

## MECHANICAL DESIGN OF MUSSEL BYSSUS: MATERIAL YIELD ENHANCES ATTACHMENT STRENGTH

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### Summary

The competitive dominance of mussels in the wave-swept rocky intertidal zone is in part due to their ability to maintain a secure attachment. Mussels are tethered to the substratum by a byssus composed of numerous extracellular, collagenous threads secreted by the foot. Each byssal thread has three serially arranged parts: a corrugated proximal region, a smooth distal region and an adhesive plaque. This study examines the material and structural properties of the byssal threads of three mussel species: *Mytilus californianus*, *M. trossulus*, and *M. galloprovincialis*. Tensile tests in general reveal similar material properties among species: the proximal region has a lower initial modulus, a lower ultimate stress and a higher ultimate strain than the distal region. The distal region also yields at a stress well below its ultimate value. In whole thread tests, the proximal region and adhesive plaque are common sites of structural failure and are closely matched

in strength, while the distal region appears to be excessively strong. We propose that the high strength of the distal region is the byproduct of a material designed to yield and extend before structural failure occurs. Experimental and theoretical evidence is presented suggesting that thread yield and extensibility provide two important mechanisms for increasing the overall attachment strength of the mussel: (1) the reorientation of threads towards the direction of applied load, and (2) the 'recruitment' of more threads into tension and the consequent distribution of applied load over a larger cross-sectional area, thereby reducing the stress on each thread. This distal region yield behavior is most striking for *M. californianus* and may be a key to its success in extreme wave-swept environments.

Key words: biomechanics, byssal thread, intertidal, material properties, mussel, *Mytilus* sp.

### Introduction

Sessile organisms inhabiting the rocky intertidal zone must withstand the hydrodynamic forces generated by breaking waves in order to survive. In this environment, water velocities of over  $10 \text{ m s}^{-1}$  are common and water accelerations are likely to exceed  $400 \text{ m s}^{-2}$  (Denny, 1985, 1988; Bell and Denny, 1994). Such water motion can generate large hydrodynamic forces on organisms that live in this environment (Denny *et al.* 1985; Gaylord *et al.* 1994). For mussels, which are one of the dominant competitors for space in the rocky intertidal zone (Seed and Suchanek, 1992), a secure attachment to the substratum is crucial to their survival.

Mussels attach to the substratum by means of a byssus, which is an extracellular, collagenous structure secreted by the foot and is composed of three distinct parts (originally described by Brown, 1952): (1) the *root* which is embedded in the byssus gland at the basal region of the foot and links the entire structure to the byssus retractor muscles, (2) the *stem* which extends outwards from the root and supports each byssal thread, and (3) the *byssal threads* which extend from the stem

in many directions and attach to the substratum. Typically, 20–60 byssal threads are involved in tethering an animal to its substratum (E. C. Bell and J. M. Gosline, in preparation).

Each byssal thread is formed within the groove of the mussel foot by a process resembling polymer injection-molding (Waite, 1992). The threads themselves are complex structures, and each can be divided into three parts: (1) the *proximal region* which has a corrugated surface and represents approximately one-third of the total thread length, (2) the *distal region* which is smoother, narrower and approximately twice the length of the proximal region, and (3) the *adhesive plaque* which attaches the thread distally to the substratum.

Each component of the thread is distinct in both its ultrastructure and protein composition (Bairati and Vitellaro-Zuccarello, 1976; Benedict and Waite, 1986). The proximal region has a corrugated sheath which covers an inner core of short, loosely arranged, coiled fibrils, while the distal region is primarily composed of bundles of densely packed filaments. The adhesive plaque consists of a coat of collagen-like fibers

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layered over an inner spongy matrix. Complementary protein gradients have been found in the thread components of *Mytilus edulis*, such that the proximal region is dominated by a protein with collagen and elastic domains (Col-P), while the distal region is dominated by a protein with collagen and silk-like domains (Col-D; Qin and Waite, 1995).

Because of these structural and compositional differences between the three regions of a byssal thread, it is not surprising that they have also been found to differ in mechanical behavior. To date, most of the studies on the mechanical properties of single byssal threads have been limited to *M. edulis* and provide little insight into the mechanical performance of a whole thread. Allen *et al.* (1976) found that the distal region was the strongest of the three thread components and inferred from observations that the proximal region was weaker than the adhesive plaque, although their strengths were not compared directly. Smeathers and Vincent (1979) and Price (1981) also found large differences in the strength of the proximal and distal regions, but did not consider the plaques. Smeathers and Vincent (1979) demonstrated the high extensibility of the proximal region relative to the distal region and the yield behavior of the thread before failure. However, it is still not well understood which region of the thread is the limiting factor in determining the overall strength of the thread structure, nor are the mechanical behavior and function of each region well described.

A number of studies have reported differences in the overall strength of byssal attachment for co-existing species of intertidal mussels that are in direct competition for space. Specifically, *M. californianus* has a stronger byssus than *M. edulis* [Harger, 1970; Witman and Suchanek, 1984 (note that *M. edulis* was probably *M. galloprovincialis* in both studies (McDonald and Koehn, 1988)], while the attachment of *M. galloprovincialis* is stronger than that of *M. edulis* (Gardner and Skibinski, 1991; Willis and Skibinski, 1992). The attachment strength within a species has also been shown to depend on season (Price, 1980, 1982) and wave exposure (Witman and Suchanek, 1984).

In each of the above studies, it is not clear whether the observed increased attachment strength was due to an increase in the number of threads forming the byssus or to an alteration of the mechanical strength of the individual threads. Byssal thread production is dependent on a number of environmental cues, such as flow, salinity and temperature (Lee *et al.* 1990; Seed and Suchanek, 1992; Dolmer and Svane, 1994), and presumably could account for the increased byssal strength, but the strength of the individual threads of different species has not been explored and consequently must not be discounted. The relative length of each region in a byssal thread has been shown to differ among species, with the distal region comprising 56% and 80% of the threads of *M. edulis* and *M. californianus*, respectively (Mascolo and Waite, 1986), and could contribute to differences in byssus strength. This study investigates the mechanical properties of the byssal threads of a typically wave-exposed species, *M. californianus*, and those of two species that typically inhabit calmer waters, *M.*

*trossulus* and *M. galloprovincialis* (Seed and Suchanek, 1992). These properties are compared with published values for *M. edulis*, a species that is morphologically and ecologically similar to *M. trossulus* and *M. galloprovincialis* (thus, all three are considered to be 'edulis-like'; McDonald and Koehn, 1988). Such a comparison of the mechanical design of threads produced by different species may provide insight into the evolution of the byssus for attachment in wave-swept environments.

This study demonstrates that the byssal threads of edulis-like species do not differ substantially in their material or structural behavior and are therefore likely to be of similar composition and structure. In comparison, threads of *M. californianus* are mechanically similar to those of the other species in the proximal region but exhibit a more dramatic yield behavior with a higher initial stiffness and ultimate strain in the distal region. For all species tested, the proximal region and plaque are shown to be the most common location of failure of whole threads, while the distal region appears to be excessively strong. We propose that the high strength of the distal region is a byproduct of a different design criterion: to yield and thereby undergo a large extension before the structure breaks. We present experimental and theoretical evidence which suggests that yielding provides two mechanisms for increased attachment strength: (1) thread reorientation towards the direction of applied load, and (2) 'recruitment' of more threads into tension, thereby distributing an applied load over a larger cross-sectional area and reducing the stress on each individual thread. The yield behavior is most pronounced in *M. californianus* and may be a key feature to its success in highly wave-swept environments.

### Materials and methods

Mussels (*Mytilus californianus* Conrad, *M. trossulus* Gould and *M. galloprovincialis* Lamark) were collected from Barkley Sound (48°50' N; 125°08' W) on the west coast of Vancouver Island, British Columbia, Canada. Animals were maintained at 10–15 °C in a gently recirculating seawater aquarium (approximate flow rate 5 cm s<sup>-1</sup>).

Thread cross-sectional areas were assumed to be circular and were calculated from thread diameter ( $\pm 1 \mu\text{m}$ ) measured with an ocular micrometer. The proximal region of a thread is approximately twice the thickness of the distal region, so diameter was measured either locally (for tests on isolated regions of thread) or at a 'characteristic' location (in the distal region approximately 3 mm from the adhesive plaque), thus yielding a 'characteristic' cross-sectional area for the entire thread.

All statistical tests were performed using SigmaStat (Jandel Scientific, San Rafael, CA, USA). The null hypothesis was rejected at a significance level (*P*) of 0.05. When analysis of variance (ANOVA) indicated a significant effect of treatment, the Student–Newman–Keuls method was used for pairwise multiple comparisons. More specific details on statistical testing are presented in each subsection below.

### Morphometry

General thread dimensions were measured on field-produced threads from 13 representatively sized mussels for each of the three species as follows. From each individual, a thread was haphazardly sampled and its unstretched length measured to the nearest millimeter. The length of the distal region was measured and expressed as a percentage of total thread length. The characteristic cross-sectional area of each thread was calculated from the diameter measurements. In addition, maximal shell length was measured to the nearest millimeter. The effect of species on the proportion of distal region in a thread was evaluated using ANOVA.

### Tensile tests

All mechanical tests were conducted in sea water at  $15 \pm 1$  °C. Two separate mechanical testing systems were employed. For tests on isolated regions of byssal thread (except those of *M. galloprovincialis*), an Instron-1122 tensometer was used at an extension rate of  $10 \text{ mm min}^{-1}$ . The resulting load on the material was detected either by a standard Instron load cell or by a more sensitive cantilever beam force transducer (constructed from stainless-steel shim stock, the deflection of which was detected by four strain gauges wired as a full Wheatstone bridge). Force (as a voltage output) was sampled at 10 Hz, converted to newtons, and stored digitally. Analog voltage output was also recorded on chart paper.

All other mechanical tests were conducted on a custom-made tensometer. An electric motor rotated a threaded shaft attached to a crosshead that extended the sample at  $6.5 \text{ mm min}^{-1}$ . The resulting load on the material was detected as described above by a cantilever beam force transducer affixed to the crosshead. The force transducer output was sampled digitally at 4 Hz as described above. Analog voltage output was lowpass-filtered (0.3 Hz) and recorded on chart paper.

### Material properties of isolated regions of byssal thread: proximal versus distal

For each of the three test species, byssal threads produced by mussels maintained in an aquarium were collected and test specimens from the distal and proximal regions were isolated. Each end of the specimen was sandwiched between short balsa strips with cyanoacrylate glue, clamped in the testing apparatus, and extended until failure occurred. Force measurements were converted to stress ( $\sigma$ , in  $\text{Nm}^{-2}$ ) by dividing by specimen cross-sectional area. Specimen length ( $l$ ) measurements were converted to strain [ $\varepsilon$ , calculated as  $(l-l_0)/l_0$ , where  $l_0$  is the unstressed length of the specimen].

For representative tests of each region and each species, initial modulus (or material stiffness,  $E_i$ , in  $\text{Nm}^{-2}$ ) was calculated as the slope of the regression line through the initial, linear portion of the stress–strain curve. Ultimate stress ( $\sigma_u$ , or material strength) and ultimate strain ( $\varepsilon_u$ , or material extensibility) may only be calculated for tests where failure does not occur prematurely as a result of clamp damage. Unfortunately, premature failure was a common occurrence for

tests on both regions. Distal region tests were repeatedly performed and only a fraction of those tests provided acceptable measures of  $\sigma_u$  and  $\varepsilon_u$ . For the proximal region, reliable estimates of  $\sigma_u$  were instead obtained from whole thread tests (described below) and  $\varepsilon_u$  was estimated by a linear extrapolation of the final portion of the stress–strain curve of the isolated tests to a mean  $\sigma_u$  for each species.

A two-way ANOVA on ranks was performed to evaluate the effects of species and thread region on initial modulus,  $\sigma_u$  and  $\varepsilon_u$ . For the distal region,  $\sigma_u$  and  $\varepsilon_u$  measurements were unobtainable for *M. galloprovincialis*, and this species was therefore omitted from the two-way ANOVA. For all three material properties tested, these preliminary analyses indicated a highly significant effect of region (i.e. the distal region was stiffer, stronger and less extensible than the proximal region for all species,  $P < 0.001$ ). A one-way ANOVA was then performed for each separate region to evaluate the effect of species on each material property. This allowed for the inclusion of *M. galloprovincialis* as well as *M. edulis* (from the literature) into the statistical analyses.

### Structural properties of whole byssal threads

For each of the three test species, individuals were placed on a bed of granite pebbles in a seawater aquarium. After 1–3 days, the mussels had produced a number of byssal threads, each attached to a single pebble. The stem was dissected from each mussel and clamped in one end of the testing apparatus. A pebble to which a single byssal thread was attached was glued (5-min epoxy, Devcon, Wood Dale, IL, USA) to a metal stub that was clamped to the other end of the testing apparatus. With this mounting technique, the structural strength of a whole byssal thread (proximal region, distal region and adhesive plaque) was not compromised by direct clamping. The stem/thread/pebble structure was extended until failure occurred, and the location of failure (e.g. proximal, distal or plaque) was noted. The occurrence of failure was noted for each location and expressed as a percentage of the number of threads tested for each species.

A second group of *M. californianus* threads that had been produced and maintained in the field was also tested to examine the effects of ‘weathering’ on thread mechanics. In the field, pieces of granite or congener shell that supported a thread were carefully chiselled away from the substratum. The animals were brought to the laboratory where the stems were dissected out and the stem/thread/substratum structure was maintained for 1–3 days in a seawater aquarium until tested as described above for laboratory-produced threads. Threads were selected on the basis of the criterion of maximal position along the stem (corresponding to older threads that were dark brown in color) yet were still healthy in appearance. The selected threads were of unknown age but were likely to be at least 3 weeks older than the pale yellow laboratory-produced threads used in this study. Thus, the field-produced threads differ from the laboratory-produced threads in that they were manufactured and maintained under natural conditions and were older at the time of testing.

The yield force and the force at which structural failure occurred were identified for each whole thread. The yield force marks the transition of the thread from a high-stiffness phase to a low-stiffness phase (Smeathers and Vincent, 1979) and was defined as the force at which a tangent to the initial portion of the force–length curve intersected a tangent to the plateau region of the curve (see Results). Note that yield, as defined in this study, marks a region of high extensibility but does not imply structural failure.

After each whole thread had been tested to structural failure, it was remounted to assess the force required to break the other regions of the structure. For example, if structural failure occurred in the plaque, that end of the thread was clamped (using balsa strips as described above) and the proximal and distal regions were tested in series and the breaking force noted. The region that did not fail (typically the distal region) was then mounted alone and tested to failure.

The characteristic cross-sectional area of each thread (in the distal region near the plaque) was measured as described above. Mean characteristic cross-sectional area was calculated for each species and used to scale all measured forces (yield, structural failure, proximal region failure, distal region failure and plaque failure) by multiplying each individual force by the ratio of mean to individual thread characteristic area. Statistical analyses were performed on these scaled forces. A paired *t*-test was performed to compare yield force with the structural failure force for each of the whole thread test groups. ANOVAs were performed to evaluate the effect of region on failure force for each of the four groups. Data were log-transformed to equalize variances when necessary.

Structural stiffness ( $k$ , in  $\text{N m}^{-1}$ ) is the force required to achieve a unit of whole thread extension. Initial structural stiffness ( $k_i$ ) of each thread was calculated as the slope of a regression through the initial, linear region of the force–length curve. Average structural stiffness ( $k_a$ ) was calculated as structural failure force divided by ultimate extension for whole threads. Ultimate structural strain ( $\varepsilon_{\max}$ ) was also calculated from whole thread extension measurements. ANOVAs were performed to compare each structural property among the four test groups.

#### Whole animal

One *M. californianus* (shell length 9.1 cm) was placed on a flat granite boulder (surface area approximately  $100 \text{ cm}^2$ ) in a gently flowing seawater aquarium. After 2 days, nine byssal threads had been produced; they were approximately 4 cm in length. The boulder with the attached mussel was then glued to a stub and clamped in the testing apparatus. A cable tie was threaded through a swivel and around the intact mussel perpendicular to its long axis. The cable tie was cinched tightly to provide a firm grip, but still allowed some gaping by the animal. The swivel was affixed to the force transducer of the testing apparatus and the animal (long axis parallel to the substratum) was pulled normal to the substratum at  $6.5 \text{ mm min}^{-1}$  until all threads were broken. The number of

threads that were load-bearing (i.e. not slack or broken) was visually determined throughout the experiment.

#### Theoretical attachment strength of mussel byssus

A mathematical model was developed to explore systematically the effect of both empirical and hypothetical byssal thread structural properties on the attachment strength of a byssus composed of multiple threads. Attachment strength was modeled for two displacement orientations relative to the substratum, normal and parallel, which correspond to the primary hydrodynamic forces experienced by mussels within a bed (lift) and by solitary mussels (drag), respectively (Denny, 1987; E. C. Bell and J. M. Gosline, in preparation). The byssus was assumed to be composed of 50 radially arranged threads initially in the same  $x,y$  plane as the substratum. The threads were modeled to have the dimensions and structural properties of laboratory-produced threads of *M. californianus*, with an  $l_0$  of 0.03 m and an ultimate force ( $F_u$ ) of 1.83 N [calculated as the product of  $l_0$  (in m),  $\varepsilon_{\max}$  and  $k_a$ , see Results]. Thus, the potential maximum strength of attachment with 50 threads was 91.5 N. Additional thread structural properties are described in the Results.

For a given mussel displacement, the force of attachment of a mussel byssus is the sum of the forces on each individual thread. For a mussel displaced normally from the substratum, all threads fail at the same displacement; thus, the peak force of attachment is the sum of the normally oriented component of force on each thread at failure ( $F_{\perp}$ ):

$$F_{\perp} = \sin\theta F_u. \quad (1)$$

$\theta$  is the angle of each thread (relative to the  $x,y$  plane) at ultimate structural strain ( $\varepsilon_{\max}$ ):

$$\theta = \arccos\left(\frac{1}{1 + \varepsilon_{\max}}\right). \quad (2)$$

The calculation of the peak force of attachment for a mussel displaced parallel to the substratum is more complex because threads are not loaded equally for a given displacement (roughly half of the threads initially become slack, etc.). Thus, attachment force was calculated for a mussel displaced along the  $x$ -axis as the sum of the  $x$ -directed component of the force on each thread,  $F_{\parallel}$ , for a given displacement,  $\Delta x$  (in m). For a thread initially oriented at an angle  $\phi_0$  relative to the  $x$ -axis, this force is:

$$F_{\parallel}(\Delta x) = k(l_{\Delta x} - l_0)\cos(\phi_{\Delta x}). \quad (3)$$

$k$  is the thread structural stiffness ( $\text{N m}^{-1}$ ), and  $l_{\Delta x}$  and  $\phi_{\Delta x}$  are

$$l_{\Delta x} = \sqrt{(l_0\cos\phi_0 + \Delta x)^2 + (l_0\sin\phi_0)^2}, \quad (4)$$

$$\phi_{\Delta x} = \arcsin\left(\frac{(l_0\sin\phi_0)}{l_{\Delta x}}\right). \quad (5)$$

Attachment force was calculated for 0.5 mm increments of  $\Delta x$  until all threads failed by exceeding  $\varepsilon_{\max}$ .

## Results

### Morphometry

The average length of the byssal threads produced by different species of mussel ranged from 1.6 to 3.1 cm (Table 1). This difference in length was largely attributable to differences in animal size: thread length was 30–40% of the shell length of each species. The proportion of the thread that was represented by distal region material was species-dependent ( $P<0.001$ ). Multiple comparisons revealed that the proportions of the distal region in the byssal threads of *Mytilus californianus* and *M. galloprovincialis* were significantly higher and lower, respectively, than that of *M. trossulus*.

### Tensile tests

#### Material properties of isolated regions of byssal thread: proximal versus distal

Typical stress versus strain curves for isolated distal and proximal regions of a byssal thread (Fig. 1) illustrate their differences in material properties. In general, the proximal region has a lower  $E_i$ , a lower  $\sigma_u$  and a higher  $\epsilon_u$  than the distal region. The distal region exhibits a striking yield behavior (a sharp loss of stiffness at approximately  $35 \text{ MN m}^{-2}$ ) and is considerably stronger than the proximal region.

Material properties of the two isolated regions are presented for the three test species in Table 2, accompanied by values for *M. edulis* from the literature. For all species, the distal region had a much higher  $E_i$  ( $100\text{--}900 \text{ MN m}^{-2}$ ) than the proximal region ( $15\text{--}30 \text{ MN m}^{-2}$ ).  $E_i$  was species-dependent in the distal region ( $P<0.005$ ), where *M. californianus* threads were 3–8 times stiffer than those of the other two species tested, but not in the proximal region ( $P=0.47$ ).

The distal region of each species was typically twice as strong as the proximal region (Table 2).  $\sigma_u$  depended on species in the proximal region ( $P<0.05$ ), but not in the distal region ( $P=0.15$ ). However, the significance level in the proximal comparison is largely dependent on the differences between the two *M. edulis* measurements and is of questionable biological significance; the range of variation between species is quite small.

The proximal region was approximately twice as extensible as the distal region for each species (Table 2).  $\epsilon_u$  was significantly influenced by species in both the distal region, where *M. californianus* was most extensible ( $P<0.001$ ), and the

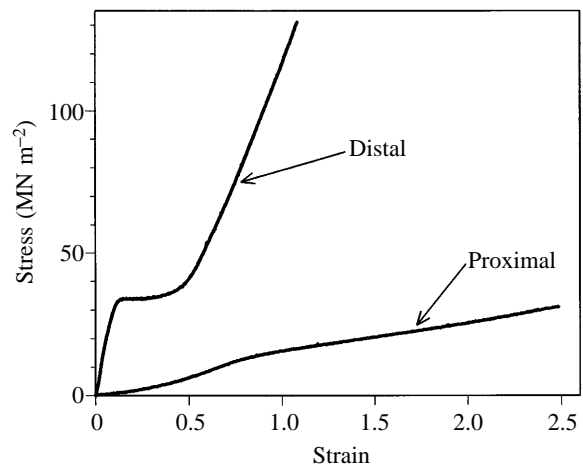


Fig. 1. Tensile tests of two regions isolated from the same laboratory-produced thread of *Mytilus californianus*. Proximal region  $l_0=5.4 \text{ mm}$ , distal region  $l_0=12.5 \text{ mm}$ , where  $l_0$  is unstressed length. Distal region failure was premature owing to grip damage. Extension rate  $10 \text{ mm min}^{-1}$ .

proximal region, where *M. edulis* was least extensible ( $P<0.05$ ).

#### Structural properties of whole byssal threads

**Typical behavior.** A typical force–length curve for a whole byssal thread has three phases (Fig. 2): (1) the structure is initially moderately stiff, then (2) exhibits a decrease in stiffness (yield) at a force of approximately half of its ultimate value, and finally (3) stiffens again before structural failure occurs. The whole thread in Fig. 2 extended a total of 2.1 cm before failure, corresponding to a strain of 0.95.

Because the extensions of the proximal and distal regions were not monitored during whole thread testing, it is unclear how they may contribute to the overall behavior of the thread structure. The material properties of isolated regions were measured in this study, however, and this information can be used to reconstruct the behavior of each region in the whole thread. This was done by replotting the original force–extension data for the isolated regions, presented as  $\sigma$  and  $\epsilon$  in Fig. 1, but with the extension data scaled to correspond to extensions that would have occurred if the regions had been as long as those expected for the 2.2 cm

Table 1. Morphometry of *Mytilus* byssal threads

	<i>M. californianus</i>	<i>M. trossulus</i>	<i>M. galloprovincialis</i>	<i>M. edulis</i> <sup>a</sup>
Thread length (cm)	3.1±0.1	1.6±0.1	2.5±0.2	3.1
Distal region length (% of total thread length)	78.8±0.9	73.6±2.1	65.2±2.6	56
Characteristic cross-sectional area (m <sup>2</sup> )	$5.94 \times 10^{-8} \pm 0.34 \times 10^{-8}$	$0.77 \times 10^{-8} \pm 0.02 \times 10^{-8}$	$0.93 \times 10^{-8} \pm 0.07 \times 10^{-8}$	–
Shell length (cm)	7.8±0.5	4.4±0.1	8.4±0.6	–

<sup>a</sup>Data from Mascolo and Waite (1986).

Animals selected corresponded to a representative size for each species.

Values are means ± S.E.M.,  $N=13$ .

Table 2. *Material properties of two regions of Mytilus byssal threads*

	Initial modulus, $E_i$ (MN m <sup>-2</sup> )	Ultimate stress, $\sigma_u$ (MN m <sup>-2</sup> )	Ultimate strain, $\epsilon_u$
Distal region			
<i>M. californianus</i>	<b>868.6±181.2 (5)</b>	73.3±5.7 (31)	<b>1.09±0.03 (28)</b>
<i>M. trossulus</i>	109.3±8.6 (5)	56.2±9.1 (17)	0.79±0.05 (14)
<i>M. galloprovincialis</i>	300.4±90.1 (3)	–	–
<i>M. edulis</i> <sup>a</sup>	–	–	0.66±0.01 (10)
<i>M. edulis</i> <sup>b</sup>	–	83±15 (8)	–
ANOVA ( <i>P</i> )	<0.005	0.15	<0.001
Proximal region			
<i>M. californianus</i>	15.6±1.3 (5)	34.6±5.8 (8)	1.97±0.21 (5)
<i>M. trossulus</i>	20.6±5.2 (5)	20.3±3.3 (11)	1.65±0.23 (5)
<i>M. galloprovincialis</i>	26.4±11.5 (3)	17.1±4.5 (3)	1.64±0.41 (3)
<i>M. edulis</i> <sup>a</sup>	–	21±3 (23)	<b>1.22±0.04 (10)</b>
<i>M. edulis</i> <sup>b</sup>	–	33±2 (60)	–
ANOVA ( <i>P</i> )	0.47	<0.05	<0.05

<sup>a</sup>Data from Smeathers and Vincent (1979); 5 mm min<sup>-1</sup> extension rate (wet threads).

<sup>b</sup>Data from Price (1981); 25 mm min<sup>-1</sup> extension rate.

Values are means ± S.E.M. (*N*).

Values in bold type are significantly different from those for the other species (one-way ANOVA with Student–Newman–Keuls multiple comparisons, *P*=0.05).

thread presented in Fig. 2 (80% of the thread is distal region; Table 1). This analysis is presented in Fig. 3 and shows that extension in the first phase of the force–length curve of a whole thread is dominated by extension in the proximal region. The second phase of the curve is dominated by the distinct yield in the distal region, where approximately 0.5 cm of extension is achieved with essentially no increase in force. The final phase of increased stiffness in the whole thread is due to a concomitant stiffening of the distal region. Notice that the distal region in Fig. 3 is capable of sustaining a force in excess of 4 N (this is a conservative value due to premature failure caused by grip damage) but, placed in the context of a whole thread, structural failure occurs at the plaque well before the distal region is ever loaded to such a force.

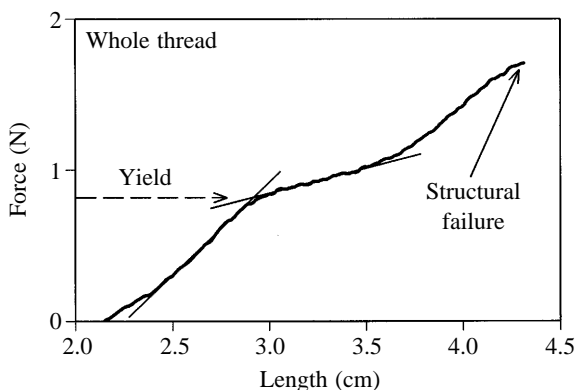


Fig. 2. A typical tensile test of a whole mussel byssal thread (produced in the laboratory by *M. californianus*). Structural failure occurred in the adhesive plaque. The intersection of the two tangents defines the yield force. Extension rate 6.5 mm min<sup>-1</sup>;  $l_0=2.2$  cm.

*Failure location.* The adhesive plaque was the most common location of failure for *M. californianus* (67% for both laboratory- and field-produced threads; Table 3), while the proximal region was the most common location of failure in the threads of *M. trossulus* and *M. galloprovincialis* (58 and 100%, respectively). Structural failure generally occurred in either the adhesive plaque or the proximal region; distal region failure was observed in only two of the 42 whole thread tensile tests.

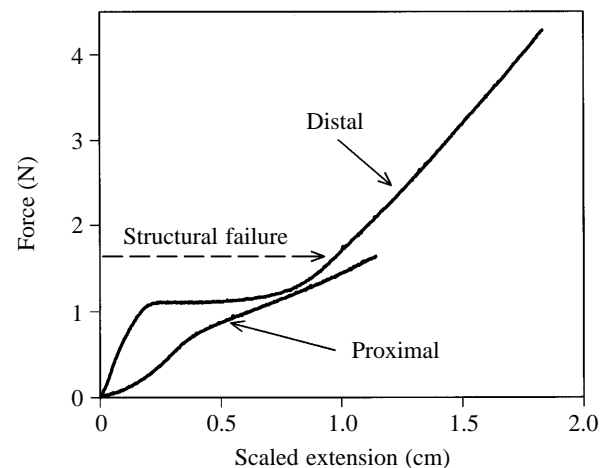


Fig. 3. Predicted force–extension behavior of proximal and distal regions in a whole thread of *M. californianus*. Force is the original data for the isolated regions presented in Fig. 1. Original regional extensions in Fig. 1 were scaled to correspond to initial lengths typical of *M. californianus* threads ( $l_0=0.5$  and 1.7 cm for proximal and distal region, respectively). The distal region breaking force is underestimated due to grip damage, but is nonetheless considerably higher than the structural failure force.

Table 3. Location of structural failure (percentage of total) of laboratory- and field-produced threads of three *Mytilus* species

	<i>M. californianus</i> , laboratory (N=15)	<i>M. californianus</i> , field (N=12)	<i>M. trossulus</i> , laboratory (N=12)	<i>M. galloprovincialis</i> , laboratory (N=3)
Proximal region	26.7	33.3	58.3	100
Distal region	6.7	0	8.3	0
Adhesive plaque	66.7	66.7	33.3	0

**Failure forces.** In 86 % of the whole thread tensile tests, a distinct yield, or decrease in stiffness, was observed before structural failure occurred (Fig. 2). On average, the yield force in the four test groups ranged from 52 to 81 % of the force required for structural failure (Table 4). The yield force was significantly lower than the structural failure force in three of the four test groups, indicating that a considerable amount of extension of the distal region was achieved before failure occurred.

In general, the forces required to break the proximal region and the adhesive plaque were not significantly different (Table 4). The distal region was generally the strongest, despite the fact that the distal measurements were probably underestimated as a result of grip damage. For both test groups of *M. californianus* as well as *M. trossulus*, the distal region had a significantly higher failure force than either the proximal region or the plaque, the failure forces of which were statistically indistinguishable. The failure forces of the three regions of *M. galloprovincialis* were not significantly different ( $P=0.32$ ), but this is probably a reflection of low sample size and underestimating distal region failure.

The mean forces associated with field-produced threads

were generally higher than those for laboratory-produced threads in *M. californianus* (Table 4). Mann–Whitney rank sum tests comparing each scaled force (laboratory *versus* field) found significant differences in the yield, distal region failure and proximal region failure, but no difference between plaque and structural failures ( $P<0.001$ , 0.05, 0.05 and  $P=0.29$  and 0.23, respectively).

**Structural properties.** All thread structural properties were species-dependent ( $P<0.001$ ; Table 5), primarily because of differences in *M. californianus* threads.  $k_i$  was significantly higher in field- than laboratory-produced threads of *M. californianus*, which in turn was significantly higher than that of *M. trossulus* threads.  $k_a$  was highest for field-produced threads of *M. californianus*, but did not differ among laboratory-produced threads of the three test species ( $P<0.001$ ; Table 5).  $\varepsilon_{\max}$  was highest for laboratory-produced threads of *M. californianus*, while values for the other test groups were indistinguishable. Note that Price's (1981) value for ultimate structural strain for *M. edulis* is in agreement with values for *M. trossulus* and *M. galloprovincialis* from the present study, but that Smeathers and Vincent's (1979) estimate is somewhat lower. For all of the structural properties measured, no

Table 4. Structural analysis of laboratory- and field-produced byssal threads of three *Mytilus* species

	<i>M. californianus</i> , laboratory (N=15)	<i>M. californianus</i> , field (N=12)	<i>M. trossulus</i> , laboratory (N=12)	<i>M. galloprovincialis</i> , laboratory (N=3)
Characteristic area (m <sup>2</sup> )	$5.89 \times 10^{-8} \pm 0.65 \times 10^{-8a}$	$5.89 \times 10^{-8} \pm 0.65 \times 10^{-8}$	$1.11 \times 10^{-8} \pm 0.18 \times 10^{-8}$	$0.77 \times 10^{-8} \pm 0.27 \times 10^{-8}$
Scaled forces (N)				
Yield	0.99±0.06	1.96±0.25 <sup>b</sup>	0.30±0.04 <sup>b</sup>	0.35±0.01
Structural failure	1.90±0.23	2.56±0.43	0.49±0.10	0.60±0.11
<i>P</i> (paired <i>t</i> -test)	0.001	0.01	0.01	0.13
Proximal region failure	2.35±0.27	3.89±0.43	0.53±0.10	0.60±0.11
Distal region failure <sup>c</sup>	<b>3.43±0.24</b>	<b>5.58±0.81</b>	<b>0.88±0.12</b>	0.83±0.17
Adhesive plaque failure	2.04±0.20	2.92±0.62	0.65±0.13	0.91±0.11
<i>P</i> (one-way ANOVA)	<0.001	0.03	0.04 <sup>d</sup>	0.32

Values are means ± S.E.M.

All forces are scaled to the characteristic area for each species.

<sup>a</sup>Laboratory- and field-produced threads pooled ( $N=27$ ); unpooled values are  $5.23 \times 10^{-8} \pm 0.73 \times 10^{-8}$  m<sup>2</sup> and  $6.70 \times 10^{-8} \pm 1.19 \times 10^{-8}$  m<sup>2</sup>, respectively.

<sup>b</sup>Yield not observed in one sample.

<sup>c</sup>Underestimate due to grip damage. Values in bold type are significantly higher than values for the proximal region or adhesive plaque failure (Student–Newman–Keuls multiple comparisons,  $P=0.05$ ).

<sup>d</sup>Data log-transformed for statistical analysis.

Table 5. Structural properties of laboratory- and field-produced byssal threads

	<i>M. californianus</i> , laboratory (N=15)	<i>M. californianus</i> , field (N=12)	<i>M. trossulus</i> , laboratory (N=12)	<i>M. galloprovincialis</i> , laboratory (N=3)	<i>M. edulis</i> , laboratory (N=23 <sup>a</sup> ) or (N=63 <sup>b</sup> )
Structural stiffness (N m <sup>-1</sup> )					
Initial, $k_i^c$ ( $P<0.001$ )	117±17	258±33↑	50±5↓	48±15	
Average, $k_a^c$ ( $P<0.001$ )	65±11	253±34↑	41±6	46±16	28 <sup>a,d</sup>
Ultimate strain, $\epsilon_{max}$ ( $P<0.001$ )	0.94±0.10	0.46±0.08↓	0.66±0.09↓	0.62±0.10	0.44±0.03 <sup>a</sup> ↓ 0.67±0.03 <sup>b</sup> ↓

Values are means ± S.E.M.

*P* denotes significance of a one-way ANOVA for each structural property.

Arrows indicate values that are significantly higher (↑) or lower (↓) than values for laboratory-produced threads of *M. californianus* (Student–Newman–Keuls multiple comparisons,  $P=0.05$ ).

<sup>a</sup>Data from Smeathers and Vincent (1979).

<sup>b</sup>Data from Price (1981).

<sup>c</sup>Statistical analysis performed on log-transformed data, omitting *M. edulis*.

<sup>d</sup>Calculated from Smeathers and Vincent's (1979) ultimate force and strain values, assuming that the unstressed length was 2.0 cm.

statistical differences were detected among the three edulis-like species.

#### Whole animal

When a whole animal was extended normally from the substratum, only three of its nine threads were initially in tension (Fig. 4). As these three threads were extended, a slight decrease in the rate of force increase was observed that was probably due to the yield behavior of the threads ( $Y_1$ ). Shortly after this first yield, three additional threads came into tension. A decrease in slope was detectable again after 2.5 min and was probably due to the yield of the second three threads ( $Y_2$ ). Once the load had risen steeply to approximately 6 N, an active response by the animal was observed: it gaped, rapidly moved its foot and repeatedly adjusted and ultimately lowered the tension in the byssus by approximately 2 N. This behavior was also observed

(to a lesser extent) at 7.5 min. A third decrease in slope ( $Y_3$ ) was detected shortly after the final three threads came into tension.

Note that all nine threads were in tension before any single thread failed. The last threads to come into tension were initially quite slack; the animal extended approximately 3.3 cm (5 min of extension at 6.5 mm min<sup>-1</sup>) before all threads were observed to be in tension. The byssus supported a peak force of nearly 15 N distributed over all nine threads before any individual thread failed. The drop in force observed when each thread failed can be interpreted to be its ultimate force. The sum of these individual forces is approximately 18 N, indicating that the observed peak force sustained by the byssus was 83% of its potential strength.

#### Theoretical attachment strength of mussel byssus

On the basis of the results of this study, thread structural

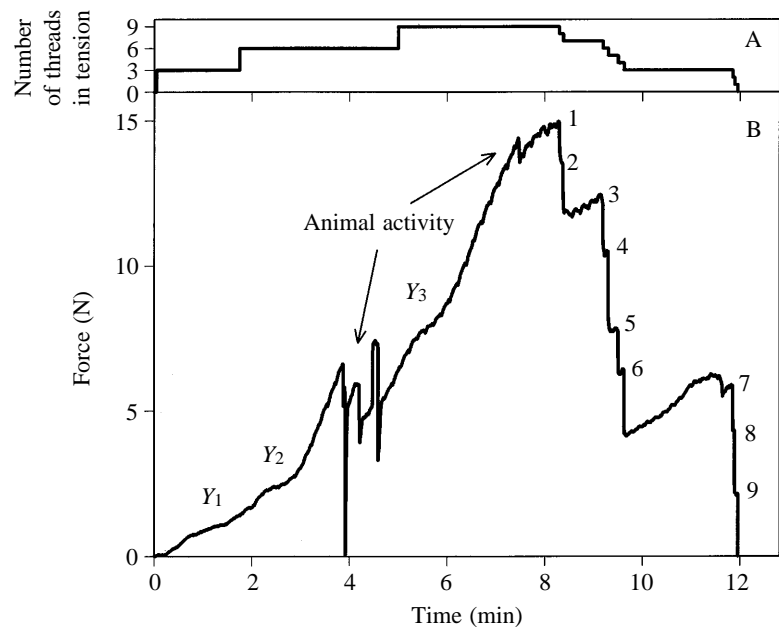


Fig. 4. Tensile test of a live mussel attached to a small granite boulder by nine byssal threads. (A) Number of threads visually determined to be load-bearing. (B) Byssal attachment force. The animal was extended away from the substratum at 6.5 mm min<sup>-1</sup> and the number of threads in tension was visually determined throughout the experiment. Putative regions of the curve corresponding to thread yield are labelled  $Y_1$ – $Y_3$ . Gaping and rapid foot movements were observed at 4 and 7.5 min. A sharp drop in force accompanied the failure of an individual thread (labelled 1–9). The small drop in force before failure of the seventh thread is an artifact.



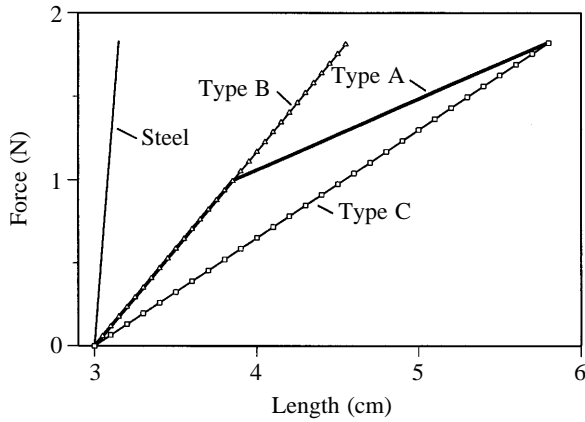


Fig. 5. Force-length behavior of threads in the byssal attachment model. Ultimate force  $F_u=1.83\text{ N}$  for all threads. Type A (bold line) approximates the behavior of laboratory-produced threads of *M. californianus*, with a yield at 1 N. Type B (triangles) and type C (squares) share the initial stiffness and ultimate strain, respectively, of type A, but lack its yield behavior. Steel thread has an ultimate strain of 0.05. See Table 6 for specific parameters.

properties were modeled in four different ways to explore various features of thread mechanics on overall attachment strength (Fig. 5; Table 6): (1) *type A*, which had the  $k_i$  and  $k_a$  of *M. californianus* laboratory-produced threads (Table 5), and a yield occurring at 1 N (Table 4;  $k$  for forces above the yield was consequently constrained to  $42\text{ N m}^{-1}$ ); (2) *type B*, which had the  $k$  of type A (initial value), but not the yield behavior, and consequently had a lower  $\epsilon_{\max}$ ; (3) *type C*, which had the same  $\epsilon_{\max}$  as type A, but no yield behavior, and consequently had a lower  $k$ ; and (4) *steel*, which illustrates the extreme case

Table 6. Input parameters and results for different thread types for the mussel byssal attachment model

	Thread type			Steel
	A	B	C	
<b>Input parameters</b>				
Yield force (N)	1	–	–	–
Structural stiffness, $k$ ( $\text{N m}^{-1}$ )	117, 42 <sup>a</sup>	117	65	1222
Ultimate strain, $\epsilon_u$	0.94	0.52	0.94	0.05
<b>Results</b>				
Maximum attachment force (N)				
Normal displacement	78.7	68.6	78.7	27.5
Parallel displacement	44.5	34.5	39.0	29.7
Percentage of potential maximum attachment force				
Normal displacement	86	75	86	30
Parallel displacement	49	38	43	32

All threads are constrained by an ultimate force of 1.83 N. See text for details.

<sup>a</sup>For loads  $\leq$  yield,  $k=117\text{ N m}^{-1}$ ; for loads  $>$  yield,  $k=42\text{ N m}^{-1}$ .

of high strength and very low strain at failure. The value of ultimate strain ( $\epsilon_{\max}=0.05$ ; Oberg *et al.* 1977) is typical of a very high-strength, tempered, steel alloy; the high modulus of steel will also produce a high  $k$ . Note that the ultimate force is the same for all four thread types.

For mussels displaced normal to the substratum, the maximum attachment force was highest for the most extensible thread types (A and C; Table 6). The percentage of the potential maximum attachment force ranged from 75 to 86%, except for a byssus of steel threads, which achieved only 30% of its potential maximum strength.

The results of the model of a mussel displaced parallel to the

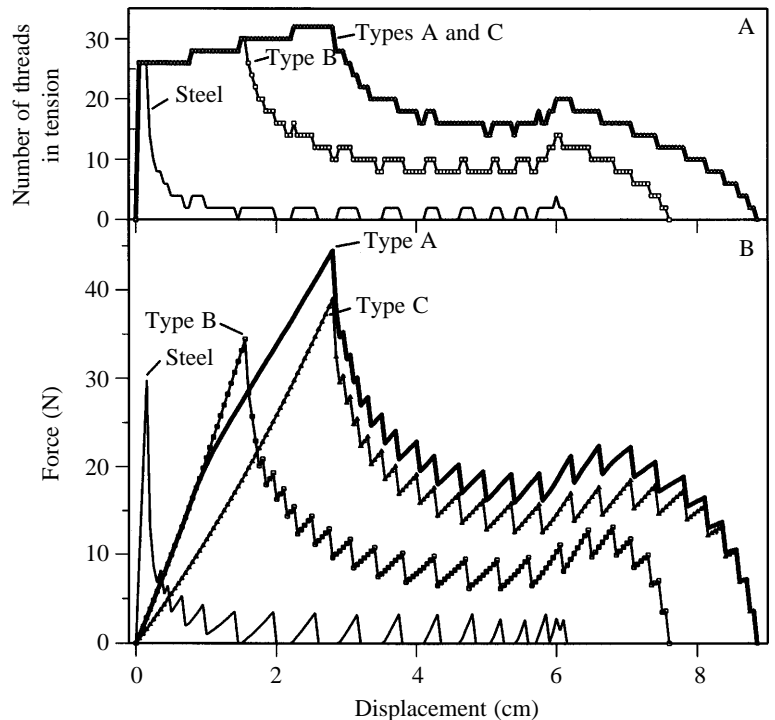


Fig. 6. Modeled byssal attachment (parallel displacement) for four different thread types. (A) Number of threads that are load-bearing. (B) Byssal attachment force. Other details are as defined in Fig. 5.

substratum are presented in Table 6 and Fig. 6. Approximately half of the threads are placed in tension as the mussel was initially displaced, regardless of thread type. The more extensible the thread type, the more new threads were recruited into tension before peak attachment force was achieved. Note that thread types A and C had the same number of threads in tension for a given displacement, but the attachment force was always greater for type A. Peak attachment strength was therefore greatest for thread type A, which attained nearly 50% of its potential maximum strength. In contrast, a byssus constructed from relatively inextensible steel threads reached only 32% of its potential maximum strength.

### Discussion

For all three species tested, the distal and proximal regions demonstrated strikingly different mechanical properties. The distal region was constructed from a material with high initial stiffness, but it showed an abrupt yield and extended at essentially constant stress before stiffness rose again to the failure point, properties that are nearly identical to those seen for hair keratins (Wainwright *et al.* 1982). The material that made up the proximal region was much more elastomeric in its behavior, with lower initial stiffness and approximately 200% extension at failure. This material, however, was about an order of magnitude stiffer and stronger than the rubber-like proteins elastin and resilin (Wainwright *et al.* 1982). Several differences between the proximal and distal materials will be crucial to the analysis of the mechanical design in the byssal apparatus that follows. The distal material had five- to 50-fold higher initial stiffness ( $E_i$ ) and a twofold greater strength ( $\sigma_u$ ) than the proximal material. Also, the distal material exhibited a very abrupt yield and large-scale deformation at a stress well below its strength, whereas the proximal region material exhibited relatively constant stiffness over the full range of extension. These differences in mechanical properties are consistent with the findings that the distal and proximal materials differ in both ultrastructure (Bairati and Vitellaro-Zuccarello, 1974, 1976; Vitellaro-Zuccarello *et al.* 1983; Benedict and Waite, 1986) and protein composition (Benedict and Waite, 1986; Mascolo and Waite, 1986; Qin and Waite, 1995).

In contrast, few differences were observed in the material properties of threads manufactured by different species of mussel. Proximal region  $E_i$ ,  $\sigma_u$ , and  $\varepsilon_u$  were essentially identical among species, which suggests that they are constructed from essentially the same material. Note that the one exception to this generalization is Smeathers and Vincent's (1979)  $\varepsilon_u$  estimates for *Mytilus edulis*, which were significantly lower than those for the other species (Table 2). Proximal region  $\sigma_u$  and  $\varepsilon_{\max}$  were also lower than those reported by Price (1981) for the same species (Tables 2, 5), and Smeathers and Vincent (1979) did not observe a final phase of increased stiffness in a whole thread force-length curve (e.g. Fig. 2). One possible explanation for the anomalous data of Smeathers and Vincent (1979) is the inclusion of tests where failure occurred at the grip, as can commonly occur with the mounting technique they describe

(Price, 1981; E. C. Bell and J. M. Gosline, personal observation). The issue of grip failure was not addressed by Smeathers and Vincent (1979) and could have resulted in an underestimate of the ultimate material properties for *M. edulis*.

Some species differences in material properties were observed in the distal region, however, where *M. californianus* was initially stiffer and ultimately more extensible than the three edulis-like species, which were in turn statistically indistinguishable. These differences are reflected in the mechanical behavior of a whole byssal thread: laboratory-produced threads of *M. californianus* had a higher  $k_i$  and  $\varepsilon_{\max}$  than those of *M. trossulus* (but not *M. galloprovincialis*, probably because of the small sample size of the latter; Table 5). The difference between  $k_i$  and  $k_a$  in laboratory-produced *M. californianus* threads is due to the dramatic yield behavior of the distal region, with a high initial modulus yet a high ultimate extensibility.

In general, the four *Mytilus* species exhibit a common byssal thread design: a short length of relatively weak, low-modulus material (the proximal region) connected in series to a longer length of stiffer, stronger material that can yield and extend (the distal region). Each thread is attached at one end to the substratum by means of an adhesive plaque and at the other end to the stem (which is ultimately rooted into the mussel tissue). A useful analogy for describing the byssus is a collection of chains (threads) arising from the stem that anchor the mussel to the substratum. Each chain is composed of three 'links' that correspond to different regions of the thread, each with its own distinct mechanical behavior: the proximal region, the distal region and the adhesive plaque. How does such a structure perform in tension?

Because a chain is only as strong as its weakest link, an optimal design would call for each link to be of equal strength. Thus, no single link is 'overdesigned' and each has an equal probability of being the location of structural failure when the chain is placed in tension. Mussels, however, have not adopted this apparently optimal design for their byssal threads. Structural failure only rarely occurred in the distal region, suggesting that this link in the chain is generally stronger than the other two. For *M. californianus*, the weak link in the chain was most commonly the plaque (followed by the proximal region), while the proximal region was the weak link for *M. trossulus* (followed by the plaque) and *M. galloprovincialis*. While these observations on the frequency of structural failure at each location identify weak links in the chain, they do not explore the relative strengths of each link. Thus, the force required to break each separate link was measured and, as expected, the link with the highest frequency of structural failure required the lowest mean force for failure. For *M. californianus*, this link was the adhesive plaque, the failure force of which was lower than, but statistically indistinguishable from, that of the proximal region. As a result, structural failure in the proximal region was less frequent, but did occur. The opposite trend was observed in *M. trossulus*, where the weak link and most frequent location of failure was the proximal region, but the adhesive plaque was found to be only moderately stronger and hence was

occasionally the location of structural failure. The sample size for *M. galloprovincialis* threads was low, but the data followed similar trends to *M. trossulus*. Thus, the strength of the whole thread structure of mussels is in general limited by the strengths of the proximal region and the adhesive plaque. These links are well matched in that they are of approximately the same strength and have high probabilities of being the location of structural failure. The distal region, however, seems to be overdesigned in that it is much stronger than it needs to be; its strength could be reduced considerably without affecting the overall strength of the structure.

Byssal thread production can represent a substantial fraction of the energy expenditure of mussels (e.g. 8% during the summer, Hawkins and Bayne, 1985), so presumably it would be advantageous to reduce the amount of unnecessary material dedicated to the distal region. One hypothesis we explored was that the distal region may be overdesigned in response to a higher rate of fatigue in nature. The transition from the proximal to the distal region occurs at the shell margin (E. C. Bell and J. M. Gosline, personal observation); thus, the proximal region is relatively protected inside the mussel shell while the exposed distal region may be more likely to accumulate flaws from sand scour, crab claws, gastropod radulas, etc. As a result, the distal region of older, presumably flawed, threads from the field could have lower breaking forces and be a more common location of structural failure than laboratory-produced threads. This was not the case, however, as the distal region was never observed to be the location of structural failure in field-produced threads of *M. californianus*. Flaws were indeed observed in the distal region of the field threads, but they were not of sufficient magnitude to affect the overall strength of the structure. Interestingly, both the proximal and distal regions of field-produced threads were stronger than those produced in the laboratory by *M. californianus*. However, despite the increased strength in these regions, the structural strength of field-produced threads was unaffected because failure most commonly occurred in the adhesive plaque, where no significant difference was detected between laboratory- and field-produced threads.

Why is the distal region so strong? We propose that the most functionally important mechanical property of the distal region is not its high ultimate strength but, instead, its ability to yield and extend. The yield force in whole threads was statistically distinguishable from the structural failure force in three of the four test groups, and yield was observed in nearly all of the samples before they failed. This implies that one consistent aspect of the distal region design is to yield before the structural failure force is reached. The molecular mechanism for the yield behavior of the distal region is, at this point, unknown, but it is possible that one byproduct of such molecular structuring is a high material strength. That is, the high (and seemingly wasted) strength observed in the distal region may simply be due to constraints imposed by the manufacture of a material capable of undergoing a yield at an appropriate force.

The function of a yield in the distal region is clear only when it is placed in the context of the geometrically complex structure

in which it operates: a single link in a chain that, with other chains, tethers the mussel to the substratum. When placed in unidirectional flow, mussels have been observed to lay down threads in the direction of flow, thereby increasing attachment strength in the most likely direction of applied load (Dolmer and Svane, 1994). It is unlikely that mussels could make use of this response in the wave-swept rocky intertidal zone, where the direction of maximum flow is highly unpredictable (Denny, 1985). This is due to the topographic complexity of the habitat, where even the most simple, orderly waves can produce complex, chaotic flows once they break at the shore. Thus, the direction of maximum flow is unpredictable from wave to wave, and the best strategy for a mussel would be to 'hedge its bets' and distribute its threads uniformly in all directions. Indeed, no preferred orientation of byssal threads has been observed for mussels in the field; they are generally evenly distributed in a radial pattern (E. C. Bell and J. M. Gosline, personal observation).

One potential drawback to such a radial orientation is that a large proportion of the byssal threads are not initially aligned in the direction of the applied load. However, because byssal threads are ultimately extensible, they can reorient towards the direction of force before failure occurs. Consequently, a modeled mussel byssus displaced normal to the substratum achieved 75–86% of its potential strength when constructed from extensible threads (types A–C), but only 30% of its potential with inextensible steel threads (Table 6). Thus, one function of the distal region yield behavior is to provide the extension necessary for thread realignment. A similar enhancement of strength is provided by the extensibility of the frame and viscid silk in spider webs (Denny, 1976, 1980).

A second function of the distal region yield is to provide thread extension for the recruitment of additional threads into tension. Consider a hypothetical case of a mussel tethered by steel threads, each with a small, variable degree of slack. When displaced parallel to the substratum, owing to the low extensibility of steel, each single thread would fail before it could extend enough to share the load with another thread. As a result, the overall strength of attachment never exceeds the strength of a single thread. This principle is illustrated by the modeled byssus of steel threads displaced parallel to the substratum (Fig. 6;  $\Delta x > 0.2$  cm).

For the live mussel in this study, the overall strength of attachment greatly exceeded the strength of a single thread, approaching the sum of the strengths of the nine individual threads. This is despite the fact that only three threads were initially in tension while the others were slack (over 3 cm). Because the threads that were initially in tension were able to yield and extend before they failed, new threads were 'recruited' (i.e. came into tension), and the load on each individual thread remained below its failure force. The yield behavior of the distal region therefore provides an important mechanism for recruiting more threads and applying a load over a larger cross-sectional area. This has the effect of reducing the stress in any individual thread, increasing the total attachment strength of the animal and decreasing the likelihood that it will be dislodged from the substratum. Note that the live

mussel displaced normal to the substratum achieved 83 % of its potential maximum attachment strength, a value that is consistent with that predicted for thread type A (Table 6).

Thread type A was modeled on laboratory-produced *M. californianus* threads in that the yield force approaches, but is less than, the force required for structural failure. If this yield force were to exceed the failure force (perhaps by increasing the thickness of the distal region), then the threads would behave like type B and maximum attachment force would be reduced by 13–22 %, depending on the displacement direction (Table 6). Similarly, if the yield force were reduced (perhaps by decreasing distal region thickness), threads would behave like type C and the peak force of attachment would be compromised by 12 % for parallel displacement, but unchanged for normal displacement. At the extreme of stiffness, steel threads have the lowest peak attachment force, and attachment is weak and unstable for parallel displacements beyond 0.2 cm. If a small, variable amount of slack were introduced into the parallel model, the peak attachment force of a byssus with steel threads would be greatly reduced because of their low extensibility.

In the model for normal displacement, realignment (provided by thread extensibility) is the primary mechanism for increased attachment strength. Thus, thread types A and C, which have the same  $\epsilon_{\max}$ , result in the same byssal attachment strength. For parallel displacement, thread recruitment is the dominant mechanism for increased attachment strength. In this orientation, a byssus composed of type A threads always has the stronger attachment (compared with type C) because newly recruited threads have a high initial structural stiffness. In this manner, both  $k_i$  and  $\epsilon_{\max}$  are important structural properties for increasing byssal attachment strength for parallel displacements, or for any orientation where threads are initially slack (as was observed in the live mussel pulled normally from the substratum).

Thus, one key feature to the high attachment strength of *M. californianus* is the existence of threads that are initially stiff, yet extensible. These are structural properties that are largely due to the material properties of the distal region. The yield and extension of this region allow for the reorientation of threads in the direction of the applied load, as well as the recruitment of new threads into tension. Threads of the three edulis-like species have a lower representation of the distal region and are less stiff and less extensible than those of *M. californianus*. This edulis-like thread design is intermediate between types A and B and results in a relatively lower attachment strength (80 % and 42 % of maximum potential strength for normal and parallel displacement, respectively; data not shown). This reduction in attachment strength due to thread structural properties is consistent with the observation that *M. trossulus*, *M. galloprovincialis* and *M. edulis* are generally restricted to calmer wave-swept habitats (note that thread size and number are also important factors, E. C. Bell and J. M. Gosline, in preparation).

The structural properties of the field-produced threads of *M. californianus* differed from those produced in the laboratory (Table 5). The threads sampled from the field were slightly thicker (see footnote, Table 4), but this does not completely

account for the observed increase in  $k_i$  and  $k_a$  in these threads. Furthermore, these field-produced threads appear to have a reduced yield behavior, demonstrated by their reduced  $\epsilon_{\max}$  and small reduction in  $k_a$  (relative to  $k_i$ ). Similar trends have been observed in laboratory-produced threads that have 'aged' in an aquarium for 1 month (E. C. Bell and J. M. Gosline, unpublished data). Thus, because the field-produced threads in this study were also well-aged (probably for at least 3 weeks), these observed differences may be age-dependent and are not necessarily attributable to differences between threads produced and maintained by mussels in nature *versus* an aquarium. Nonetheless, the structural properties of field-produced threads approach those of type B and, because of their lower extensibility, attachment strength is reduced even though individual thread strength is unchanged (Fig. 5; Table 6). If this loss of extensibility is age-related, the overall attachment strength of a mussel would be enhanced if old threads were continually replaced with newer, more extensible ones.

Interestingly, the production of many threads is a good strategy for increasing the likelihood that a single thread will form a solid attachment to a substratum of variable strength. Much of the primary intertidal substratum on the west coast of North America is granitic and it is often friable in patches due to weathering (E. C. Bell and J. M. Gosline, personal observation). When mussels are placed in tension in the field, it is often the substratum that fails, rather than the thread (E. C. Bell and J. M. Gosline, in preparation). By producing many small threads angled away from the shell, the mussel is able to 'sample' a larger area of substratum and thereby to increase the likelihood of encountering a good, stable patch of substratum for attachment. One potential difficulty with a tethering system constructed from many threads is in removing the 'slack' in each thread so that each is placed in tension. Mussels have largely avoided this potential problem by constructing their tensile elements from extensible materials.

How are byssal threads likely to be loaded in nature? The hydrodynamic forces encountered by mussels in the rocky intertidal have been modeled by E. C. Bell and J. M. Gosline (in preparation). For a typically sized *M. californianus* (length 8 cm), moderate wave action ( $5 \text{ m s}^{-1}$  water velocity) can generate 10–30 N of force, while heavy wave action ( $>10 \text{ m s}^{-1}$ ) can produce forces in excess of 50 N. If this load is distributed uniformly over 50 threads, such wave action can produce 0.2–0.6 N and more than 1 N of force per thread for moderate and heavy wave action, respectively. Thus, it is likely that byssal threads are most frequently loaded below their yield force and that mussels are fairly rigidly attached to the substratum (because their threads are loaded in the stiff region of their force–extension curve). For extreme flows, which are infrequent but do occur, threads are likely to be loaded beyond their yield force and can extend to recruit additional threads into tension.

The tensile tests presented in this study were conducted at relatively low extension rates and are probably most representative of the loading generated in mussel–mussel interactions within dense aggregations (e.g. Harger, 1971). Extension rates are probably much higher for mussels exposed

to breaking waves. Preliminary experiments conducted at higher extension rates (up to  $1 \text{ m min}^{-1}$ ) indicated a modest increase in  $k_i$  and an increase in yield force. The relative differences between yield and structural failure force, however, were unaffected by strain rate. Thus, the general conclusions on the mechanical and structural behavior of byssal threads presented in this study are also likely to apply to higher extension rates.

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