Attachment strength of the mussel *Mytilus galloprovincialis*: effect of habitat and body size

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Abstract

Marine organisms adapt to a wide variety of environments, often altering their morphology and behaviour in response to local habitat. This study addressed the effects of habitat (wave exposure) and body size on the morphology and byssal attachment of mussels within the same estuary. Tenacity of the mussel *Mytilus galloprovincialis* was higher at the exposed site, particularly for the smaller size classes. This was largely due to differences in thread thickness; mussels from the exposed site produced thicker and stronger byssal threads. For a given shell length, exposed mussels also produced thicker and smaller shells and had lower gonadal condition. In laboratory flume experiments, both thread production and mechanical performance (strength and extensibility) decreased with increased flow, suggesting flow alone does not explain tenacity differences between sites. Altogether, these analyses suggest that mussels at exposed sites allocate resources to reducing risk of dislodgment (smaller and thicker shell, stronger byssal threads) instead of growth and reproduction, and these allocation differences between sites are less apparent in larger size classes. The lack

of the largest size class (8 cm) at the exposed site may reflect an upper limit to size imposed by wave induced mortality, where attachment strength does not keep pace with hydrodynamic loading.

Introduction

Environmental characteristics greatly influence aspects of the life histories of marine organisms, such as growth, reproduction or spawning periods (Seed and Suchanek 1992). In the case of estuarine tidal zones, environmental factors like temperature, salinity, aerial exposure and hydrodynamics represent key elements that influence population dynamics. Specifically, disturbances created by wave-generated hydrodynamic forces have a controlling influence in structuring mussel bed communities as mussels become dislodged and new space is created for colonization (Hunt and Scheibling 2001; Carrington et al. 2009). The risk of dislodgment increases with flow speed and mussel size and decreases with mussel tenacity, or attachment strength (Carrington 2002).

Mussels are sessile and gregarious organisms capable of withstanding strong flows as consequence of their ability to secrete an extracellular structure called byssus, a bunch 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 the byssus apparatus has to be replaced continuously because threads decay over time (about 2 to 8 weeks; Carrington 2002; Moeser and Carrington 2006) and byssus production can represent up to 8-15% of the mussel’s total energy expenditure (Hawkins and Bayne 1985). Another important structural feature mussels manufacture is shell; greater shell mass and thickness provides protection from aerial exposure, wave action and predation, and may represent also a high metabolic cost, up to 25-50% of the total energy (Gardner & Thomas 1987).

Different environments may induce morphological changes in mussels, such as shell dimensions (Raubenheimer and Cook 1990; Akester and Martel 2000; Steffani and Branch 2003; Beadman et al. 2003;

Babarro and Carrington 2011), and may also alter energy allocation to other vital structures such as soft tissue growth or byssus secretion. Energy allocation can also shift with body size, as larger animals mature reproductively. While many biotic and abiotic factors are known to influence byssal attachment strength of mussels, body size represents an endogenous parameter that is not often considered explicitly. For example, factors influencing mussel attachment strength, like byssal thread thickness and production (Bell and Gosline 1997; Zardi et al. 2007; Babarro et al. 2008; 2010) may vary also as a function of the individual's size. Moreover, Moeser et al. (2006) reported seasonal variation in attachment strength reflected changes in the mechanical properties of the threads themselves, perhaps due energetic shifts to reproduction.

The mechanical properties of mussel byssus have been quantified in several studies (Smeathers and Vincent 1979; Bell and Gosline 1996; Carrington and Gosline 2004; Brazee and Carrington 2006; Babarro and Carrington 2011 among others). Breaking force can be estimated as the maximum force supported by an individual thread and the breaking strain, or extensibility, is the distance a thread can extend before failure divided by its resting length (Moeser and Carrington 2006). Generally speaking, strength of the entire byssal structure increases for stronger and more extensible threads; higher extensibility allows individual threads to stretch and realign within the byssal complex and recruit more threads to resist an applied load (Bell and Gosline 1996).

Byssal attachment strength generally increases linearly with body size of *Perna perna* and *Mytilus galloprovincialis* (Zardi et al. 2006) and *Mytilus* spp. (*Mytilus trossulus* and *Mytilus edulis*; Kirk et al. 2007; Hunt and Scheibling 2001). This may be due to changes in the mechanical properties of the byssal threads, but may reflect the rate of thread production and decay (Moeser et al. 2006). To date, several studies have reported on the effect of body size on thread secretion (see review of Clarke and McMahon 1996; van Winkle 1970; Lee et al. 1990; Eckroat et al. 1993; Seed and Richardson 1999), with conflicting patterns. Babarro et al. (2008) observed significantly lower rate of byssus thread secretion in large mussels (> 8.5 cm shell length) as compared to juveniles (2.5 cm) in calm water and suggested a metabolic limitation to thread production in larger size classes. One aim of this study is evaluate how byssal thread performance varies with mussel size, the extent to which it depends on metabolic aging, and its implications for the mussels' ability to resist dislodgement.

The mussel species we studied was *Mytilus galloprovincialis*, a widely distributed and commercially important bivalve. Our field locations were selected along the coastline of Rías Gallegas (NW Spain), where individuals may tolerate occasionally abrupt abiotic variability between outer and inner locations of the Ría (Babarro and Carrington 2011). Here, we examine the influence of habitat within the same estuary (Ría de Vigo) on mussel morphometry and byssal attachment strength over the body size range encountered *in situ*. We chose two very different intertidal locations (inner sheltered vs. outer exposed) which supported mussel patches that clearly differed in the upper limit of its size distribution frequency (smaller at the exposed site). We tested the hypotheses that (1) habitat would influence the scaling relationship of attachment strength with mussel size, (2) morphometric differences in the byssus secreted by different size classes would account for differences in attachment, and (3) the quality and quantity of the byssus secreted decreases with mussel size.

Material and Methods

Environment

Field sampling was conducted at two littoral sites of Ría de Vigo (NW Spain) with strong environmental differences. A detailed comparison of the conditions at each site is described in Babarro and Carrington (2011) and is briefly summarized here. Both experimental sites are located near the city of Vigo and are 30 km apart (Figure 1): one site at the outer exposed Ría in Cabo Estay (CE) and the other at the inner sheltered zone in the Ensenada San Simón (SS). The rocky shore at both sites is mainly composed of granitic rocks although a muddy-granitic bottom is more frequent in the sheltered SS site. Mussels, however, are attached only on hard granitic substrate and grow in numerous patches at both sites as free-living monolayer beds at a tidal height of 20% aerial exposure. Solitary individuals were not considered; mussel patches had similar density (~400 ind m^{-2}). Mussels from the interior part of the patches attached to hard rocks were selected for strength measurements and byssus collection. It was assumed mussels in patches experience primary lift because neighbours shield individuals from drag (Denny 1987; Bell and Gosline 1997). Environmental differences between outer exposed and inner sheltered sites include wave exposure, salinity, temperature and littoral vegetation. Mussels living at the exposed site face wave impact directly whereas a bed of vegetation (i.e. *Fucus sp*.) protects those at the sheltered site during aerial exposure. Mussels were sampled in early September 2007.

Attachment strength

Attachment strength was measured as described by Bell and Gosline (1997) and Babarro and Carrington (2011). A mussel was connected to a spring scale (Kern MH, resolution of 0.01N) with a thin monofilament fishing line through a 0.2-cm diameter hole drilled through the shell valves, close to the posterior margin. The spring scale was pulled perpendicular (normal) to the substratum until dislodgement occurred and the peak dislodgment force was recorded. Sample size was approximately 100 mussels per site spanning size classes of mussels ranging of 2 - 8 cm shell length, at 0.5 cm intervals; mussels smaller than 2 cm shell length were not included because collection would damage their byssus structure. Following Carrington et al. (2009), attachment strength was divided by mussel size (planform area) to obtain tenacity in N m^{-2}.

Morphometrics: animals and byssus

After dislodgment, individual shell dimensions were measured along the antero-posterior (shell length), dorso-ventral (shell height) and lateral axis (shell width) to the nearest millimeter with vernier callipers. Shell planform area was approximated as an ellipse with shell height and width as major and minor axes, respectively (Bell and Gosline 1997). Image analysis (IA) was performed for shell area using the software

QWin (© Leica Imaging Systems) on a PC (AMD Athlon XP 3000+) connected to a video camera (Leica IC A) on a stereo microscope (Leica MZ6). Camera and light settings were established at the beginning of the analysis and kept constant throughout the whole analysis. Shell thickness was estimated as shell mass *versus* surface area ratio (Beadman et al. 2003).

Byssal threads were collected from mussels adjacent to those used for dislodgement measurements. Thread thickness secreted by the mussels *in situ* was measured by Image Analysis (IA), performed on 20-30 threads per size class of individuals (2 - 8 cm shell length, at 1 cm intervals). Here, thread thickness is the diameter of the major axis of the distal region (Bell and Gosline 1997).

Gonadal index

Gonadal index of mussels used for tenacity measurements was the proportion of mussel biomass composed of mantle tissue (site of gametogenesis in *Mytilus*; Carrington 2002; Babarro and Carrington 2011). Wet mantle was dissected from the wet body and together with the rest of organs were freeze-dried for 48 hours. Samples 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).

Byssus secretion in the laboratory: the effect of flow speed and body size

To explore possible causes for the observed patterns in tenacity between sites (see Results), we conducted a laboratory study to investigate how byssus production and strength varies with flow speed for different mussel size classes (4, 6 and 8 cm shell length). Mussels were carefully collected from a raft culture in the Ría de Vigo and transported to the laboratory and maintained in an open flow system following Babarro and Fernández Reiriz (2010). Briefly, an input flow was distributed into the series of four 19-litre experimental tanks at 0.10 cm s^{-1}. The tanks were of open flow design using filtered (10 μm) seawater (Cartridge CUNO Super Micro-Wynd 10 μm) with controlled salinity and temperature values of 35.5‰ and 15ºC, respectively. The filtered seawater was supplemented with a mixture of microalgae (Tahitian *Isochrysis* aff. *galbana*, T-

ISO) and sediment from the seafloor below the rafts (40:60 microalgae:sediment, by weight) supplied with a peristaltic pump at constant flow, so that particulate material load was maintained at 1.0 mg l^{-1} with an organic content of 50%, simulating the mean values of food availability for the animals in their natural environment of Galician Rías (Babarro et al. 2000).

Animals of different size were exposed to several water velocities for 24-h in a custom flume. The flume volume was 1720 L (from above: 320 cm length x 60 cm width × 40 cm water depth). The rectangular working section was 80 cm x 60 cm x 40 cm (L x W x H). The water flowed through a system of collimators (PVC pipes 2 cm opening diameter × 100 cm length), positioned at 40 cm from the inlet and 40 cm upstream of the working section, removing large-scale turbulence. Flow in the chamber was generated by a variable speed axial flow pump and was measured to the nearest cm s^{-1} using a flow meter (2D-ACM Falmouth Scientific, Inc. Cataumet, MA 02534 USA). The flume used filtered seawater (Cartridge CUNO Super Micro-Wynd 10 μm) with controlled salinity and temperature values of 35.5‰ and 15ºC, respectively. Phytoplankton and sediment were pulsed in daily, as in the maintenance tanks. Care was taken to ensure that the microalgae added as food for the mytilids were well mixed in the chamber and that the chamber was operating at the average tested velocity. In the working section of the flume, animals were fixed to vertical posts using 5 minute epoxy (Imedio S.A. Madrid, Spain) and suspended 0.6 cm above a slate tile platform with the posterior end facing upstream, as shown by Carrington et al. (2008). Two platforms were used for each trial, covering the flume tank width. Mussels were mounted near the anterior portion of the post to reducing flow obstruction and were separated by one shell length.

Twelve mussels from each size class were exposed to a range of unidirectional water velocities, from 3 to 52 cm s^{-1}. Velocity in the vicinity of the experimental mussels was measured for each experimental trial. Thread production was monitored for 7 velocities for each animal size class and the order of these velocities was randomized among trials. In order to avoid continuous exposure of the same animals to consecutive flows that could weaken their condition, animals used for one trial were returned to the maintenance system and new set of animals were used for the following one. The seawater of the flume was aerated, maintained at 15

±1ºC and renovated every two days. After 24-h trial, threads produced by each mussel were counted and carefully cut from the stem for morphometric analysis of the whole byssus.

The tensile properties of byssal threads secreted by different size class mussels in each flow trial were tested according to Bell and Gosline (1996), using an Instron-5565 tensometer. Maximum load (N), strain at maximum load, initial modulus (MPa), yield force (N) and scaled (by thread thickness) force to break (N) were measured for whole threads. All mechanical tests were conducted in seawater at 15 ±1ºC and an extension rate of 1 cm min^{-1}.

Statistical analyses

Analysis of slopes and intercepts of the linear relationships between shell parameters (and gonadal index) against shell length of individuals of both mussel populations were performed following Zar (1999). Shell area data were log transformed before analyses. Least squares regression equations describing the dependence of mussel tenacity, shell morphometrics and distal byssus thickness on mussel size were used to estimate relative differences in scaling relationships between the two experimental populations. Mussel attachment force (in newtons) was plotted against the square of the byssus thickness values.

Two-way ANOVA was used to test for the effects of mussel size and flow speed on production rate and mechanical properties of byssus secreted in the laboratory flume. Two-way ANOVA was also used to estimate the effects of experimental location and mussel size on the byssus thickness secreted by the individuals in the field. Independency of the cases was assumed and normality was checked with Shapiro-Wilk tests. Homoscedasticity was established using Levene’s test and homogenous groups among experimental mussels could be established *a posteriori* using Tukey and Fisher tests. When variances were not homogenous, non-parametric tests Kruskal-Wallis and Mann-Whitney were used. All analyses were performed using STATISTICA 6.0 (Statsoft Inc. USA).

Results

Attachment strength

Mussel tenacity as a function of individual size and habitat is illustrated in Figure 2A. Tenacity varied regardless of the mussel size ($p>0.05$) in the sheltered population with mean values of 5.8 ±1.8 N m^{-2} x 10^{-4} (Figure 2A). However, a decrease in tenacity was reported with increased size in the exposed mussel population (Figure 2A). Consequently, magnitude of differences in mussel tenacity between sites decreased with body size of mussels, from 59% stronger tenacity in small size classes (2-3.5 cm shell length) for the exposed population to 33% for larger size classes (4-6 cm shell length; Figure 2A).

A significant inverse relationship was obtained for the mussel tenacity and gonadal index when all samples are combined (Figure 2B). Mussel from the exposed population tended to have higher tenacity and lower gonad condition.

Mussel morphometry and gonadal index

Mussel morphometry measurements for different size classes are presented in Figure 3A-D. Mussels at the exposed site were more cylindrical, with lower ($p<0.001$; Table 1) but wider shells (for mussels > 3 cm shell length, $p<0.001$; Table 1) as compared to the sheltered population (Figure 3A-B). Differences in shell height between populations were independent of mussel size as indicated by the similar slope value (20% lower shells for the exposed population as mean value; Figure 3A; Table 1) but differences in shell width increased with size of individuals up to 9% wider shells for larger size classes of the exposed population (4-6 cm shell length; Figure 3B) according to significantly higher slope value (Table 1). Projected area of the shell increased with size of individuals but distinctly depending on mussel population as reported by the different slope value of the linear relationships (Table 1; Figure 3C). Consequently, differences between populations in shell area of individuals decreased with mussel size from 25% smaller shells for 2-3.5 cm shell length size classes of the exposed mussels to 13% smaller shells for 4-6 cm shell length size classes (Figure 3C). Shell thickness increased linearly with size and was significantly higher in the exposed population over the entire size range (Figure 3D) ($p<0.001$ for the intercept analysis; Table 1).

Gonadal index increased linearly with mussel size in both populations; values were approximately 2-fold higher in the sheltered site ($p<0.001$ for the intercept analysis; Table 1) compared to the exposed population (Figure 3E).

Byssus (field): thickness

Thickness of the byssus secreted in the field by different mussel sizes is presented in Figure 4A. Mussel size and habitat were significantly correlated with the distal thread diameter secreted by the mussels. Byssus distal thickness increased with mussel size in both exposed and sheltered populations ($p<0.001$; Kruskal-Wallis test; Figure 4A). The effect of site was also significant for the whole mussel size range analysed ($p<0.001$; Mann-Whitney test; Figure 4A) with distal sections of the byssus 28% (2-3 cm shell length) and 14% (4-6 cm shell length) thicker in the exposed mussels as compared to sheltered population (Figure 4A). A significant relationship between byssus distal diameter (as transformed values to the square of thickness) and attachment force (in Newtons) was obtained for the two mussel populations with equal pattern (Figure 4B).

Byssus (laboratory): amount of threads, thickness and mechanical properties

The amount of byssus secreted by different mussel size groups maintained in the laboratory decreased with flow speed ($p<0.001$; Table 2A; Figure 5A). However, the latter decrease in byssus production was not equal for each mussel size, as shown by the interaction term (size x flow; $p<0.05$; Table 2A). Large mussels (8 cm shell length) secreted fewer byssal threads as compared to smaller size classes (6 and 4 cm shell length) with a steady value of 14 ±2 threads within the velocity range of 3-36 cm s^{-1} and a drop in byssus secretion at higher flow speeds (Figure 5A). On the contrary, a continuous decrease in byssus secretion with increased flow speed was observed for 4 cm and 6 cm shell length size class animals ($p<0.001$; Figure 5A). For these smaller two size classes, thread production ranged 20-25 threads secreted in calm waters (3 cm s^{-1}) and decreased to 6-8 threads at the highest flow speed tested (52 cm s^{-1}, Figure 5A).

Mechanical properties of the byssus secreted by different mussel size groups exposed to a range of flow speeds in the laboratory are reported in Tables 3-4 and Figure 5B. Mussel size strongly affected all tensile properties of byssal threads ($p<0.001$) and for the specific case of scaled force to break such effect was also dependent on flow regime (see interaction terms in Table 2B). Scaled force values were highest for the largest mussel size (8 cm shell length) facing calm waters (1.24 N) although a significant drop was observed with flow speed increased (Figure 5B). Scaled force of the byssus secreted by the other mussel size classes did not vary significantly with regard to flow speed ($p>0.05$) and represented mean values of 0.57 ±0.07 (range: 0.48-0.65 N) and 0.27 ±0.05 (range: 0.23-0.33 N) for 6 cm and 4 cm shell length classes, respectively (Figure 5B).

Maximum load and strain values of the byssus varied according to mussel size and flow speed (Table 3). Both load and strain values of the byssus increased with size of individuals and were highest for the largest mussels facing calm seawater (Table 3). In contrast, an increase in the flow speed caused a significant drop in both mechanical properties (Table 3).

Distal yield and modulus of the byssus secreted in the laboratory varied regardless of flow speed but as a positive (yield) and negative (modulus) function of mussel size ($p<0.001$; Table 4). Distal byssus thickness increased with size of individuals ($p<0.001$; Kruskal-Wallis test; Table 4) but varied regardless of flow speed tested in the laboratory ($p=0.050$; Kruskal-Wallis test).

Overall, we note that mussel size had a larger impact on thread mechanics than flow speed. There was an increase of load, strain and yield of the byssus with mussel size whereas stiffness dropped significantly. Flow speed, however, caused a decrease in maximum load and extensibility values as well as scaled force to break for the specific case of large mussels.

Discussion

Shape of individuals was clearly modified by habitat within the same estuary. Mussels living at the rougher exposed site produced lower and wider shells (Figure 3A-B), perhaps due to strong differences in the hydrodynamic forcing between the experimental sites (see Babarro and Carrington 2011). For a given water velocity, reduced mussel shell area would cause a minor hydrodynamic force acting on the mussel (Denny 1995; Zardi et al. 2006). By modifying their shape, mussels living at the exposed site would offer better resistance to wave dislodgement (Price 1980; 1982; Bell and Gosline 1997; Hunt and Scheibling 2001; Carrington 2002; Steffani & Branch 2003; Babarro and Carrington 2011).

Mussels from the exposed population allocated relatively more energy to protective tissues (byssal attachment and shell thickness; Figures 2A and 3D) and less energy to soft tissue growth (i.e. gonadal index; Figure 3E). Similar trade-off patterns were previously reported by Raubenheimer and Cook (1990), Carrington (2002) and Moeser and Carrington (2006). Shell thickness was significantly higher for the exposed population (Figure 3D), which would promote the ability to withstand the destructive, erosive effects of wave action. However, the influence of other factors, like predation and age, may also influence shell thickness. First, we can note that distribution of the gastropod *Nucella lapillus*, one of the greatest predators on littoral mussel populations in Ría de Vigo, is similar between exposed and sheltered sites (Barreiro et al. 1999). Second, although age can affect inter-population variation in shell morphology (Raubenheimer and Cook 1990), shell thickness differences in the present survey were reported for the whole size range analysed (Figure 3D) and are most likely associated to differences in wave-action stress because both intertidal mussel seed populations are subjected to similar aerial exposure (see Materials and Methods) and would come from the same early summer spawning season. The significant negative relationship between mussel tenacity and gonadal index reported here for the exposed population (Figure 2B) suggests these mussels cannot afford to investing energy simultaneously to both byssus and reproductive tissues; natural resources available in the sheltered site, along with a calmer water motion, would have allowed these animals to channel energy to attachment strength and gametogenesis with no restrictions. Our results agree with the study of Zardi et al. (2007) that highlighted a negative relationship between mussel attachment and gonadosomatic index despite the latter authors also indicated that such a link could be coincidental and have no biological meaning. The strong relationship found in our survey between attachment strength and gonadal index might be a consequence of considering juveniles (not sexually mature) and adults in the same analysis which might have masked the competing strategies between byssus secretion and reproduction.

Tenacity of mussels living at the exposed site was significantly higher than the sheltered site, particularly for the smaller size classes (Figure 2A). Moreover, tenacity of the exposed individuals dropped significantly with mussel size whereas values for the sheltered population kept a rather constant pattern (Figure 2A). The ability of mussels to adjust the secretion rate of byssal threads represents a key parameter for explaining attachment strength variability. The counting of byssus filaments *in situ*, however, is difficult because of the interconnection of byssus among tightly clustered individuals. Theoretically, one might expect that higher attachment strength of the exposed mussels would be consequence of higher thread secretion. Indeed, Seed and Suchanek (1992) suggested that "*Mytilus* detects and responds to movement by wave energy …by the production of increased numbers of byssal threads". However, such hypothesis was not confirmed in the present survey. Byssus secretion per individual declined with increased flow speed in the flume (Figure 5A), indicating flow inhibited rather than stimulated thread secretion (see also Moeser et al. 2006 and Carrington et al. 2008). Carrington and co-workers suggested that flow would impose physical limitation for the foot organ to be extended properly beyond the margin of the shell long enough to mold and attach a new thread.

Increased byssal thread thickness is another way of increasing tenacity, and is often quantified in the distal section (Figure 4A-B; Bell and Gosline 2007). Variation in distal byssus thickness was previously reported in *M. galloprovincialis*, either for mussels of different size and condition kept in laboratory (Babarro et al. 2008; Babarro and Fernández Reiriz 2010) or linked to different field sites (Babarro and Carrington 2011). In this study, mussel attachment force increased with byssus thickness (Figure 4B), although this does not entirely account for differences in mussel tenacity between sites especially for smaller size classes (Figure 2A, 4A). This gap might be filled with other factors like mechanical properties of the byssus which would allow mussels to secrete stronger and stiffer threads in wave exposed sites (Babarro and Carrington 2011). This idea is extended in Figure 6. Differences in tenacity and distal byssus thickness between exposed and sheltered mussels were evident for lower size classes but not for large size classes. For a given size class, differences in shell area also tended to disappear in large mussels (Figure 6). Consequently, tenacity differences between populations were high enough to compensate the increase in shell projected area of growing individuals although for a given mussel size > 6 cm shell length, we can hypothesize that differences between mussel populations would be narrower (Figure 6). This would mean that the exposed site would be a restricted environment for larger size mussels and might represent the basis to explain their absence in the field.

The amount of byssus secreted dropped with mussel size and flow speed in the laboratory flume experiments (Figure 5A). This result, along with the mechanical properties of the byssus (Tables 3-4; Figure 5B) allowed us to evaluate both size and flow speed as key parameters for explaining relatively weaker attachment of larger animals facing rougher conditions. Large mussels (8 cm shell length) generally secreted fewer, but mechanically superior byssal threads. However, high flow decreased thread mechanical performance (lower extensibility and scaled force to break values), which would make these animals weaker in high energy environments (Table 3; Figure 5B). Moeser et al. (2006) highlighted that seasonal variability in attachment strength based on thread secretion may not match always changes in wave action, suggesting that other factors like thread decay and material properties of filaments would play a role. We can assume that wave action in nature may be even far more important than flow for byssus formation and consequently, it is possible to hypothesize that field exposed site may limit the maximum size of mussels by constraining their ability to produce a byssus strong enough to resist dislodgment.

Wave action has been suggested to be the strongest predictor of byssal attachment strength of bivalves (Hunt and Scheibling 2001; Lachance et al. 2008) and represents a qualitative term that refers to small-scale turbulence superimposed on a directional current exposing mussels to different potential stimuli for byssus secretion (e.g. mean flow, acceleration and hydrodynamic loading of the byssal retractor muscle; see Moeser et al. 2006). From these stimuli, flow has been reported to be the primary cue for increased thread production in *M. edulis* (Van Winkle, 1970 and Lee et al., 1990 among others) and therefore, it was considered in the present survey as valid hydrodynamic indicator of high energy environment assuming most likely differences between mean flow tested in the laboratory and wave action in nature. Differences in the wave activity between exposed and sheltered populations within Ría de Vigo (Babarro and Carrington 2011) are likely related to mussel tenacity differences documented here. Nevertheless, it is plausible to hypothesize that wave action itself, in our environment, would be not sufficient to explain the absence of larger mussel sizes (> 6 cm shell length) at the rougher sites (see Material and Methods). According to equations that relate predicted scaled hydrodynamic forces as a function of water velocity (see Figure 6 in Zardi et al. 2006), *M. galloprovincialis* in our survey should have experienced seawater flows of 13-15 m s^{-1} to rupture the strength value generated in the field which is actually very unlikely to occur in Ría de Vigo.

Here, we report large animals are more vulnerable to wave action as consequence of lower byssus quantity and quality secreted in high flow environments. Larger size mussels under high flow produced weaker and less extensible byssus, key properties for enhancing attachment strength of mussels in nature (Bell and Gosline 1996). Our results illustrate the importance of environmental factors within an embayment that modifies mussel morphology through shifts in energy allocation between protective (byssus, shell parameters) and soft tissues. Distal byssus thickness represents a key value to explain attachment strength differences in the habitat and mussel size comparisons.

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Table 1. Regressions of height, width, projected area and thickness values of the shell as well as gonadal index against shell length of individuals.

Linear regressions Y = a + b X (values of shell projected area were log transformed to obtain linear functions)

		a = intercept (SD)	b =slope (SD)	n	r
shell height	exposed	0.585 (0.151)	0.366 (0.036)	9	0.918
	sheltered	0.767 (0.184)	0.451 (0.036)	12	0.969
		t = 5.781; df = 18; P<0.001	t = 1.444; df = 17; ns		
			common slope: 0.427		
shell width	exposed	-0.131 (0.073)	0.451 (0.017)	9	0.995
	sheltered	0.256 (0.078)	0.327 (0.015)	12	0.989
			t = 4.843; df = 17; P<0.001		
shell projected area	exposed	-1.493 (0.080)	1.784 (0.058)	9	0.989
	sheltered	-1.013 (0.105)	1.563 (0.068)	12	0.978
			t = 2.233; df = 17; P<0.05		
shell thickness	exposed	50.486 (19.781)	47.535 (4.706)	9	0.967
	sheltered	-1.462 (4.308)	42.449 (7.872)	12	0.863
		t = 4.231; df = 18; P<0.001	t = 0.427; df = 17; ns		
			common slope: 43.884		
gonadal index	exposed	-2.562 (0.4.394)	3.544 (1.045)	9	0.788
	sheltered	7.152 (2.930)	2.486 (0.572)	12	0.808
		t = 3.086; df = 18; P<0.001	t = 0.924; df = 17; ns		
			common slope: 2.785		

The standard deviation on the slopes and intercepts are given between parentheses and the r^2 estimates the proportion of the total variation explained by the regression model. ns, not significant.

In case slopes are different, analysis of intercepts was not performed (see Zar, 1999)

Table 2. Two-way ANOVA of byssal thread secretion by *M. galloprovincialis* as a function of mussel size (fixed: 4, 6 and 8-cm shell length) and flow (fixed: 3-52 cm s^{-1}). ns: not significant . A) Number of threads secreted (see Fig 5A). B) Scaled force to break (see Fig 5B)

A

Factor	DF	MS	F	P
size	2	0.246	1.048	ns
flow	6	1.479	6.312	<0.001
size x flow	12	0.464	1.982	<0.05
Error	202	0.234		

B

Factor	DF	MS	F	P
size	2	19.157	140.087	<0.001
flow	6	0.277	2.025	ns
size x flow	12	0.455	3.330	<0.001
Error	165	0.137		

Table 3. Ultimate mechanical properties of laboratory produced byssal threads of *Mytilus galloprovincialis* as a function of size (SL = shell length) and flow speed. Values are means ± SD (N=10-14).
Results of a two-way ANOVA of the data are presented below. ns: not significant

	load (N)			strain (mm/mm)		
SL (cm)	4	6	8	4	6	8
Flow (cm s^{-1})						
3	0.36 ±0.13	0.78 ±0.13	1.15 ±0.36	1.13 ±0.57	1.91 ±0.44	2.69 ±0.71
8	0.49 ±0.26	0.61 ±0.21	1.12 ±0.34	1.02 ±0.32	1.17 ±0.37	2.13 ±0.59
18	0.29 ±0.09	0.54 ±0.16	0.96 ±0.22	1.05 ±0.40	1.49 ±0.63	1.66 ±0.53
24	0.36 ±0.21	0.51 ±0.12	0.94 ±0.37	0.96 ±0.55	1.16 ±0.35	1.84 ±0.68
36	0.30 ±0.08	0.56 ±0.24	0.94 ±0.41	1.00 ±0.32	1.40 ±0.48	1.51 ±0.65
47	0.44 ±0.10	0.58 ±0.16	0.91 ±0.25	0.71 ±0.34	1.41 ±0.48	1.62 ±0.67
52	0.35 ±0.12	0.55 ±0.21	0.84 ±0.24	0.93 ±0.42	1.57 ±0.36	1.73 ±0.70

ANOVA	DF	MS	F	P	DF	MS	F	P
size	2	16.398	139.741	<0.001	2	7.867	41.898	<0.001
flow	6	0.373	3.179	<0.01	6	0.650	3.463	<0.01
size x flow	12	0.120	1.024	ns	12	0.220	1.171	ns
Error	192	0.117			188	0.188		

Table 4. Selected mechanical (yield, modulus) and morphological (distal thickness) properties of laboratory produced byssal threads of *Mytilus galloprovincialis* as a function of size (SL=shell length). Values are means ± SD (N=10-14 and 35-45 for mechanical and morphological values, respectively). No significant effect of flow speed was observed.

SL (cm)	4	6	8
yield (N)	0.23 ± 0.03	0.37 ± 0.06	0.54 ± 0.04
modulus (MPa)	138.24 ± 23.19	78.28 ± 21.86	62.11 ± 15.19
distal thickness (µm)	66.21 ± 8.47	109.51 ± 9.08	141.50 ± 8.66

Figure Legends

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

Figure 2. A) Tenacity of field collected mussels as a function of size (shell length) and habitat. Symbols are means ± SD (N=3-5 for each mussel size class). Lines are linear regressions (continuous line CE: $y = -2.169x + 18.61$; $r^2 = 0.76$; $P<0.001$ and dashed line SS: $y = -0.044x + 6.38$; $r^2 = 0.002$; ns). B) Mussel tenacity decreases exponentially with gonadal index when the two mussel populations are pooled according to the exponential function: $y = 39.26\ x^{-0.647}$; $r^2 = 0.56$; $P<0.05$.

Figure 3. Mophological relationships, scaled to shell length, of mussels collected from the two field sites. A-D) shell height, shell width, shell area and shell thickness. E) gonadal index of mussels as a function of shell length and field site. Symbols are means ± SD (N=3-5 for each mussel size class). Lines are linear regressions (see legend of Figure 2 A for explanation). Slope and intercept values of these linear relationships are presented in Table 1.

Figure 4. Scaling relationships of field-produced byssal threads from the two sites. A) Thread thickness (measured in the distal section) increases with shell length and trend is elevated in the exposed site. B) Relationship between attachment strength of the mussels and thread diameter of the distal portions (as square values of thread thickness) considering both mussel populations.

Figure 5. Summary of laboratory produced byssal threads as a function of mussel size and flow speed. Symbols are means ± SD (N=10-14). A) Number of threads produced in 24 hours. B) Scaled force to break byssal threads.

Figure 6. A comparison of key biomechanical and morphological scaling relationships between the two field sites. Small mussels from the exposed site have relatively stronger tenacity, thicker byssal threads and smaller shell area (exposed relative to sheltered). The relative differences between sites decrease with increasing shell length.

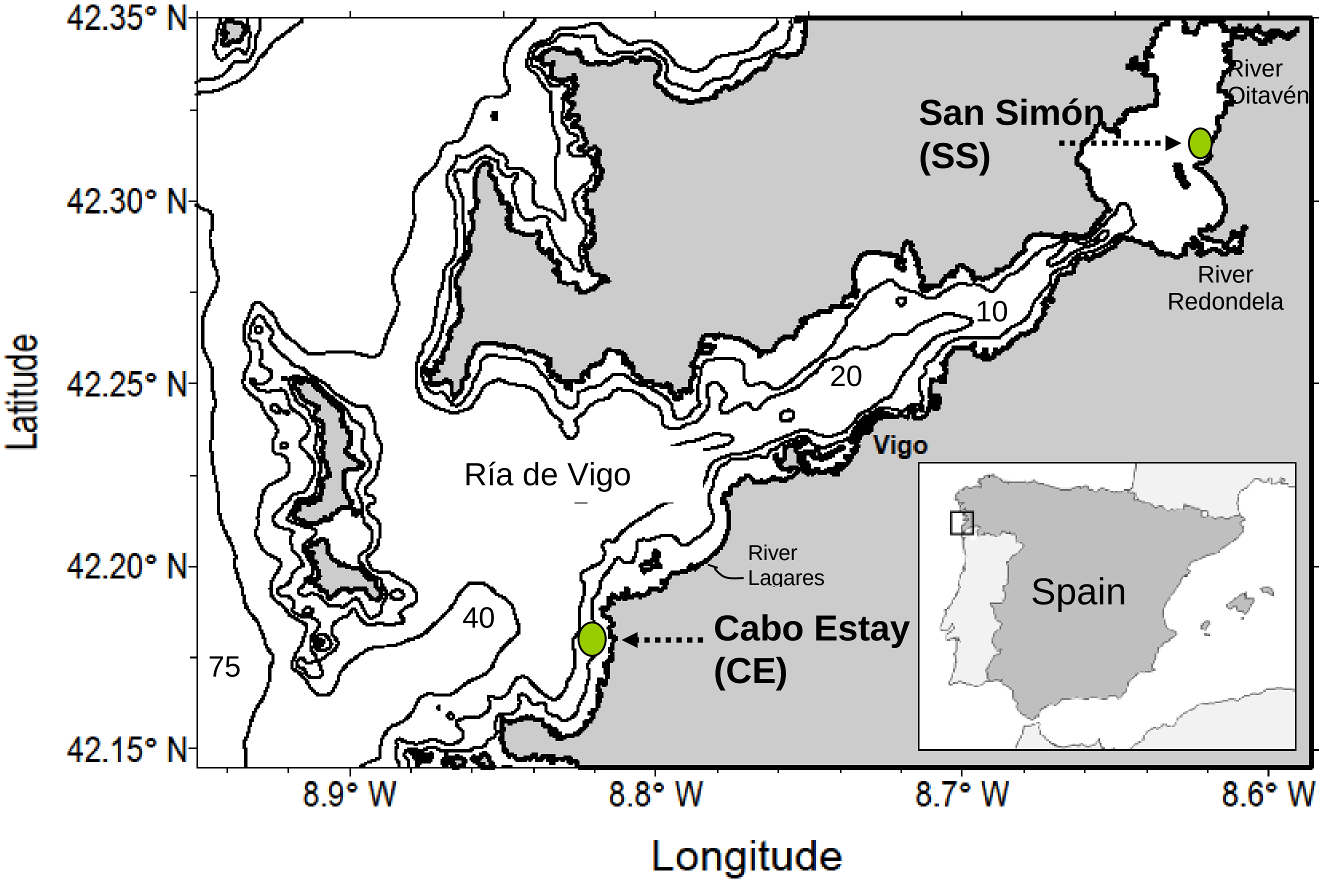
42.35° N
42.30° N
42.25° N
42.20° N
42.15° N
Latitude
8.9° W
8.8° W
8.7° W
8.6° W
Longitude
San Simón
(SS)
River
Oitavén
River
Redondela
10
20
Vigo
Ría de Vigo
River
Lagares
40
75
Cabo Estay
(CE)
Spain

Figure 1

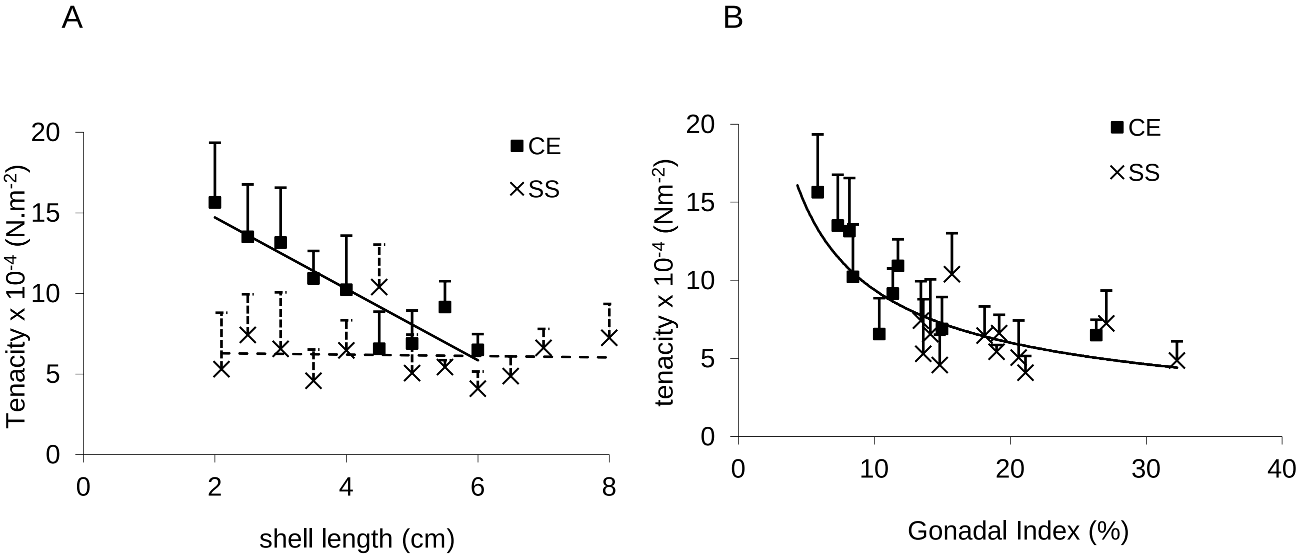
A
B
Tenacity x 10^-4 (N.m^-2)
shell length (cm)
CE
SS
0
5
10
15
20
0
2
4
6
8
tenacity x 10^-4 (Nm^-2)
Gonadal Index (%)
0
10
20
30
40

Figure 2

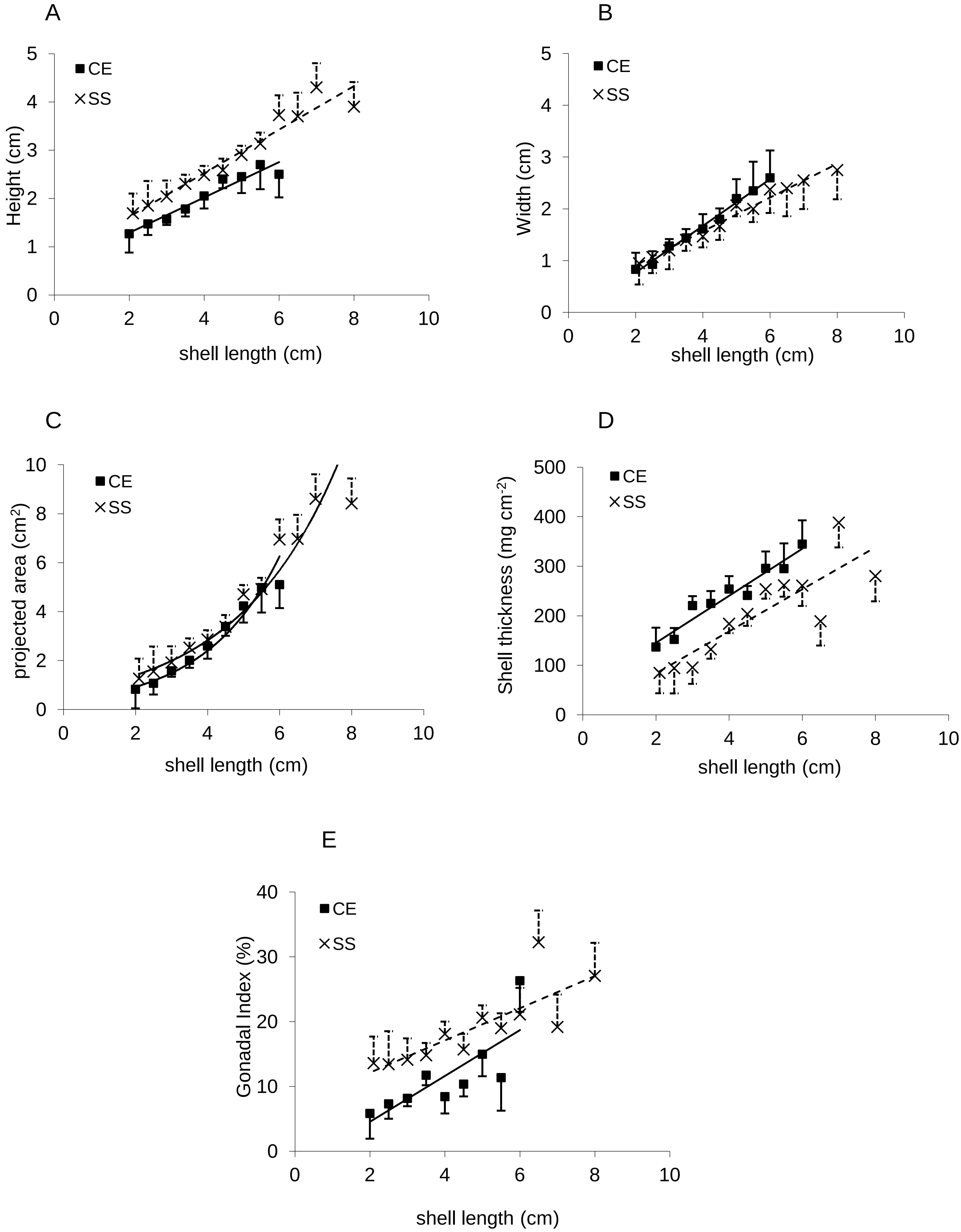
A
CE
SS
Height (cm)
0
1
2
3
4
5
0
2
4
6
8
10
shell length (cm)
B
CE
SS
Width (cm)
0
1
2
3
4
5
0
2
4
6
8
10
shell length (cm)
C
CE
SS
projected area (cm^2)
0
2
4
6
8
10
0
2
4
6
8
10
shell length (cm)
D
CE
SS
Shell thickness (mg cm^{-2})
0
100
200
300
400
500
0
2
4
6
8
10
shell length (cm)
E
CE
SS
Gonadal Index (%)
0
10
20
30
40
0
2
4
6
8
10
shell length (cm)

Figure 3

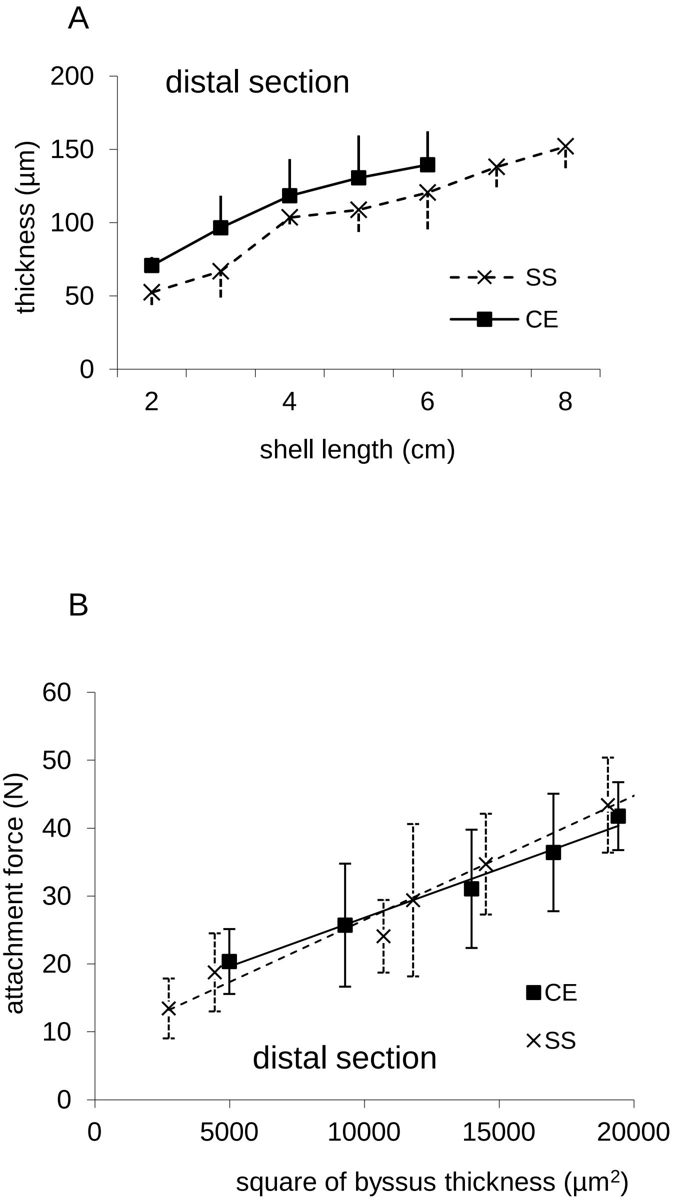
A
distal section
200
150
100
50
0
thickness (µm)
2
4
6
8
shell length (cm)
SS
CE
B
60
50
40
30
20
10
0
attachment force (N)
0
5000
10000
15000
20000
square of byssus thickness (µm2)
CE
SS
distal section

Figure 4

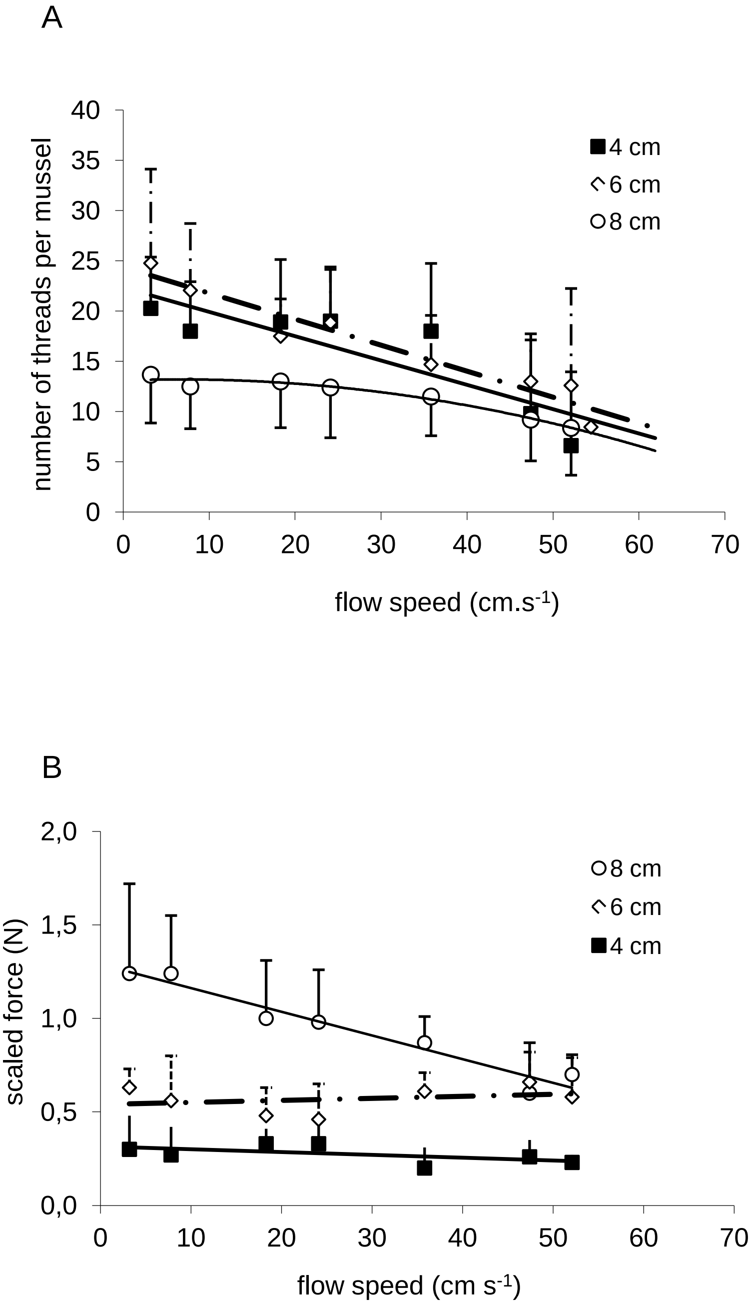
A
number of threads per mussel
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
flow speed (cm.s-1)
■ 4 cm
◇ 6 cm
○ 8 cm
B
scaled force (N)
2,0
1,5
1,0
0,5
0,0
0
10
20
30
40
50
60
70
flow speed (cm s-1)
○ 8 cm
◇ 6 cm
■ 4 cm

Figure 5

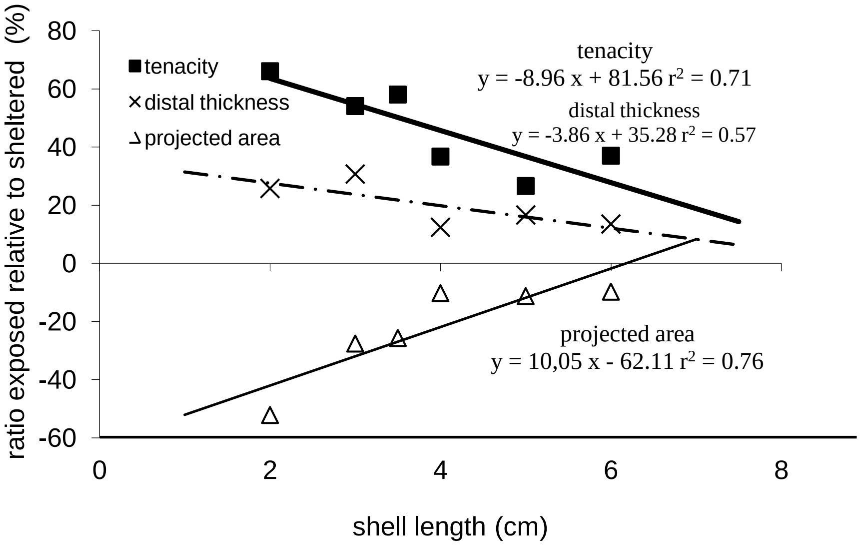
ratio exposed relative to sheltered (%)
80
60
40
20
0
-20
-40
-60
0
2
4
6
8
shell length (cm)
■ tenacity
× distal thickness
△ projected area
tenacity
y = -8.96 x + 81.56 r² = 0.71
distal thickness
y = -3.86 x + 35.28 r² = 0.57
projected area
y = 10,05 x - 62.11 r² = 0.76

Figure 6