm
390 for all mussel populations (Table 5). However, both site ($P<0.05$) and origin ($P<0.001$) factors as

391 well as its interaction term ($P<0.001$) were significant to explain variability in distal section of the
392 byssus secreted by the mussels (Table 5). Distal thickness of the threads secreted by the exposed
393 CE→CE native animals was significantly higher than those secreted by the sheltered SS→SS
394 natives (Table 5). After 3 months of transplant from sheltered SS to exposed CE conditions, threads
395 secreted by the mussels showed an increase in the thickness up to similar native values (CE→CE)
396 (Table 5). The contrary way of transplant (from exposed CE to sheltered SS) surprisingly did not
397 show any significant change as compared to CE→CE natives (Table 5) which established
398 asymmetry for the plasticity of this morphological feature of the byssus structure. Length of byssus
399 secreted by the mussels was significantly affected by the site factor ($P<0.05$) but not by the origin of
400 individuals (Table 5). Mussels located in the sheltered SS (both native SS→SS and transplanted
401 CE→SS) secreted significantly longer byssal threads ($P<0.05$) as compared to mussels located in
402 the exposed CE (native CE→CE and transplanted SS→CE) (Table 5).
403
404 In general, mechanical properties of the byssus collected from the transplant experiment followed
405 similar pattern of that presented for distal thread thickness. Byssus load values varied significantly
406 with the origin of mussels ($P<0.05$) and the interaction term site x origin ($P<0.01$; Table 5). Load
407 values are significantly higher in the exposed native CE→CE mussels as compared to the sheltered
408 native SS→SS (Table 5). After 3 months of transplant from sheltered SS to exposed CE conditions,
409 threads secreted by the mussels showed an increase in load that can be sustained up to similar native
410 values (CE→CE). Mussels transplanted in the opposite direction, from exposed CE to sheltered SS
411 continued to produce strong threads similar to those of the original site CE→CE (Table 5) (see
412 asymmetry for the plasticity of this parameter). Strain varied within the range of 0.56-0.73 mm/mm
413 for all mussel populations but no significant effect of site or origin could be detected (ANOVA;
414 Table 5). Similarly to load, yield values were also significantly higher in the exposed native
415 CE→CE as compared to the sheltered native SS→SS (Table 5). After three months of
416 transplantation, there was an increase in the yield values of the threads secreted by the sheltered

417 population transplanted to the exposed site (SS→CE) up to similar values between both mussel
418 populations though the contrary transplant did not cause any change according to the original site
419 (Table 5). Modulus value of the byssus was significantly affected by site ($P<0.05$) with no influence
420 of the origin of mussels in the transplant experiment, values being significantly higher for the
421 threads secreted at the exposed CE location (by both natives and transplanted) as compared to the
422 sheltered SS (Table 5). Scaled force values of the threads were significantly higher in the exposed
423 native mussels CE→CE as compared to the sheltered native mussels SS→SS (Table 5). After 3
424 months of transplant from sheltered SS to exposed CE conditions (SS→CE), threads secreted by the
425 mussels showed an increase in scaled force up to similar values than native mussels (CE→CE). As
426 was the case for other mechanical properties, scaled force of the byssus secreted by mussels
427 transplanted from exposed CE to sheltered SS remained higher similar to those of the original site
428 CE→CE (Table 5) which is represented by the asymmetry pattern for this value.
429
430 Specific amino acid compositional analyses of the acid-hydrolysed distal and proximal regions of
431 the threads secreted by the mussels in the transplant experiment are illustrated in the Figure 7. Here,
432 we can report no differences by site or origin of mussels in quantity of byssal collagen, according to
433 specific amino acids hydroxyproline and hydroxylysine for native and transplanted mussels
434 (ANOVA; data not shown) (Figure 7A). Distal and proximal values of both hydroxyproline plus
435 hydroxylysine residues were 11-12.7% and 10-11.5%, respectively (Figure 7A). The sum of
436 histidine and lysine residues in the proximal byssus showed no differences by site but a significant
437 effect of the origin of mussels transplanted (ANOVA; data not shown). Histidine and lysine residues
438 remained similar in the comparison native exposed CE and sheltered SS mussels ($P>0.05$) although
439 values in proximal sections of the threads were significantly higher (3.1-4%) than those reported for
440 the distal sections (2.0-2.2%) ($P<0.01$) (Figure 7B). Transplanted mussels showed a significant drop
441 in histidine plus lysine residues of the proximal byssus and regardless of its original site (Figure

7B). No effect of both site and origin (and its interaction term) was reported for the histidine and lysine residues in the distal section of the byssus (ANOVA; data not shown) (Figure 7B).

DISCUSSION

The main aim of the present study was to investigate how a specific scenario within a single Ría can affect *Mytilus galloprovincialis* performance with regard to attachment strength and associated parameters. The abiotic environment of the selected sites differed in the hydrodynamics and salinity gradients within the Ría of Vigo and clearly modified the shape/morphology of the individuals and their byssus secretion *in situ*. Individuals presented a more hydrodynamic shape to withstand rougher sea in the outer exposed site CE of Ría of Vigo and energy allocation patterns shifted to protective structures such as shell thickness and byssus secretion. Indeed, hydrodynamic stress depends not only on water velocity but also on shell shape and the area over which the force acts (Denny 1995; Zardi et al. 2006) and consequently, outer exposed mussels at CE would offer better resistance to dislodgment by modifying their shape, making lower and wider shells similar to the patterns reported by Bell and Gosline (1997). Steffani & Branch (2003) have also shown that shells of *M. galloprovincialis* tended to be lower at more exposed sites in order to reduce the area over which hydrodynamic lift acts preventing the risk of dislodgement.

The more energy allocated to protective structures (i.e. shell thickness, shell tissue and byssus), the lower energy available for soft tissue growth, as can be seen in the lower condition/gonadal index for the outer exposed CE population and regardless of vertical zonation. Similar trade-off patterns between soft tissues growth and/or byssus secretion values were reported by Raubenheimer and Cook (1990), Carrington (2002), and Moeser and Carrington (2006).

467 An increase in shell thickness is required to withstand the destructive and erosive effects of wave
468 action. With our experimental design, however, we cannot entirely exclude predation as important
469 factor to explain shell thickness differences. However, due to the fact that we observed similar
470 distributions of the gastropod *Nucella lapillus*, one of the major predators on littoral mussel
471 populations in this region (Barreiro et al. 1999), we assumed that differences encountered in our
472 survey can be considered mainly driven by abiotic variability (Figure 3-4). Shell thickness and
473 mussel tenacity values were 20-26% higher in the mussels placed at the exposed CE site as
474 compared to the sheltered SS individuals in the outplant experiment but surprisingly, no effect was
475 observed for intertidal exposure (20-0% emersion). We have considered such exposure degree of
476 the individuals to emersion in the field based on the actual space mainly occupied by the mussels
477 and such degree clearly did not affect animal behaviour here. The absence of any significant effect
478 for the vertical zonation in our survey was observed not only on the whole mussel tenacity but also
479 on those parameters that eventually confer byssal strength, i.e. byssus morphology, mechanical
480 properties and compositional values (Tables 1-2-3).
481
482 Although high-energy shores can be advantageous in terms of tidal exchange and higher food
483 availability compared to more sheltered environments (Steffani and Branch 2003), here mussels
484 inhabiting the more benign SS environment benefited from the fact that less energy had to be
485 channelled into the byssus and shell thickness/mass formation. We found significantly higher
486 condition/gonadal indices for the sheltered SS mussel populations, which can be linked to both less
487 energy channelled to byssus and shell thickening but also to higher seawater load (particulate
488 matter) in this environment in both experiments (Figure 3F; Figure 4E-F). Although chlorophyll-a
489 values are occasionally higher at the highly exposed site CE, total particulate matter is 3-fold higher
490 in the inner sheltered SS, suggesting many other fractions of particulate material or food resources
491 might be used by the individuals in the sheltered site of the Ría, such as mixture of suspended
492 micro-phytobenthos and phytoplankton of marine origin. The benefit that bivalves can obtain from

493 these seston fractions has been confirmed by using stable isotopes for *Crassostrea gigas* (Riera and
494 Richard 1997) and *Cerastoderma edule* (Kang et al. (1999) in the Bahía de Marennes–Oléron and
495 for *Macoma balthica*, *Scrobicularia plana* and *Mytilus edulis* in la Bahía de Aiguillon (Riera et al.
496 1999).
497
498 Studies concerning to plasticity and/or ability of mussels to alter compositional and/or mechanical
499 properties of the byssus secretion under changing environments are not abundant. On one hand, we
500 have reported here the importance of the site factor on a number of parameters associated with
501 byssus secretion: morphology of byssus, compositional values, and mechanical properties.
502 Differences in the wave activity (flow regime, wave height and/or turbulence) between our
503 experimental locations must be clearly related to the latter pattern, as was suggested by Hunt and
504 Scheibling (2001) and Lachance et al. (2008). The higher tenacity reported for the exposed CE
505 mussels was not only due to the thicker byssal threads these animals secreted, specifically at its
506 distal sections, but also as a consequence of the fact that these threads were mechanically more
507 effective (Table 3) and with higher amount of basic amino acids histidine and lysine (Table 1).
508 The importance of these basic amino acids histidine and lysine in the byssal collagen of the mussel
509 *Mytilus galloprovincialis* has been recently reported when animals are subjected to endogenous
510 stress such as spawning events (Babarro and Fernández Reiriz 2010) and manifest the ability of
511 mussels to carry out compositional changes for increasing attachment force when needed. The latter
512 authors have established such changes in the amino acid composition of the distal section of the
513 byssus. Here, we extended this information also to the proximal sections of the byssus secreted by
514 the experimental mussels (Table 1). Residues of both lysine and histidine produce cross-links,
515 joining two or more molecules; histidine in particular has a pronounced effect on metal chelation
516 and/or cross-link ability (Waite et al. 1998) as well as the capacity to form a significant part (up to
517 22 mol% in protein mcfp-4) of the junction between collagen fibres and foam-like adhesive plaques
518 in the mussel *Mytilus californianus* (Zhao and Waite 2006). Whenever histidine-rich domains occur

519 in proteins, they usually bind with metal; the byssal collagen of *Mytilus galloprovincialis* has been
520 reported to contain additional histidine residues in their flanking domains that can help to utilise
521 more metal chelate cross-link for byssal stability and integrity (Lucas et al. 2002). Plasticity patterns
522 in the mussel byssus were also reported by McDowell et al. (1999), with an increased formation of
523 quinone-derived cross-links in mussel byssal plaques when individuals were exposed to higher flow
524 regimes which might lead to establish better attachment of the individuals to the substratum. It is
525 also important to note that when threads yield, the stress softening is reversible in a time-dependent
526 manner (Carrington and Gosline 2004) and that histidine-metal interactions are reported as the
527 perfect candidate for a sacrificial cross-link because break and reform reversibly and are weaker
528 than covalent bonds (Schmitt et al. 2000).
529 The distal section of the byssus has been established as a main factor involved in the tenacity
530 variability of mussels (Bell and Gosline 1997; Brazee and Carrington 2006; Babarro and Fernández
531 Reiriz 2010). Similar patterns were observed here, in contrast to a rather constant value for the
532 proximal section diameter (Table 2). Nevertheless, mechanical properties of the byssus followed, in
533 the present survey, similar patterns than that of distal byssus diameter (see Table 2-3) and are
534 primarily responsible for the differences in mussel's tenacity. Thickness of the proximal sections of
535 the byssus were narrower (159-170µm) in all mussel populations of the first outplant survey, but the
536 fact that compositional values i.e. the amount of residues histidine and lysine were also significantly
537 higher in the most exposed CE mussels (38%) regardless of byssal sections (proximal and distal)
538 suggests that both thread diameter but also amino acid composition do influence mussel's tenacity
539 significantly.
540
541 Animals transplanted between intertidal sites showed similar values to native individuals for a
542 number of parameters, such as tenacity, endogenous (gonadal and condition) indices, byssus
543 morphology (length and thickness) and mechanical properties, although this pattern depended on the
544 transplantation direction. A complete shift was observed in mussels transplanted to the most

exposed CE environment, suggesting that the increase in the byssus strength was necessary to cope with a rougher habitat. This was accomplished by the increased byssus diameter (distal) and a number of mechanical properties (Table 5). The opposite direction of the transplant (from the exposed CE to the sheltered SS) resulted in a shift in tenacity, endogenous gonadal/condition indices and thread length (Table 5; Figure 5C-D; Figure 6) but neither thread diameter nor mechanical properties showed any significant change (Table 5). Surprisingly, animals transplanted to inner sheltered SS location continued to secrete thick (and strong) byssus despite the relatively benign environment and established a clear asymmetry for parameters like distal thread thickness, maximum load, yield and scaled force (Table 5). Most likely, in the latter case, tenacity change of the whole animal may have been achieved by a drop in the number of byssal threads secreted, thereby saving energy for other purposes i.e. soft tissue growth (Figure 5C-D). In other words, the secretion of thick and strong byssus by those mussels originally from the exposed CE site and transplanted to the sheltered SS site (CE→SS) must be energetically not that costly, at least when compared to the secretion of more new threads, and specially when such environmental change offer the animals better food resources (Figure 4E-F). Here, we may consider the significant influence of the *past history* or *ecological memory* (Suhkotin and Pörtner 1999) according to which animals have a record of the past events in the their natural environments that may introduce a temporal dependence in their new responses when facing abrupt environmental changes. Accordingly, such influence of the *past history* for the animals living at the most exposed site would be significant when transplanted to more benign waters and would need longer time to completely adapt to this new sheltered habitat. For the opposite transplant direction, from the sheltered to the exposed site, animals would be undoubtedly force to secrete thicker and stronger byssus in a much shorter-term for a better performance.

Surprisingly, modulus values showed the opposite pattern between outplant and transplant experiments in the sites comparison (Table 3 and 5) and were the only mechanical property of the

byssus that presented complete symmetry regardless of the direction of the transplant (Table 5). Modulus values were higher in the exposed CE mussels in fall 2007 (Table 3) but this pattern was reversed in spring-summer 2008 (Table 5). The mechanism for the observed differences in modulus is not clear, but might be dependent on numerous environmental and physiological factors (Moeser and Carrington 2006). Modulus has been related to the degree of cross-linking or crystallinity in a material (Vaccaro and Waite 2001) which in turn establish the degree of structural order in a fibre. The presence of metals i.e, iron and manganese are necessary for the cross-link of byssal threads proteins (Waite et al. 2002) and changes in the water chemistry might result also in molecular interactions variability in a byssal thread and consequently, in their mechanical behaviour (Sun et al. 2001). The possibility to investigate the way how this may affect the thread quality would be an interesting aspect for future research. The seasonal distribution of metals in Rías Gallegas and, specifically in Ría of Vigo has been associated to the input of rivers, more significant in fall-winter periods, and the specific concentration of metals like Al, Fe and Mn appeared to be associated to these fresh water flows into the Rías (Villares et al. 2002). This abiotic impact, together with the endogenous variability of mussels seasonally, must be on the basis to explain differences encountered for this thread mechanical value (modulus) between outplant and transplant experiments carried out in two different seasons.

Overall, this study reports a highly dynamic value for the byssus secretion in mussels as well as plasticity patterns for the attachment strength associated with abrupt changes in the environment. Animals have the ability to modify byssus size and mechanical properties to ensure attachment strength but this response would depend on the degree of environmental change. Animals can re-allocate more energy for other vital structures like gonadal/soft tissues growth in more benign environments, shifting energy from the production of a protective byssus and shell.

ACKNOWLEDGEMENTS

We would like to thank E. Silva Caride for technical assistance in the field and laboratory and C. Craig for mechanical analysis of the byssus. JMF Babarro also thanks support and help provided by José Luis Garrido. This study was partly funded by "National Science Foundation EF1041213 to E. Carrington. JMF Babarro acknowledges the funding of the projects AGL2006-06986/ACU and AGL2010-16464 (Ministerio de Ciencia e Innovación, Spanish Government).

REFERENCES

Akester RJ, Martel AL (2000) Shell shape, dysodont tooth morphology and hinge-ligament thickness in the bay mussel *Mytilus trossulus* correlate with wave exposure. Can J Zool 78:240-253

Babarro JMF, Fernández-Reiriz MJ, Labarta U (2008) Secretion of byssal threads and attachment strength of *Mytilus galloprovincialis*: the influence of size and food availability. J Mar Biol Ass U.K. 88(4):783-791

Babarro JMF, Fernández Reiriz MJ (2010) Secretion of byssal threads in *Mytilus galloprovincialis*: quantitative and qualitative values after spawning stress. J Comp Physiol B 180:95-104

Barreiro R, Quintela M, Ruiz JM (1999) Aphally and imposex in *Nucella lapillus* from Galicia (NW Spain): incidence, geographical distribution and consequences for the biomonitoring of TBT contamination. Mar Ecol Prog Ser 185:229-238

Beadman HA, Caldow RWG, Kaiser MJ, Willows RI (2003) How to toughen up your mussels: using shell morphological plasticity to reduce predation losses. Mar Biol 142:487-494

Bell EC, Gosline JM (1996) Mechanical design of mussel byssus: material yield enhances attachment strength. J Exp Biol 199:1005-1017

Bell EC, Gosline JM (1997) Strategies for life in flow: tenacity, morphometry, and probability of dislogement of two *Mytilus* species. Mar Ecol Prog Ser 159:197-208

Brazee SL, Carrington E (2006) Interspecific comparison of the mechanical properties of mussel byssus. Biol Bull 211:263-274

623 Brown CH (1952) Some structural proteins of Mytilus edulis L. Quart J Micros Sci 93:487-502
624 Carrington E (2002) Seasonal variation in the attachment strength of blue mussels: causes and
625 consequences. Limnol Oceanogr 47(6):1723-1733
626 Carrington E, Gosline JM (2004) Mechanical design of mussel byssus: load cycle and strain rate
627 dependence. Am Malacol Bull 18:135-142
628 Coyne KJ, Qin XX, Waite JH (1997) Extensible collagen in mussel byssus: a natural block
629 copolymer. Science 277:1830-1832
630 Denny MW (1995) Predicting physical disturbance: mechanistic approaches to the study of
631 survivorship on wave-swept shores. Ecol Monogr 65:371-418
632 Freeman KR (1974) Growth, mortality and seasonal cycle of Mytilus edulis in two Nova Scotian
633 embayments. Department of the environment, Fisheries and Marine Service, Canada, Technical
634 Report No. 500, p. 112
635 Gardner JPA, Thomas MLH (1987) Growth, mortality and production of organic matter by a rocky
636 intertidal population of *Mytilus edulis* in the Quoddy Region of the Bay of Fundy. Mar Ecol Prog
637 Ser 39:31-36
638 Harrington MJ, Waite JH (2007) Holdfast heroics: comparing the molecular and mechanical
639 properties of *Mytilus californianus* byssal threads. J Exp Biol 210:4307-4318
640 Hawkins AJS, Bayne BL (1985) Seasonal variation in the relative utilization of carbon and nitrogen
641 by the mussel *Mytilus edulis*: budgets, conversion efficiencies and maintenance requirements. Mar
642 Ecol Prog Ser 25:181-188
643 Hunt H, Scheibling RE (2001) Predicting wave dislodgement of mussels: variation in attachment
644 strength with body size, habitat, and season. Mar Ecol Prog Ser 213:157-164
645 Kang CK, Sauria PG, Richard P, Blanchard GF (1999) Food sources of the infaunal suspension-
646 feeding bivalve *Cerastoderma edule* in a muddy-sandflat of Marennes-Oléron Bay, as determined
647 by analyses of carbon and nitrogen stable isotopes. Mar Ecol Prog Ser 187:147-158

Krause I, Bockhardt A, Neckermann H, Henle T, Klostermeyer H (1995) Simultaneous determination of amino acids and biogenic amines by reverse-phase high-performance liquid chromatography of the dabsyl derivatives. J Chromatogr A 715:67–79

Lachance AA, Myrand B, Tremblay R, Koutitonsky V, Carrington E (2008) Biotic and abiotic factors influencing attachment strength of blue mussels *Mytilus edulis* in suspended culture. Aquat Biol 2:119-129

Lucas JM, Vaccaro E, Waite JH (2002) A molecular, morphometric and mechanical comparison of the structural elements of byssus from *Mytilus edulis* and *Mytilus galloprovincialis*. J Exp Biol 205:1807-1817

McDowell LM, Burzio LA, Waite JH, Schaefer J (1999) Rotational echo double resonance detection of cross-links formed in mussel byssus under high-flow stress. J Biol Chem 274 (29):20293-20295

Moeser GM, Leba H, Carrington E (2006) Seasonal influence of wave action on thread production in *Mytilus edulis*. J Exp Biol 209:881-890

Moeser GM, Carrington E (2006) Seasonal variation in mussel thread mechanics. J Exp Biol 209:1996-2003

Pinho O, Ferreira IMPLVO, Mendes E, Oliveira BM, Ferreira M (2001) Effect of temperature on evolution of free amino acid and biogenic amine contents during storage of Azeitão cheese. Food Chem 75:287–291

Pujol JP, Rolland M, Lasry S, Vinet S (1970) Comparative study of the amino acid composition of the byssus in some common bivalve molluscs. Comp Biochem Physiol 34:193-201

Pujol JP, Bocquet J, Borel JP (1976) Le byssus de Mytilus: etude electrophoretique de fractions proteiques riches en hydroxyproline extraites de la ´glande du collagene´. C.R. Seances Acad Sci, Paris 283 D:555-558

Qin XX, Waite JH (1998) A potential mediator of collagenous block copolymer gradients in mussel byssal threads. Proc Nat Acad Sci 95:10517-10522

674 Raubenheimer D, Cook P (1990) Effects of exposure to wave action on allocation of resources to
675 shell and meat growth by the subtidal mussel, *Mytilus galloprovincialis*. J Shellfish Res 9(1):87-93
676 Riera P, Richard P (1997) Temporal variation of $\delta^{13}C$ in particulate organic matter and oyster
677 *Crassostrea gigas* in Marennes-Oléron Bay (France): effect of freshwater inflow. Mar Ecol Prog
678 Ser 147:105–115
679 Riera P, Stal LJ, Nieuwenhuize J, Richard P, Blanchard G, Gentil F (1999) Determination of food
680 sources for benthic invertebrates in a salt marsh (Aiguillon Bay, France) by carbon and nitrogen
681 stable isotopes: importance of locally produced sources. Mar Ecol Prog Ser 187:301–307
682 Smeathers JE, Vincent JFV (1979) Mechanical properties of mussel byssus threads. J moll Stud
683 45:219-230
684 Schmitt L, Ludwig M, Gaub HE, Tampe R (2000) A metal-chelating microscopy tip as a new
685 toolbox for a single-molecule experiments by atomic force microscopy. Biophys J 78:3275-3285
686 Steffani CN, Branch GM (2003) Growth rate, condition, and shell shape of *Mytilus*
687 *galloprovincialis*: responses to wave exposure. Mar Ecol Prog Ser 246:197-209
688 Sukhotin AA, Portner HO (1999) Habitat as a factor involved in the physiological response to
689 environmental anaerobiosis of White Sea Mytilus *edulis*. Mar Ecol Prog Ser 184:149-160
690 Sun C, Vaccaro E, Waite JH (2001) Oxidative stress and the mechanical properties of naturally
691 occurring chimeric collagen-containing fibers. Biophys J 81:3590-3595
692 Sun CJ, Waite JH (2005) Mapping chemical gradients within and along fibrous structural tissue,
693 mussel byssal threads. J Biol Chem 280 (47):39332-39336
694 Vaccaro E, Waite JH (2001) Yield and post-yield behaviour of mussel byssal thread: a self-healing
695 biomolecular material. Biomacromolecules 2:906-911
696 Villares R, Puentea X, Carballeira A (2002) Seasonal variation and background levels of heavy
697 metals in two green seaweeds. Environ Pollut 119:79–90

698 Waite JH (1992) The formation of mussel byssus: anatomy of a natural manufacturing process. In:
699 Results and Problems in Cell Differentiation, vol. 19, Biopolymers (ed. S.T. Case), Berlin, Springer-
700 Verlag, pp 27-54
701 Waite JH, Qin XX, Coyne KJ (1998) The peculiar collagens of mussel byssus. Matrix Biol 17:93-
702 106
703 Waite JH, Vaccaro E, Sun C, Lucas JM (2002) Elastomeric gradients: a hedge against stress
704 concentration in marine holdfasts? Philos Trans R Soc Lond B 357:143-153
705 Zardi GI, Nicastro KR, McQuaid CD, Rius M, Porri F (2006) Hydrodynamic stress and habitat
706 partitioning between indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) mussels:
707 constraints of an evolutionary strategy. Mar Biol 150:79-88
708 Zardi GI, McQuaid CD, Nicastro KR (2007) Balancing survival and reproduction: seasonality of
709 wave action, attachment strength and reproductive output in indigenous *Perna perna* and invasive
710 *Mytilus galloprovincialis* mussels. Mar Ecol Progr Ser 334:155-163
711 Zhao H, Waite JH (2006) Proteins in load-bearing junctions: the histidine-rich metal-binding protein
712 of mussel byssus. Biochemistry-US 45:14223-14231
713
714
715
716
717
718
719
720
721
722
723

Figures Legends

Figure 1. Location of the experimental sites in Ría of Vigo (NW Spain). Cabo Estay (CE) and San Simón (SS) indicate the outer exposed and inner sheltered experimental locations of the survey, respectively.

Figure 2. Out planting experimental mussels. Disposition of individuals on slates in the field.

Figure 3. Outplant experiment. Daily maximum values of temperature (A;C), daily maximum and minimum values of salinity (B), current speed (D), mean daily wave height values (E) of the air/seawater and, chlorophyll-a and total particulate matter (F) in the seawater reported for the sampling period at both outer exposed CE and inner sheltered SS sites.

Figure 4. Transplant experiment. Weekly average temperature (A), salinity (B), current speed (C), wave height (D), chlorophyll-a (E) and total particulate matter (F) values of the seawater reported for the sampling period at both outer exposed CE and inner sheltered SS intertidal sites.

Figure 5. Transplant experiment. Shell thickness (A-B) and, gonadal and condition indices (C-D) values of the experimental mussels after their transplantation between exposed CE and sheltered SS intertidal sites.

Figure 6. Transplant experiment. Tenacity values of the whole mussel after their transplantation between exposed CE and sheltered SS intertidal sites.

Figure 7. Transplant experiment. Hydroxyproline plus hydroxylysine (A) and histidine plus lysine (B) residues of the hydrolysed byssal thread portions (proximal and distal) secreted by the experimental mussels after their transplantation between exposed CE and sheltered SS intertidal sites.

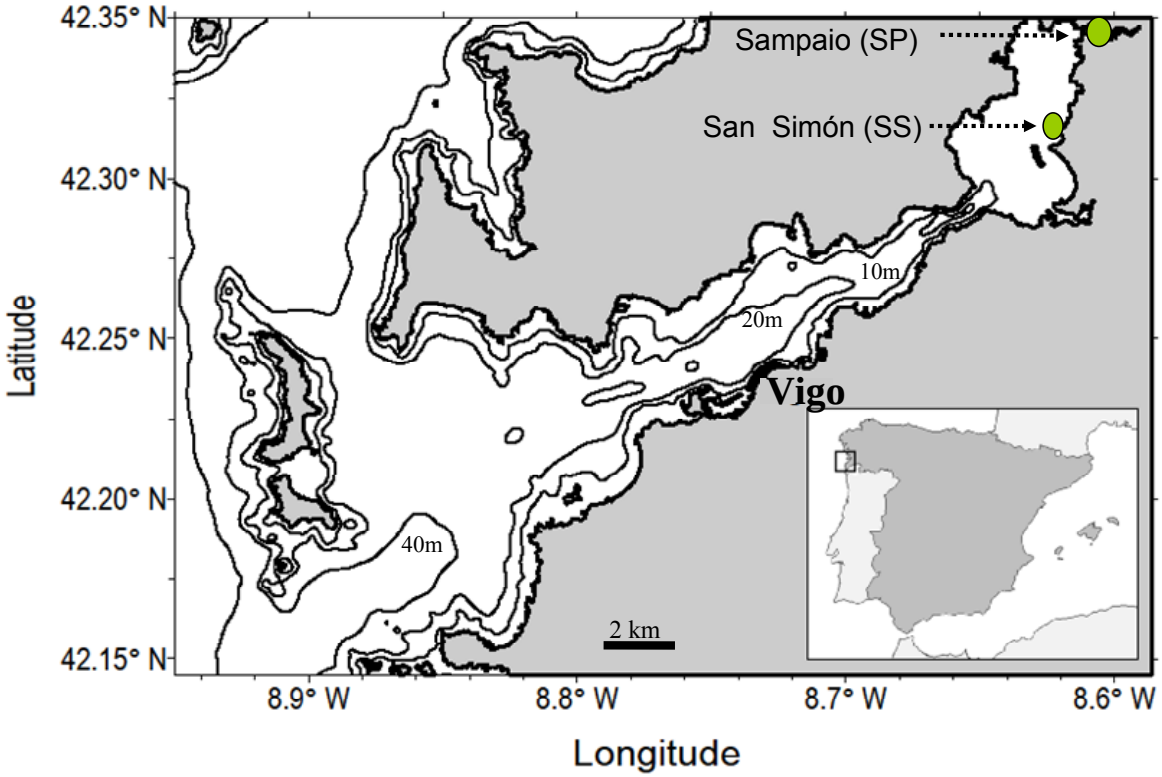
42.35° N
42.30° N
42.25° N
42.20° N
42.15° N
Latitude
8.9° W
8.8° W
8.7° W
8.6° W
Longitude
Sampaio (SP)
San Simón (SS)
10m
20m
40m
Vigo
2 km

Figure 1

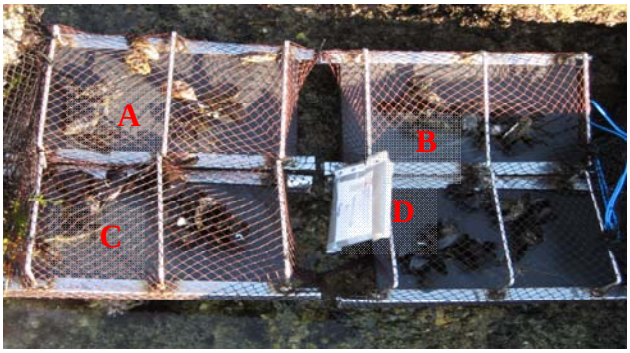

Figure 2

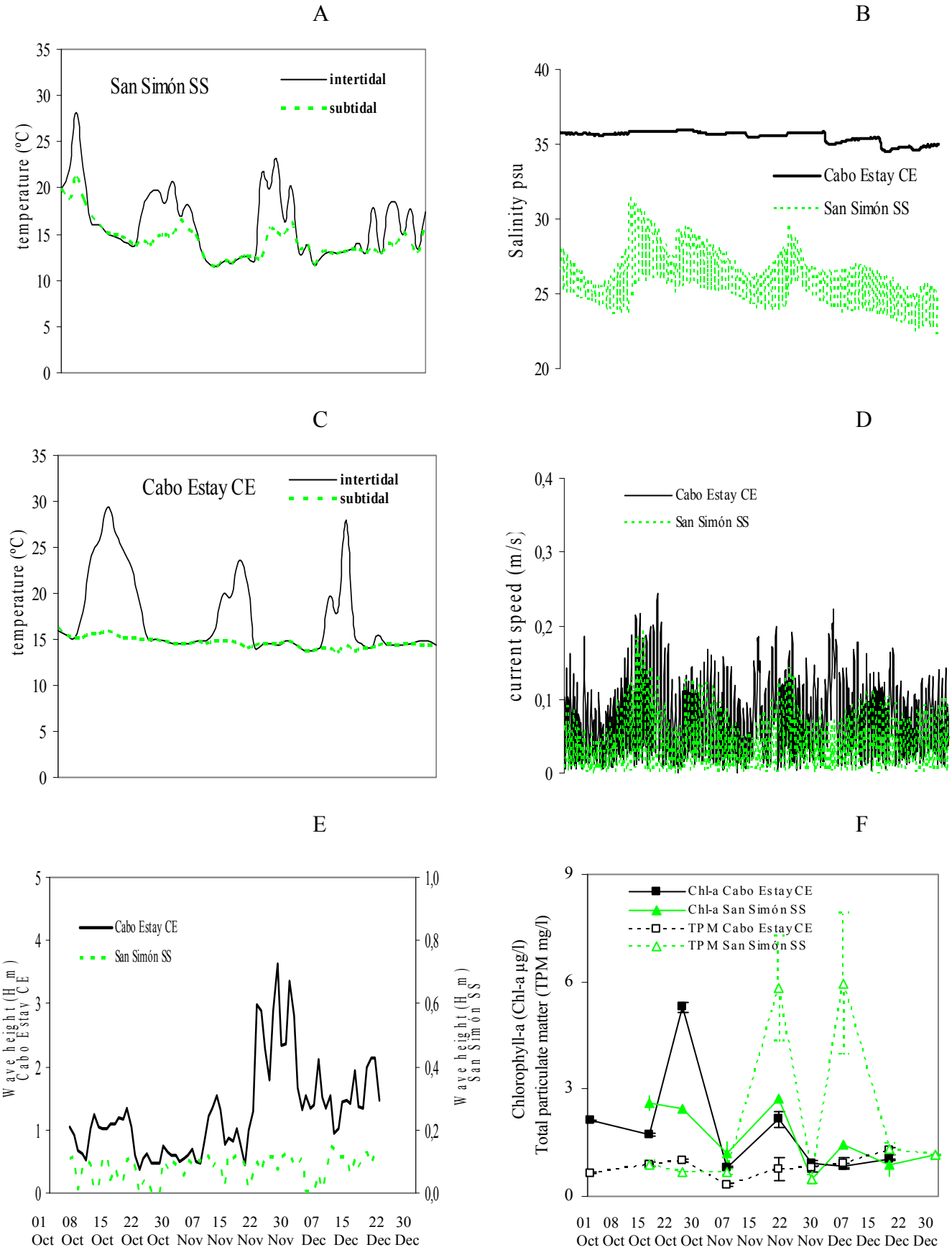
A
B
San Simón SS
intertidal
subtidal
temperature (ºC)
Salinity psu
Cabo Estay CE
San Simón SS
C
D
Cabo Estay CE
intertidal
subtidal
temperature (ºC)
current speed (m/s)
Cabo Estay CE
San Simón SS
E
F
Cabo Estay CE
San Simón SS
Wave height (Hm) Cabo Estay CE
Wave height (Hm) San Simón SS
Chl-a Cabo Estay CE
Chl-a San Simón SS
TPM Cabo Estay CE
TPM San Simón SS
Chlorophyll-a (Chl-a µg/l)
Total particulate matter (TPM mg/l)
01 Oct 08 Oct 15 Oct 22 Oct 30 Oct 07 Nov 15 Nov 22 Nov 30 Nov 07 Dec 15 Dec 22 Dec 30 Dec

Figure 3

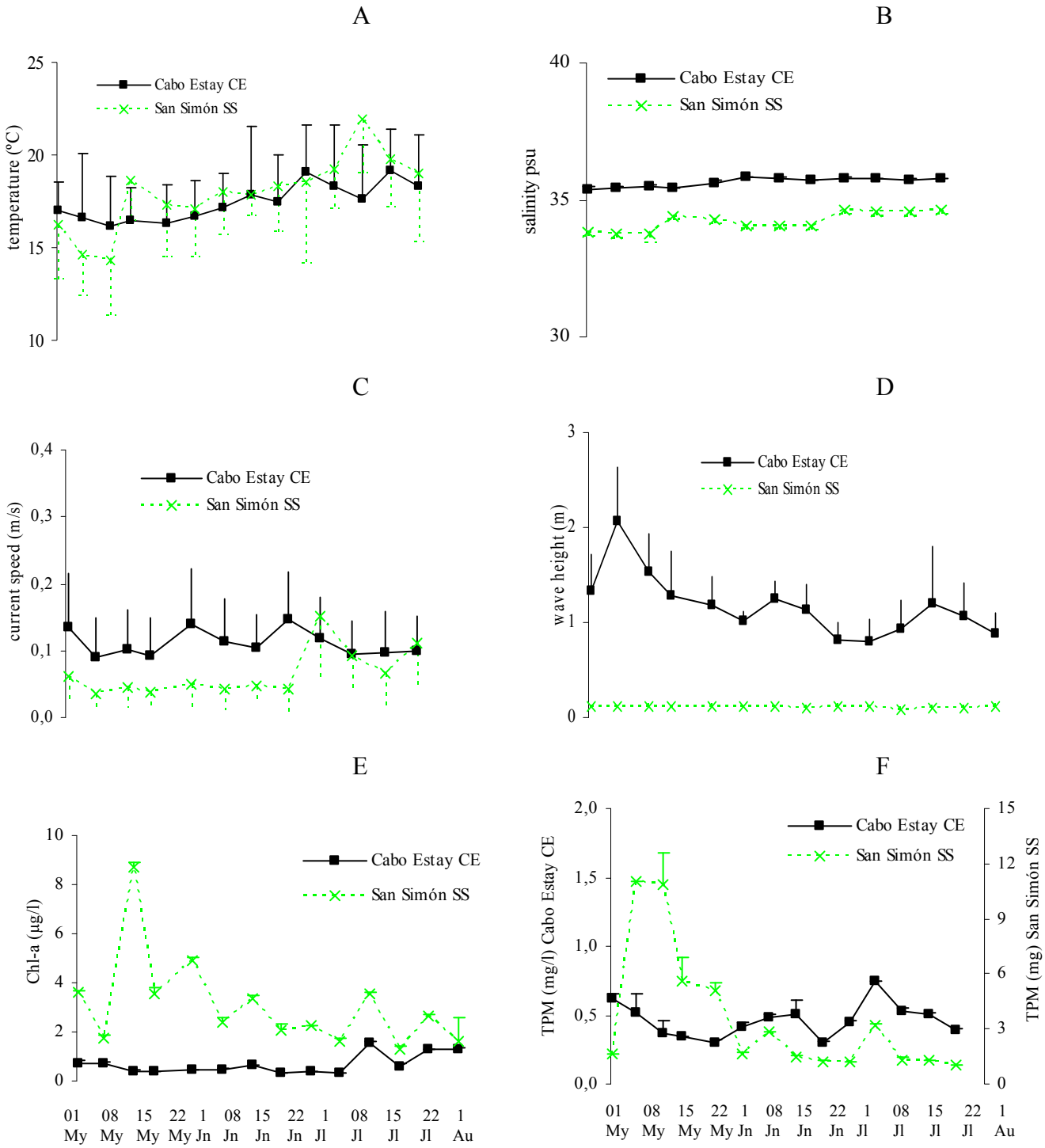
A
B
C
D
E
F
temperature (ºC)
salinity psu
current speed (m/s)
wave height (m)
Chl-a (µg/l)
TPM (mg/l) Cabo Estay CE
TPM (mg) San Simón SS
Cabo Estay CE
San Simón SS
01 My
08 My
15 My
22 My
1 Jn
08 Jn
15 Jn
22 Jn
1 Jl
08 Jl
15 Jl
22 Jl
1 Au

Figure 4

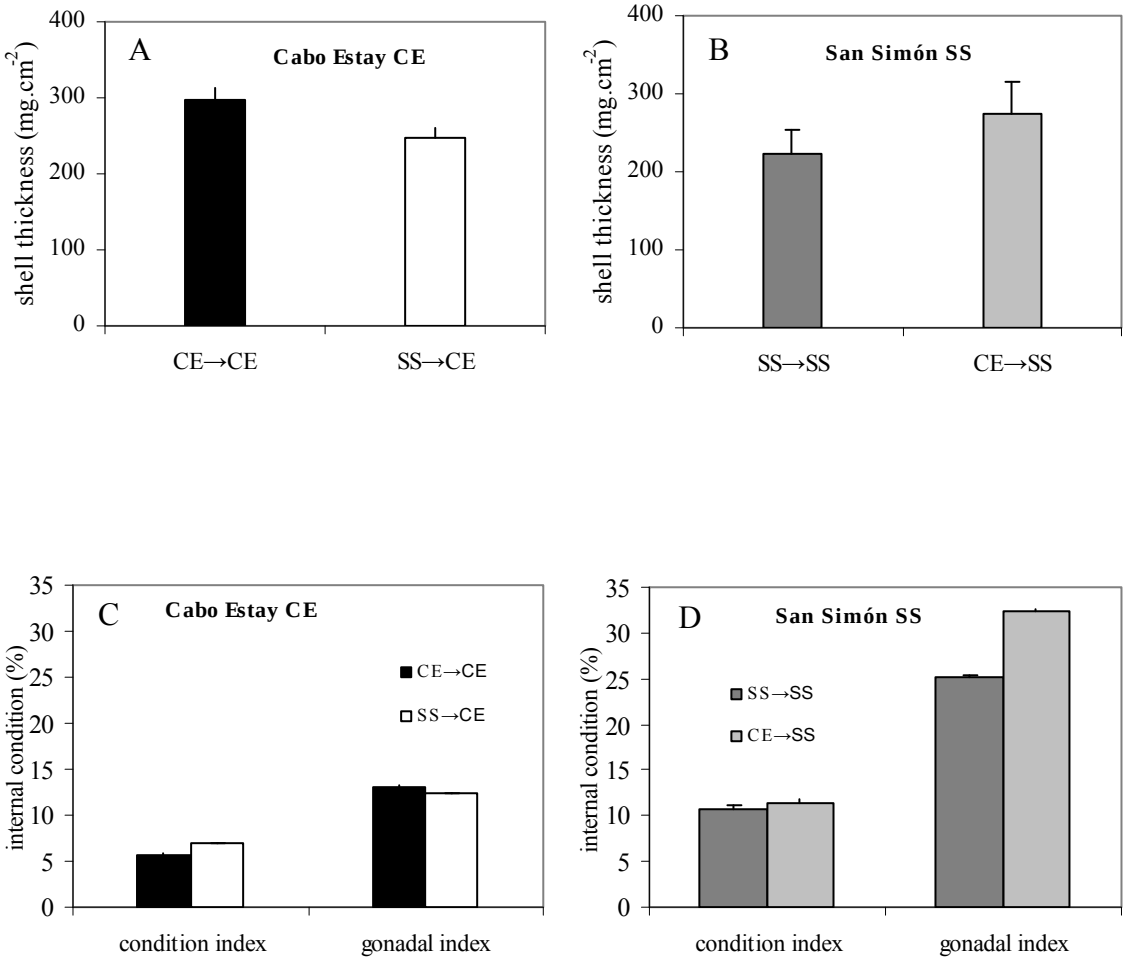
A
Cabo Estay CE
shell thickness (mg.cm-2)
400
300
200
100
0
CE→CE
SS→CE
B
San Simón SS
shell thickness (mg.cm-2)
400
300
200
100
0
SS→SS
CE→SS
C
Cabo Estay CE
internal condition (%)
35
30
25
20
15
10
5
0
CE→CE
SS→CE
condition index
gonadal index
D
San Simón SS
internal condition (%)
35
30
25
20
15
10
5
0
SS→SS
CE→SS
condition index
gonadal index

Figure 5

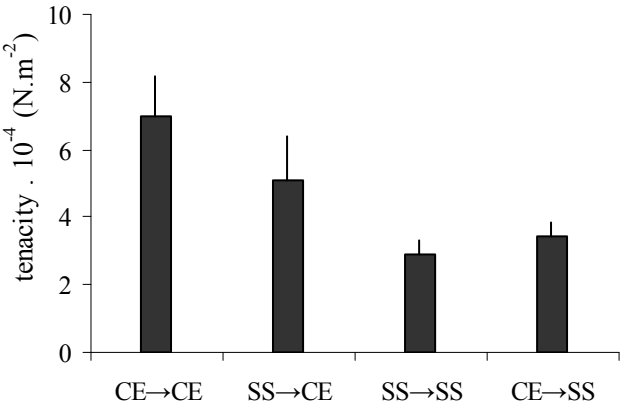

Figure 6

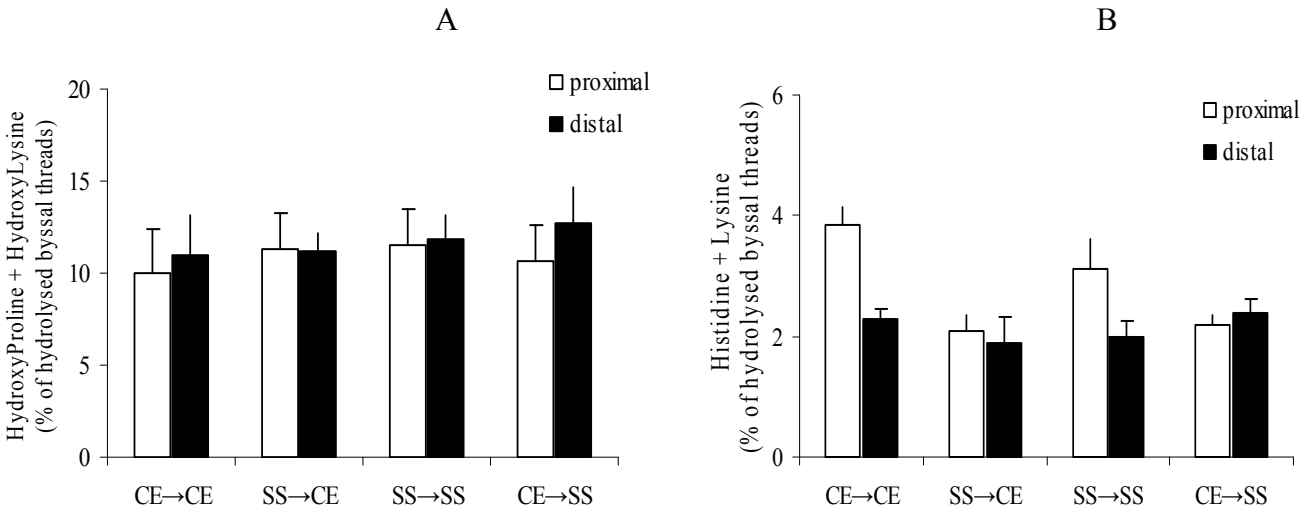
A
B
proximal
distal
HydroxyProline + HydroxyLysine
(% of hydrolysed byssal threads)
Histidine + Lysine
(% of hydrolysed byssal threads)
0
5
10
15
20
2
4
6
CE→CE
SS→CE
SS→SS
CE→SS

Figure 7

974
975
976
977
978
979
980

Table 1. Mussel shape and morphological characteristics of the individuals in the outplant experiment. Mean values ±SD (N=9)

	shell width (cm)	shell height (cm)	shell area (cm^2)	shell thickness ($mg.cm^{-2}$)
Outplant experiment				
exposed CE subtidal	2.31 ±0.17	2.20 ±0.12	5.43 ±0.38	154.66 ±9.63
exposed CE intertidal	2.11 ±0.07	2.26 ±0.15	4.51 ±0.35	145.78 ±11.74
sheltered SS subtidal	1.76 ±0.05	2.82 ±0.15	5.01 ±0.29	118.93 ±22.32
sheltered SS intertidal	1.77 ±0.07	2.74 ±0.08	4.83 ±0.25	125.76 ±6.69

continued

	gonadal index (%)	condition index (%)	Hydroxyproline + Hydroxylysine (% hydrolysed byssal threads) N=3		Histidine + Lysine (% hydrolysed byssal threads) N=3	
			proximal	distal	proximal	distal
Outplant experiment						
exposed CE subtidal	18.95 ±3.12	13.49 ±1.37	8.31 ±0.81	10.20 ±1.01	4.14 ±0.71	2.71 ±0.36
exposed CE intertidal	13.69 ±3.37	12.55 ±0.95	8.01 ±0.56	10.81 ±0.82	5.01 ±0.45	3.15 ±0.32
sheltered SS subtidal	25.17 ±3.22	17.57 ±1.68	8.12 ±0.81	11.10 ±0.88	2.61 ±0.21	1.68 ±0.13
sheltered SS intertidal	25.81 ±3.98	18.45 ±1.49	9.10 ±0.36	12.01 ±1.10	3.01 ±0.12	2.02 ±0.20

Table 2. Mussel tenacity and morphological (thickness and length) values of the byssus secreted by the individuals in the outplant experiment. Mean values ±SD (N=7 for tenacity and thread´s length; N=30-40 for byssus thickness). NS: not significant

	Tenacity x10^{-4} (N.m^{-2})	proximal byssus thickness (µm)	distal byssus thickness (µm)	thread length (mm)
Outplant experiment				
exposed CE subtidal	4.4 ±1.2	165.9 ±12.9	97.5 ±8.3	17.7 ±2.2
exposed CE intertidal	4.5 ±1.5	158.8 ±16.1	106.0 ±6.9	15.8 ±3.1
sheltered SS subtidal	2.9 ±0.6	158.7 ±21.4	89.7 ±6.3	19.0 ±4.6
sheltered SS intertidal	3.6 ±0.7	170.1 ±18.9	95.4 ±8.4	20.3 ±5.6

2-way ANOVA	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P
site	1	0.609	10.360	<0.01	1	0.0007	0.2	NS	1	0.079	10.28	<0.01	1	0.145	3.10	NS
tidal exposure	1	0.074	1.257	NS	1	0.015	4.0	NS	1	0.029	3.83	NS	1	0.007	0.142	NS
site x tidal exposure	1	0.092	1.566	NS	1	0.011	3.1	NS	1	0.002	0.26	NS	1	0.057	1.216	NS
Error	24	0.059			119	0.0036			151	0.008			24	0.047		

Table 3. Mechanical properties of *in situ* - produced byssal threads of *Mytilus galloprovincialis* during the outplant experiment. Mean values ±SD (N=7). NS: not significant

Populations	load (N)	strain (mm/mm)	yield (N)	modulus (MPa)	proximal strength (MPa)	distal strength (MPa)	scaled force (N)
exposed CE							
subtidal	0.650 ±0.192	0.615 ±0.190	0.396 ±0.111	140.07 ±28.34	30.03 ±13.26	87.04 ±25.30	0.618 ±0.241
intertidal	0.643 ±0.201	0.701 ±0.214	0.418 ±0.145	106.01 ±34.29	35.67 ±7.82	72.86 ±24.01	0.524 ±0.153
sheltered SS							
subtidal	0.486 ±0.124	0.801 ±0.150	0.288 ±0.127	86.39 ±18.34	24.54 ±8.37	74.80 ±24.74	0.412 ±0.107
intertidal	0.390 ±0.104	0.664 ±0.173	0.266 ±0.109	87.34 ±19.83	17.14 ±4.48	54.52 ±14.25	0.327 ±0.091

2-way ANOVA	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P
site	1	0.888	5.672	<0.05	1	0.169	0.986	NS	1	1.287	4.612	<0.05	1	0.823	10.553	<0.01	1	1.013	6.475	<0.05	1	0.197	1.258	NS	1	1.379	17.442	<0.01
tidal exposure	1	0.054	0.346	NS	1	0.016	0.096	NS	1	0.001	0.005	NS	1	0.187	2.404	NS	1	0.089	0.57	NS	1	0.380	2.428	NS	1	0.280	3.542	NS
site x tidal exposure	1	0.082	0.521	NS	1	0.219	1.277	NS	1	0.001	0.005	NS	1	0.148	1.900	NS	1	0.344	2.200	NS	1	0.051	0.328	NS	1	0.016	0.201	NS
Error	24	0.156			24	0.172			24	0.279			24	0.078			24	0.156			24	0.156			24	0.079		

Table 4. Transplant experiment. Length, height, width and area values of the shell for the experimental mussels after their transplantation between exposed and sheltered intertidal sites. Mean values ±SD (N=9). Statistics corresponded to T-test (homogeneity of variances) and N-par. (Non parametric: Kolmogorov-Smirnov, Mann and Whitney U tests when variances are not homogeneous). NS: not significant

	shell length (cm)	shell height (cm)	shell width (cm)	shell area (cm^2)
exposed CE				
CE t=0	4.49 ±0.19	2.26 ±0.19	1.88 ±0.13	4.25 ±0.38
CE→CE	4.49 ±0.14	2.30 ±0.15	1.90 ±0.12	4.38 ±0.43
CE→SS	4.92 ±0.25	2.46 ±0.17	2.06 ±0.14	5.05 ±0.41
sheltered SS				
SS t=0	4.51 ±0.07	2.71 ±0.15	1.74 ±0.10	4.71 ±0.33
SS→SS	4.89 ±0.25	2.80 ±0.16	1.93 ±0.12	5.41 ±0.45
SS→CE	4.57 ±0.12	2.74 ±0.16	1.83 ±0.09	5.03 ±0.39
Statistics				
CE t=0 vs. CE→CE	NS	NS	NS	NS
CE t=0 vs. CE→SS	P<0.001 (T-test)	P<0.001 (T-test)	P<0.001 (T-test)	P<0.001 (T-test)
SS t=0 vs. SS→SS	P<0.001 (N-par.)	NS	P<0.001 (T-test)	P<0.001 (N-par.)
SS t=0 vs. SS→CE	NS (N-par.)	NS	P<0.001 (T-test)	P<0.01 (T-test)

Table 5. Changes in mussel tenacity, byssus morphometrics and mechanical properties for the cross-transplant experiment between the inner sheltered (SS) and the outer exposed (CE) sites.
Mean values ±SD (N=35-45 for thread thickness; N=9 for thread length and mechanical properties). NS: not significant

	thread thickness proximal	thread thickness distal	thread length	load	strain	yield	modulus	scaled force
native SS→SS	120.3 ±15.8	69.3 ±6.6	16.8 ±3.0	0.38 ±0.1	0.56 ±0.1	0.19 ±0.1	163.6 ±53.8	0.18 ±0.1
transplanted CE→SS	135.2 ±20.6*	93.4 ±10.1	17.8 ±3.2*	0.67 ±0.2	0.68 ±0.1*	0.33 ±0.1	167.0 ±48.6*	0.40 ±0.1
native CE→CE	123.9 ±25.2	87.7 ±10.0	14.1 ±1.7	0.57 ±0.2	0.70 ±0.1	0.34 ±0.2	122.6 ±26.2	0.37 ±0.2
transplanted SS→CE	134.8 ±23.7*	88.1 ±6.1*	14.7 ±3.1*	0.52 ±0.1*	0.73 ±0.2*	0.25 ±0.1*	127.2 ±35.7*	0.41 ±0.1*

2-way ANOVA	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P
site	1	0.00014	0.005	NS	1	0.023	6.4	<0.05	1	0.327	4.233	<0.05	1	0.006	0.037	NS	1	0.066	0.537	NS	1	0.297	4.115	NS	1	0.525	7.323	<0.05	1	0.302	2.188	NS
origin	1	0.089	3.423	NS	1	0.259	72.1	<0.001	1	0.003	0.042	NS	1	0.754	4.774	<0.05	1	0.378	3.089	NS	1	0.311	4.312	<0.05	1	0.003	0.048	NS	1	2.838	20.557	<0.001
site x origin	1	0.003	0.123	NS	1	0.300	83.4	<0.001	1	0.008	0.103	NS	1	1.153	7.297	<0.01	1	0.009	0.072	NS	1	1.167	16.154	<0.001	1	0.015	0.211	NS	1	1.484	10.749	<0.01
Error	150	0.026			174	0.004			34	0.077			34	0.158			34	0.122			34	0.072			34	0.072			34	0.138		
		symmetry				asymmetry				symmetry				asymmetry				symmetry				asymmetry				symmetry				asymmetry		

* transplanted mussels do not significantly differ from natives

1076