

A comparative study of the mechanical properties of Mytilid byssal threads

Trevor Pearce^{1,*} and Michael LaBarbera²

¹Committee on Evolutionary Biology and ²Department of Organismal Biology and Anatomy, the University of Chicago, Chicago, IL 60637, USA

*Author for correspondence (e-mail: trpearce@uchicago.edu)

Accepted 6 February 2009

SUMMARY

Mytilid bivalves employ a set of threads (the byssus) to attach themselves to both hard and soft substrates. In this study, we measured the mechanical properties of byssal threads from two semi-infaunal mytilids (*Geukensia demissa* Dillwyn and *Modiolus modiolus* Linnaeus) and two epifaunal mytilids (*Mytilus californianus* Conrad and *Mytilus edulis* Linnaeus). We compared material properties with and without the assumption that changes of length and area during tensile testing are insignificant, demonstrating that previous researchers have overestimated extensibility values by 30% and may also have underestimated strength values. We detected significant differences in thread properties among tested mytilid species, contrary to previous findings. Threads from semi-infaunal species were significantly thinner than those from epifaunal species, perhaps to allow the production of a greater number of threads, which form a dense network within the substrate. *Geukensia demissa* threads were weaker than those of the other species, and had a significantly lower stiffness at failure. *Modiolus modiolus* threads were significantly stiffer than *M. edulis* threads but also significantly less extensible, suggesting a trade-off between stiffness and extensibility. The only thread property that did not show significant differences across species was toughness – even when byssal threads differ in strength or stiffness, they seem to absorb similar amounts of energy per unit volume prior to failure. This study reveals notable differences between the byssal thread properties of different mytilid bivalves and provides a reliable and thorough methodology for future comparative studies.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/212/10/1442/DC1>

Key words: mussel, byssus, byssal threads, Mytilidae, biomechanics, material properties.

INTRODUCTION

A number of marine bivalves attach themselves to the substrate by means of a set of secreted threads, collectively termed the byssus. Although byssal threads seem to have initially evolved to aid in larval dispersal and post-larval settlement, many groups also use the byssus for adult attachment (Yonge, 1962; Stanley, 1972; Sigurdsson et al., 1976; De Blok and Tan-Maas, 1977; Lane et al., 1985). In a recent survey of tropical marine bivalves, 25% of genera (counting the Anomiidae) were identified as being exclusively byssally attached (Todd, 2001).

Early endobysate (infaunal or semi-infaunal species with byssal attachment) and epibysate (epifaunal species with byssal attachment) bivalves appear to have evolved successively from burrowing taxa, although some endobysate groups may be secondary soft-bottom dwellers (Stanley, 1972; Seilacher, 1984). Evolutionary trends have often involved the disappearance of byssate groups. In the Paleozoic, endobysate taxa declined, and the Mesozoic saw a reduction in exposed byssate suspension feeders (Stanley, 1972; Stanley, 1977; Skelton et al., 1990). These trends away from byssate forms may have been driven by increased predation pressure, which can push groups toward greater mobility or purely infaunal life habits (Vermeij, 1983; Aberhan et al., 2006; Harper, 2006).

This evolutionary history provides a rich background for comparisons of life habits and byssal properties among the modern Bivalvia. There are few extant endobysate taxa, whereas most epifaunal bivalves are byssally attached. Several orders within the Pteriomorpha contain both epibysate and endobysate taxa, but among these the Mytiloidea stand out for two reasons. First, the

mytiloid byssus always takes the form of numerous threads. Among the Arcoidea, in contrast, the byssus of semi-infaunal and infaunal species consists of a small number of threads, and the byssus of epifaunal species takes the form of a sheet or plug (Oliver and Holmes, 2006). Second, the mytiloid byssus is collagenous, which distinguishes it from the byssal threads of pinnids, anomniids and dreissenids (Jackson et al., 1953; Pujol et al., 1970; Mascolo and Waite, 1986; Anderson and Waite, 1998).

There have been several comparative studies of mytilid byssal thread properties. *Mytilus californianus* Conrad threads are stiffer and more extensible than those of *Mytilus trossulus* Gould and *Mytilus galloprovincialis* Lamarck, which may be a factor in the dominance of *M. californianus* on wave-swept shores (Bell and Gosline, 1996; Bell and Gosline, 1997; Carrington and Gosline, 2004). Recently, a comparison of the thread properties of endobysate and epibysate mytilids found no significant differences between species (Brazee and Carrington, 2006). This seems to indicate that thread material properties are not tailored to a specific flow regime or environment, and it could be argued on that basis that the number and size of byssal threads are more important than the threads' inherent material properties.

However, previous comparative studies suffer from a number of methodological problems. Because of the high variance in measured properties among different threads of the same species, a large sample size is often needed to obtain statistical power. More importantly, although it is known that the length and area of byssal threads, due to their high extensibility, change substantially during measurement of tensile properties, previous researchers have consistently reported material property values that assume negligible

changes in length and area (Smeathers and Vincent, 1979; Price, 1981; Bell and Gosline, 1996; Vaccaro and Waite, 2001; Lucas et al., 2002; Carrington and Gosline, 2004; Brazee and Carrington, 2006; Moeser and Carrington, 2006; Harrington and Waite, 2007). This assumption is violated to different degrees, depending on the extensibility of the sample in question. Thus, if thread extensibility varies significantly between species, and/or exceeds 10%, comparisons of material property values that rely on the assumption of negligible changes in area or length during testing can be misleading. Finally, a number of biologically interesting variables, e.g. toughness (energy absorbed before failure), have never been measured.

In this comparative study of four mytilid species, we have sought to avoid these methodological problems to the greatest extent possible. We employ 'logarithmic' strain, which assumes neither constant length nor constant volume. In calculating stress values, we rely on the assumption that thread volume remains constant during the testing procedure. Although there may be inaccuracies in our results (in proportion to any changes in sample volume), the constant volume assumption is more conservative than the constant area assumption. Our revised methodology allows a more powerful test of whether significant differences exist between the properties of the byssal threads of mytilid species with different life habits living in different environments. These new and likely more accurate measurements for mytilid threads provide a clear baseline for future comparisons with the threads of species outside the Mytilidae [see accompanying paper (Pearce and LaBarbera, 2009)].

MATERIALS AND METHODS

Mytilus edulis Linnaeus and *Modiolus modiolus* Linnaeus specimens were ordered from Gulf of Maine Inc. (Perry, ME, USA), and kept in a tank at 5°C, the approximate temperature at the collection site. *Geukensia demissa* Dillwyn specimens were ordered from Gulf Specimen Marine Laboratories Inc. (Panacea, FL, USA), and kept in a tank at room temperature, approximately 18°C. *Mytilus californianus* Conrad specimens were collected at Tatoosh Island in Washington state; this species was housed in the 5°C tank. Tank salinity was maintained at approximately 31–32 p.p.t. by adding either tap water or Instant Ocean® (Aquarium Systems, Inc., Mentor, OH, USA) sea salt mixture as necessary. Animals were fed daily on an artificial phytoplankton substitute (Kent Marine®, PhytoPlex™, Franklin, WI, USA); individuals produced byssal threads and survived without obvious ill effects for over 6 months. *Perna canaliculus* Philipsson byssal threads were collected from live animals at Cape Foulwind and Paia Point, New Zealand. Because the *P. canaliculus* threads dried and had to be re-hydrated (compromising their mechanical properties), we only include data on thread diameter for this species.

We measured the shell length of all *M. edulis*, *M. modiolus*, *G. demissa* and *M. californianus* specimens using digital calipers. Unfortunately, thread size could not be reliably correlated with individual shell length because of animal movement within the tanks. Shell length of the *P. canaliculus* specimens was measured onsite in New Zealand.

The animals were kept on glass plates at the bottom of each tank. To harvest threads, we transferred one of the plates and any attached animals (continuously submerged) to a smaller tray, which could be lifted out of the tank. We then snipped each thread with iris scissors at the proximal end, and separated the distal plaques from the glass plate using a razor blade. To allow collection of threads without dissection, and to ensure comparability with non-mytilid threads (which do not have the two distinct regions in the thread

typical of mytilids), we collected only the distal region of each thread for testing. All samples were stored in salt water (31–32 p.p.t.) at 5°C until testing.

Thread mechanical properties were measured using a custom-built tensile tester. The apparatus consisted of a lower grip at the bottom of a Plexiglas tank and an upper grip that could be displaced by turning a crank on a dovetail slider (Velmex, Bloomfield, NY, USA; Model A6027K1M-S6). The upper grip was attached to a 10 lb (~45 N full scale) force transducer (OmegaDyne®, Sunbury, OH, USA; Model LC703-10). The four strain gauges in the transducer were set up as a full Wheatstone bridge supplied with a constant 5 V excitation; the excitation and amplification of the voltage output of the bridge circuit were performed by a bridge amplifier (Vishay® Micro-Measurements, Shelton, CT, USA; Model 2120A). We calibrated the voltage output of the amplifier to determine a voltage-to-force conversion factor. A linear variable differential transformer (Pickering Controls, Plainview, NY, USA; Model 7308-X2-A0) powered by a constant 5 V DC from an external power supply converted the displacement of the upper grip into a voltage, which could then be converted back into a displacement value following calibration. The voltage was digitized using a GW Instruments (Somerville, MA, USA) Model 100B analog-to-digital converter.

We limited each testing run to 10–15 byssal thread samples to minimize drying during preparation. Between one and six byssal threads from each individual were tested, with a total sample of about 20–25 threads per species. To ensure proper gripping, we sandwiched each end of each thread between two small squares of 100% rag paper using a drop of cyanoacrylate adhesive (Loctite® 'Gel Control' super glue; Henkel Consumer Adhesives, Inc., Avon, OH, USA) to maximize adhesion. Before testing, we measured the length of each byssal thread sample with digital calipers.

Prior to each test, we secured one end of the thread in the upper grip of the tester and the other end in the lower grip at the base of the tank; the entire thread was immersed in sea water for the duration of the test. The tank was filled with sea water from the 5°C tank (salinity 31–32 p.p.t.) during all tests but the tank was maintained at room temperature. Once the thread was secured, we initiated data capture in the application instruNet World Mac (GW Instruments) and displaced the upper grip at approximately 0.5 mm s⁻¹ until thread failure. At the outset of the test, the samples were slack; the beginning of the tensile test was taken to be the point at which there was a non-negligible force on the sample.

Following testing, we inspected the broken ends of each byssal thread under a dissecting microscope to assess the failure mode (e.g. smooth break, fraying, etc.) and checked to ensure that all of the samples came from the smooth-surfaced distal thread region. We took digital photographs (Nikon D100 camera back) of each broken end through the dissecting microscope at approximately ×100, and measured thread diameter using ImageJ (NIH). Initially we measured the minimum thread diameter before testing, but discovered that the samples invariably broke at a different (and wider) location, presumably a cryptic weak point in the structure. Thus the diameter at failure was used in all calculations of strain to ensure consistency, although this undoubtedly results in underestimation of the inherent strength of byssal thread material.

The stress (force per unit area) and strain (displacement per unit length) for each test were plotted in Microsoft® Excel® to produce a stress–strain curve. Because strains were always in excess of 50%, it was clear that byssal thread cross-sectional area and length changed significantly during the test. Thus instead of 'engineering' strain ($\epsilon_E = \Delta L/L_0$, where L is length and subscript 0 indicates initial) we

Table 1. Diameter, shell length and breaking force

Species	Thread diameter (μm)	Range in shell length (mm)	Breaking force (N)
<i>Geukensia demissa</i> (S)	37.6 \pm 2.2 (32)	62.2–76.9 [69.1 \pm 1.6] (11)	0.074 \pm 0.008 (19) ^A
<i>Modiolus modiolus</i> (S)	46.3 \pm 2.4 (28)	49.2–72.3 [57.9 \pm 2.0] (12)	0.218 \pm 0.009 (20) ^A
<i>Mytilus californianus</i> (E)	149.6 \pm 6.6 (30)	49.4–91.4 [70.0 \pm 6.1] (7)	1.636 \pm 0.129 (21) ^B
<i>Mytilus edulis</i> (E)	132.3 \pm 6.0 (55)	58.6–88.4 [72.6 \pm 3.4] (10)	1.018 \pm 0.074 (25) ^C
<i>Perna canaliculus</i> (E)	129.7 \pm 8.2 (34)	50.0–70.0 [62.3 \pm 2.7] (7)	–

Values are means \pm s.e.m., followed by the sample size (N). The threads of semi-infaunal species, marked 'S', were significantly thinner than those of epifaunal species, marked 'E' (ANOVA, Scheffe test: $P < 0.0001$; Kruskal–Wallis: $P < 0.0001$). Shell length ranges of sampled individuals were strongly overlapping. In the 'Breaking force' column, values marked with the same superscript letter are not significantly different from one another (Scheffe test: $P < 0.0001$). *M. californianus* threads broke at significantly higher forces than *M. edulis* threads.

used 'true' or 'logarithmic' strain [$\epsilon_T = \ln(L/L_0)$], which does not assume constant length or constant volume. Stress is always calculated assuming a certain value for Poisson's ratio (ν), which is defined in this case as the negative of the ratio of transverse to axial strain. The instantaneous diameter of the thread is given by $d = d_0 \exp(-\nu \epsilon_T)$. There are two possible approaches. (1) 'Engineering' stress (σ_E) assumes constant area: $\nu = 0$, thus $d = d_0$ and $\sigma_E = F/A_0$ (where F is force and A is cross-sectional area). (2) 'True' stress (σ_T) assumes constant volume: $\nu = 0.5$, and $\sigma_T = \sigma_E \exp(\epsilon_T)$. We conservatively assumed constant volume rather than constant area (see Discussion). A number of different mechanical properties can be determined from the stress–strain curve. In almost all cases, there was a sharp drop in stiffness at a characteristic stress level – the yield stress. The slope of the stress–strain curve represents the stiffness of the material; thread stiffness was determined both for the initial loading of the thread and at thread failure. We also measured extensibility, or strain at failure, and strength, or maximum stress – the latter was equivalent to the failure stress in all but two cases. Finally, by fitting a polynomial to the curve and integrating over the total strain, the area under the stress–strain curve was determined; this area is the energy absorbed per unit volume, or the toughness of the material.

A small percentage of the byssal thread stress–strain curves for each species differed dramatically from the characteristic shape of the curve for that species. In almost all cases, the discrepancy appeared to result from splitting and fraying of the thread prior to failure; we did not include the data from these samples in the analysis.

We analyzed the data using StatView 5.0 (SAS Institute, Cary, NC, USA). First, we conducted an ANOVA on the threads of each individual, followed by an ANOVA of all threads of each species, split by individual. Because no significant differences were detected, we then pooled the individuals within each species and ran an overall ANOVA, split by species. We performed *post-hoc* Scheffe tests to determine the specific differences detected by the ANOVA. We also ran a Kruskal–Wallis test (a non-parametric version of a standard ANOVA), as a normal distribution of the data could not be assumed.

To compare 'engineering' stress and strain values with 'true' stress and strain values, we ran paired t -tests as well as the non-parametric equivalent, paired sign tests.

RESULTS

For all measured variables, ANOVA revealed no significant differences between threads of a given individual or between individuals of a given species; thus all threads for each species were pooled in the overall analysis.

Shell lengths of all species fell into a similar size range, 60–70 mm on average. Despite this, thread diameters for the three epifaunal species, *M. californianus*, *M. edulis* and *P. canaliculus*, were 3–4 times greater than those for the two semi-infaunal species, *M. modiolus* and *G. demissa* – a statistically significant difference (Table 1; $P < 0.0001$). *M. californianus* threads failed at forces 60% greater than those required to break *M. edulis* threads (Table 1; $P < 0.0001$), which appears to conflict with the fact that they do not differ significantly in diameter or inherent material strength (Tables 1 and 3). This conflict disappears, however, when only those threads for which force and strength data were measured are included in the analysis; in this restricted data set, *M. californianus* threads were more than 25% thicker than *M. edulis* threads (ANOVA, Scheffe test: $P = 0.0014$), explaining their higher breaking force values.

Using the 'engineering' definition of strain, the failure strain exceeded 75% for all threads. Because this value far exceeds the range (5–10%) where the assumptions underlying the 'engineering' approximation hold, 'true' strain values were also calculated (see Materials and methods). Stress values were calculated using both the 'engineering' and 'true' approaches, assuming constant area and constant volume, respectively. Table 2 illustrates the discrepancy between the calculated values of stress and strain using the two definitions of each variable.

Values for byssal thread strength (maximum stress) calculated using the 'true' stress approach were almost twice the values calculated using the 'engineering' definition. True values for thread extensibility (strain at failure), by contrast, were around 30% lower than those calculated using the 'engineering' definition (Table 2).

Table 2. 'Engineering' versus 'true' stress and strain

Species (N)	Maximum 'engineering' stress (MPa)	Maximum 'true' stress (MPa)	Δ	'Engineering' strain at failure	'True' strain at failure	Δ
<i>Geukensia demissa</i> (19)	75.1 \pm 10.4	140.8 \pm 18.7	+88%	0.897 \pm 0.034	0.637 \pm 0.018	–29%
<i>Modiolus modiolus</i> (20)	161.3 \pm 19.8	287.8 \pm 35.6	+78%	0.781 \pm 0.045	0.571 \pm 0.024	–27%
<i>Mytilus californianus</i> (21)	111.0 \pm 11.7	215.3 \pm 25.3	+94%	0.901 \pm 0.032	0.639 \pm 0.016	–29%
<i>Mytilus edulis</i> (25)	109.2 \pm 8.6	216.9 \pm 18.8	+99%	0.958 \pm 0.034	0.669 \pm 0.017	–30%

Values given are means \pm s.e.m. For both stress and strain and for all species, 'true' values were significantly different from 'engineering' values, allowing us to reject the hypothesis that changes in thread area and length during the testing procedure were insignificant (paired t -test: $P < 0.0001$; paired sign test: $P < 0.0001$). Note that the discrepancy (Δ) between 'engineering' and 'true' values increases as strain at failure increases.

These large discrepancies arise from the high strains that byssal threads can undergo before failure – the greater the strain, the greater the discrepancy.

‘True’ stress and strain were used to construct the stress–strain curves for all of the byssal thread samples. The curve for a representative thread sample from each species is given in Fig. 1. As previously reported (Brazee and Carrington, 2006), *M. modiolus* byssal threads exhibit two distinct yield points, one in the same range as the yield points of the threads of the other species in this study, and one at a higher stress and strain.

Geukensia demissa byssal threads were less stiff and weaker than those of the other species. The extensibility of *G. demissa* threads did not differ significantly from that of the threads of the other species, but *G. demissa* threads uniformly exhibited the lowest values for all other material properties and each was significantly different from at least a subset of the thread properties of the other species (Fig. 1; Table 3).

Modiolus modiolus byssal threads were significantly stronger than those of *G. demissa*. Although *M. modiolus* threads were significantly stiffer than *M. edulis* threads, they were also significantly less extensible (Table 3). The byssal threads of *M. californianus* and *M. edulis* did not differ significantly in any of their material properties, despite *M. californianus* threads breaking on average at higher forces (Table 1, Table 3). ANOVA revealed significant differences between species for all mechanical properties except toughness, the energy absorbed by the material before failure (Table 3). (For a complete list of property values for all the individual threads tested in this study, see supplementary material Table S1.)

DISCUSSION

As biomechanics researchers have often pointed out, biologists can easily be led astray by the formulas engineers have developed to understand the behavior of common structural materials like metals. Biological materials have quite different properties, and often violate the assumptions that were used to generate the formulas (Vogel, 2003). Biologists are often more interested in the properties of structures than in the properties of materials. In this study, however, we have focused on the inherent material properties of byssal threads – this is what is meant by the shorthand ‘thread properties’. Thus, even though *M. edulis* threads can support greater forces than *M. modiolus* threads, this is only because the former are much thicker; there is no significant difference in inherent strength between the materials that make up the threads of these two species (Table 1, Table 3).

Byssal threads produced in aquaria may have different properties to byssal threads produced in the wild, given that byssal thread chemistry and mechanical properties can be greatly affected by external conditions (Moeser and Carrington, 2006). Comparisons between the breaking force of laboratory-produced and field-produced threads have revealed significant differences, although it is unknown whether these translate into significant differences in inherent material properties (Bell and Gosline, 1996). One advantage of testing laboratory-produced threads, however, is that the different species produce their threads under relatively similar circumstances – the controlled environments of aquaria. The results in this study can thus be used as a baseline of comparison for future studies of field-produced byssal threads.

The results presented in Table 2 indicate that previous studies of byssal thread material properties have overestimated the inherent extensibility of thread material by almost 30%. Although there is an even larger discrepancy between the values for ‘engineering’ and

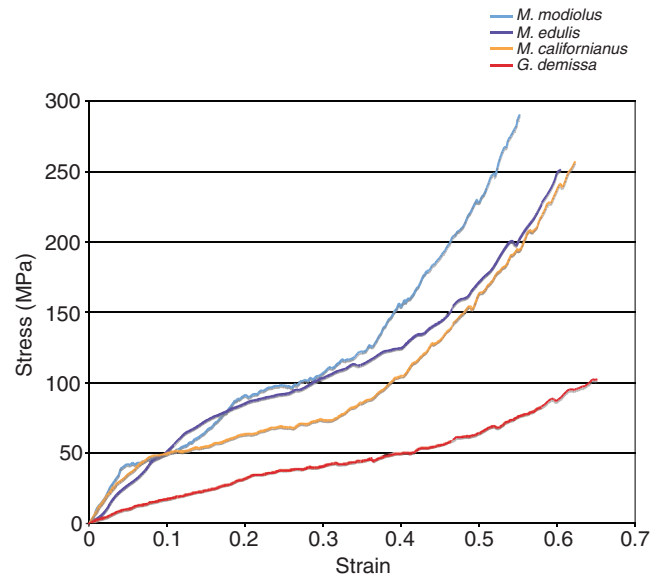


Fig. 1. Byssal thread stress–strain curves. A typical stress–strain curve was chosen for each species. Note that the *Modiolus modiolus* curve has two distinct yield points. It is also apparent that the final stiffness (final slope of the curve) of *Geukensia demissa* threads was lower than that of the other species, as was their strength.

‘true’ stress – the latter are 75–100% greater than the former – the calculation of ‘true’ strain does not assume constant volume during tensile testing while the calculation of ‘true’ stress does. Hence, in the comparison between ‘engineering’ and ‘true’ stress as measures of the inherent strength of a material, we are faced with two competing assumptions: either (1) the Poisson’s ratio is assumed to be zero, i.e. constant area during testing is assumed; or (2) the Poisson’s ratio is assumed to be 0.5, i.e. constant volume during testing is assumed (see Materials and methods).

We think ‘true’ stress is a superior measure of the strength of byssal thread materials because it makes the more conservative assumption. It is known that thread area is reduced during testing, and that this reduction is substantial given that byssal threads can be stretched to almost twice their length prior to failure. However, it is not known whether thread volume remains constant during testing; the Poisson’s ratio (ν) of byssal threads has never been measured. If $\nu=0.00-0.25$, ‘engineering’ stress is more accurate than ‘true’ stress, whereas if $\nu=0.25-0.50$, ‘true’ stress is more accurate. For materials with complex architecture, it is possible to have $\nu>0.5$, but although both ‘engineering’ stress and ‘true’ stress become more inaccurate as ν enters this higher range, calculations based on the strains in our study show that ‘true’ stress is more accurate for $\nu=0.25-0.90$ (supplementary material Fig. S1). Moreover, published data for similar materials suggest that the constant volume assumption may hold for byssal threads: the Poisson’s ratio of rubber is 0.5, and spider silk fibers do not change in volume during tensile testing (Vogel, 2003; Guinea et al., 2006). Employing the proper formulas, it is straightforward to convert ‘true’ stress into ‘engineering’ stress, and also to breaking force given thread area, and thus it is straightforward to compare new results with those of previous researchers. (We have provided complete values for all of these variables for each individual thread tested in supplementary material Table S1.)

The benefits of using ‘true’ stress and strain are many. The instantaneous stiffness can be calculated at any point on the

Table 3. Byssal thread material properties

Species (N)	Yield stress (MPa)	Strength (MPa)	Initial stiffness (MPa)	Final stiffness (MPa)	Extensibility	Toughness (J m ⁻³)
<i>Geukensia demissa</i> (19)	23.9±4.2 ^A	140.8±18.7 ^A	324.7±60.8 ^A	319.1±45.2 ^A	0.637±0.018 ^{A,B}	43.3±5.6
<i>Modiolus modiolus</i> (20)	35.5±5.8 ^{A,B}	287.8±35.6 ^B	593.3±94.6 ^B	1039.6±129.0 ^B	0.571±0.024 ^A	67.4±8.5
<i>Mytilus californianus</i> (21)	33.0±2.9 ^{A,B}	215.3±25.3 ^{A,B}	432.3±45.5 ^{A,B}	810.0±93.9 ^B	0.640±0.016 ^{A,B}	51.7±5.9
<i>Mytilus edulis</i> (25)	44.4±6.6 ^B	216.9±18.8 ^{A,B}	328.6±30.8 ^A	784.4±62.7 ^B	0.669±0.017 ^B	56.9±6.3
ANOVA, <i>P</i> -value	0.0298	0.0023	0.0068	<0.0001	0.0042	0.1060
Kruskal–Wallis, <i>P</i> -value	0.0057	0.0044	0.0079	<0.0001	0.0030	0.1828
Scheffe test, <i>P</i> -value	0.0345	0.0023	<0.0310	<0.0040	0.0050	n.a.

Values given are means ± s.e.m. *P*-values in bold are significant. For each property apart from toughness, the null hypothesis of similar values across species was robustly rejected (ANOVA and Kruskal–Wallis). In each column, values marked with the same superscript letter are not significantly different from one another (Scheffe test). Because the yield point was not obvious in all tests, only 12 *M. edulis*, 13 *M. modiolus* and 20 *M. californianus* data points were used in the analysis for yield stress.

stress–strain curve by taking the first derivative to find the slope at that point, and the inherent toughness of the material, or the energy absorbed by the material prior to failure, can be accurately calculated (Table 3). In comparative studies both within and outside the Bivalvia the use of ‘true’ stress and strain is especially important, since the discrepancy between ‘engineering’ and ‘true’ values is dependent on the inherent extensibility of the material in question. For example, the methodology of the current study allows accurate comparison, for the first time, of bivalve byssal thread and the dragline silk of spiders – the former is more extensible, but not as stiff, strong or tough as the latter (Swanson et al., 2006). Of course, if the Poisson’s ratio of byssal thread materials were to vary greatly between bivalve species, this would also compromise comparisons; thus, we hope that these ratios will be measured by future workers.

The correlation between life habit and thread diameter found here – that epifaunal species have thicker threads – was not found in another recent comparative study (Brazeo and Carrington, 2006). One likely reason for this discrepancy is that the *M. modiolus* and *G. demissa* specimens used in the earlier study were larger, and the *M. edulis* specimens smaller, than those used here; thus the relationship between life habit and thread size for mussels of similar shell lengths could not be observed. Although the threads of semi-epifaunal species are thinner than those of epifaunal species, it has been shown that *M. modiolus* produces many more threads than *M. edulis*, especially for substrate particle sizes from 250 to 2000 µm (Meadows and Shand, 1989). This relationship between life habit and thread number also seems to hold for *M. californianus* and *G. demissa* (T.P. and M.L., personal observation). Producing a smaller number of larger diameter threads, then, may be beneficial for mussels with epifaunal life habits, or *vice versa* for mussels with semi-epifaunal life habits. Thread diameter measurements for epifaunal *Ctenoides mitis* Lamarck and semi-epifaunal *Atrina rigida* Lightfoot support the relationship reported here between life habit and byssal thread diameter [see accompanying paper (Pearce and LaBarbera, 2009)]. Because overall attachment strength is a function not just of material properties but also of thread size and thread number, future work should consider measuring the fraction of proteinaceous nitrogen devoted to the byssal apparatus in different bivalves. This would indicate whether certain species achieve a greater attachment strength with a similar investment of resources.

There were no significant differences in material properties between the threads of the two *Mytilus* species examined here, despite recent claims that *M. californianus* threads are ‘mechanically superior’ to *M. edulis* threads (Carrington and Gosline, 2004; Harrington and Waite, 2007). The evidence for such claims in the literature is slight. Bell and Gosline combined their *M. californianus* data with the *M.*

edulis data of Smeathers and Vincent, purporting to show that both the distal and proximal regions of *M. californianus* threads are significantly more extensible than those of *M. edulis* threads (Smeathers and Vincent, 1979; Bell and Gosline, 1996). However, both studies used the ‘engineering’ definitions of stress and strain and each used a different strain rate during tensile testing; moreover, Smeathers and Vincent only provided 10 data points on extensibility for *M. edulis*, which is problematic given the high variance in thread properties within a given species. Citing Bell and Gosline, Harrington and Waite also claim that *M. californianus* threads are 2–3 times stiffer than *M. edulis* threads – this is a mistake, as no stiffness values for *M. edulis* threads were presented in the earlier paper (Bell and Gosline, 1996; Harrington and Waite, 2007). Nonetheless, several differences in the sequences of the proteins making up the distal regions of *M. californianus* and *M. edulis* threads have recently been discovered, and thus more research is needed to determine whether these molecular differences translate into significant differences in thread material properties (Harrington and Waite, 2007). *Mytilus edulis* threads do seem to recover more slowly than *M. californianus* threads following cyclical loading, but the functional importance of this difference is unclear (Carrington and Gosline, 2004). While *M. californianus* may be better adapted to wave-swept shores, and may have a greater overall attachment strength than *M. edulis*, our findings indicate that the latter is likely to be due to differences in the number or size of threads rather than to any inherent mechanical superiority of the material in *M. californianus* threads.

The stress–strain curves presented here are qualitatively similar to those found in earlier studies. It has been suggested that homogeneous threads, i.e. those lacking two distinct regions (proximal and distal), have less complex stress–strain behavior (Brazeo and Carrington, 2006). However, there were clear yield points for almost all threads of all species tested here, despite the fact that samples were taken only from the distal region. The extraordinary double-yield behavior of *M. modiolus* threads was consistently produced when testing only the distal portion of threads. Although *G. demissa* threads did not have two clear yield points, new results for *A. rigida* threads suggest that double-yield behavior may be correlated with endobysal life habits (Pearce and LaBarbera, 2009). It would be interesting to re-examine existing molecular analyses of *M. modiolus* and *A. rigida* threads in light of this unusual yield pattern, which seems to imply an underlying two-phase molecular structure (Mascolo and Waite, 1986; Rzepecki et al., 1991).

Early comparative studies demonstrated that there are significant differences in mechanical properties between the threads of different *Mytilus* species (Bell and Gosline, 1996). However, it has been

suggested more recently that material properties of mytilid threads tend to be similar across a range of life habits and environments (Brazee and Carrington, 2006). The data presented here confirm that there are indeed significant mechanical differences between the threads of different mytilid bivalves. One of the most interesting findings is that, although threads of different species tend to differ in strength, stiffness and extensibility, ANOVA indicates that they absorb similar amounts of energy prior to failure, i.e. they are equally tough. One explanation for this observation may be that species with stronger threads, e.g. *M. modiolus*, tend to have less extensible threads, and toughness is a function of both strength and extensibility. In many engineered materials, there is a trade-off between strength or stiffness and extensibility – think of ceramics, which are extremely stiff and strong, but are minimally extensible before failure. Likewise, although *M. edulis* threads are significantly more extensible than *M. modiolus* threads, the latter are significantly stiffer. However, this trade-off between stiffness and extensibility does not divide semi-infaunal from epifaunal species, as might be expected given the difference in experienced fluid forces. Semi-infaunal *G. demissa* threads have mechanical properties similar to those of epifaunal *Mytilus* threads, apart from a lower final stiffness. Another hypothesis for the similar toughness of all threads might simply be that energy absorption by byssal threads is the most important variable when it comes to attachment or predator resistance, and the different species simply achieve this toughness via different combinations of other mechanical properties.

Despite the fact that semi-infaunal and epifaunal species do not group along any of the mechanical variables measured here, the two semi-infaunal species investigated each stand out, albeit for different reasons. *Geukensia demissa* threads have a significantly lower stiffness at failure than the threads of other mytilids; moreover, for all species but *G. demissa*, final stiffness was significantly greater than initial stiffness (paired *t*-test, $P < 0.0004$; paired sign test, $P < 0.0001$). The low strength and stiffness of threads produced by *G. demissa* may be related to its habitat – it usually lives in low intertidal peat marshes, and attaches to the stems and roots of grasses (Stanley, 1970). The underground network of roots and threads, together with the peat surrounding its shell, may enhance overall attachment strength, eliminating any selection pressure for stronger or stiffer threads. To assess this hypothesis, one could compare the properties of *G. demissa* threads with those of the threads of *Modiolus americanus* Leach, which also frequently attaches to stems and roots in seagrass meadows (Peterson and Heck, 2001). This comparison would be especially interesting, as the *M. modiolus* threads measured here were significantly stronger than those of *G. demissa*.

Most studies of bivalve byssal threads have focused on the effect of abiotic or biotic ecological variables on the size and number of threads produced (Meadows and Shand, 1989; Dolmer and Svane, 1994; Côté, 1995; Clarke and McMahon, 1996; Leonard et al., 1999; Cheung et al., 2006; Moeser et al., 2006). Recently, however, it has been shown that thread material properties vary with the seasons, indicating that more controlled studies investigating the influence of ecological factors on thread biomechanics would produce interesting results (Moeser and Carrington, 2006). Given recent data demonstrating significant variation in thread properties among the Mytilidae, the time is ripe for a systematic comparative study involving ecological, taxonomic and biomechanical variables. A first step in this direction would involve measuring the mechanical properties of byssal threads outside the Mytilidae, to find out whether they suggest any evolutionary patterns (see Pearce and LaBarbera, 2009).

This study has illustrated the fruitfulness of comparative work in byssal thread biomechanics. There are significant differences in strength, stiffness and extensibility between different mytilid species living in different environments. Further research, following the methodology outlined here, has the potential to reveal patterns in the evolutionary history of this biomechanical variation.

We thank J. Timothy Wootton for collecting the *Mytilus californianus* specimens and Mark Novak for collecting *Perna canaliculus* byssal threads and measuring the lengths of the animals which produced them.

REFERENCES

- Aberhan, M., Kiessling, W. and Fursich, F. T. (2006). Testing the role of biological interactions in the evolution of mid-Mesozoic marine benthic ecosystems. *Paleobiology* **32**, 259-277.
- Anderson, K. E. and Waite, J. H. (1998). A major protein precursor of zebra mussel (*Dreissena polymorpha*) byssus: deduced sequence and significance. *Biol. Bull.* **194**, 150-160.
- Bell, E. C. and Gosline, J. M. (1996). Mechanical design of mussel byssus: material yield enhances attachment strength. *J. Exp. Biol.* **199**, 1005-1017.
- Bell, E. C. and Gosline, J. M. (1997). Strategies for life in flow: tenacity, morphometry, and probability of dislodgment of two *Mytilus* species. *Mar. Ecol. Prog. Ser.* **159**, 197-208.
- Brazee, S. L. and Carrington, E. (2006). Interspecific comparison of the mechanical properties of mussel byssus. *Biol. Bull.* **211**, 263-274.
- Carrington, E. and Gosline, J. M. (2004). Mechanical design of mussel byssus: load cycle and strain rate dependence. *Am. Malacol. Bull.* **18**, 135-142.
- Cheung, S. G., Luk, K. C. and Shin, P. K. S. (2006). Predator-labeling effect on byssus production in marine mussels *Perna viridis* (L.) and *Brachidontes variabilis* (Krauss). *J. Chem. Ecol.* **32**, 1501-1512.
- Clarke, M. and McMahon, R. F. (1996). Effects of hypoxia and low-frequency agitation on byssogenesis in the freshwater mussel *Dreissena polymorpha* (Pallas). *Biol. Bull.* **191**, 413-420.
- Côté, I. M. (1995). Effects of predatory crab effluent on byssus production in mussels. *J. Exp. Mar. Biol. Ecol.* **188**, 233-241.
- De Blok, J. W. and Tan-Maas, M. (1977). Function of byssal threads in young postlarval *Mytilus*. *Nature* **267**, 558.
- Dolmer, P. and Svane, I. (1994). Attachment and orientation of *Mytilus edulis* L. in flowing water. *Ophelia* **40**, 63-74.
- Guinea, G. V., Pérez-Rigueiro, J., Plaza, G. R. and Elices, M. (2006). Volume constancy during stretching of spider silk. *Biomacromolecules* **7**, 2173-2177.
- Harper, E. M. (2006). Dissecting post-Palaeozoic arms races. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **232**, 322-343.
- Harrington, M. J. and Waite, J. H. (2007). Holdfast heroics: comparing the molecular and mechanical properties of *Mytilus californianus* byssal threads. *J. Exp. Biol.* **210**, 4307-4318.
- Jackson, S. F., Kelly, F. C., North, A. C. T., Randall, J. T., Seeds, W. E., Watson, M., Wilkinson, G. R. and Randall, J. T. (1953). The byssus threads of *Mytilus edulis* and *Pinna nobilis*. In *The Nature and Structure of Collagen* (ed. J. T. Randall), pp. 106-116. London: Butterworths Scientific.
- Lane, D. J. W., Beaumont, A. R. and Hunter, J. R. (1985). Byssus drifting and the drifting threads of the young post-larval mussel *Mytilus edulis*. *Mar. Biol.* **84**, 301-308.
- Leonard, G. H., Bertness, M. D. and Yund, P. O. (1999). Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. *Ecology* **80**, 1-14.
- Lucas, J. M., Vaccaro, E. and Waite, J. H. (2002). A molecular, morphometric and mechanical comparison of the structural elements of byssus from *Mytilus edulis* and *Mytilus galloprovincialis*. *J. Exp. Biol.* **205**, 1807-1817.
- Mascolo, J. M. and Waite, J. H. (1986). Protein gradients in byssal threads of some marine bivalve mollusks. *J. Exp. Zool.* **240**, 1-7.
- Meadows, P. S. and Shand, P. (1989). Experimental analysis of byssus thread production by *Mytilus edulis* and *Modiolus modiolus* in sediments. *Mar. Biol.* **101**, 219-226.
- Moeser, G. M. and Carrington, E. (2006). Seasonal variation in mussel byssal thread mechanics. *J. Exp. Biol.* **209**, 1996-2003.
- Moeser, G. M., Leba, H. and Carrington, E. (2006). Seasonal influence of wave action on thread production in *Mytilus edulis*. *J. Exp. Biol.* **209**, 881-890.
- Oliver, P. G. and Holmes, A. M. (2006). The Arcoidea (Mollusca: Bivalvia): a review of the current phenetic-based systematics. *Zool. J. Linn. Soc.* **148**, 237-251.
- Pearce, T. and LaBarbera, M. (2009). Biomechanics of byssal threads outside the Mytilidae: *Atrina rigida* and *Ctenoides mitis*. *J. Exp. Biol.* **212**, 1449-1454.
- Peterson, B. J. and Heck, K. L. (2001). Positive interactions between suspension-feeding bivalves and seagrass: a facultative mutualism. *Mar. Ecol. Prog. Ser.* **213**, 143-155.
- Price, H. A. (1981). Byssus thread strength in the mussel, *Mytilus edulis*. *J. Zool.* **194**, 245-255.
- Pujol, J. P., Rolland, M., Lasry, S. and Vinet, S. (1970). Comparative study of amino acid composition of byssus in some common bivalve molluscs. *Comp. Biochem. Physiol.* **34**, 193-201.
- Rzepecki, L. M., Chin, S. S., Waite, J. H. and Lavin, M. F. (1991). Molecular diversity of marine glues: polyphenolic proteins from five mussel species. *Mol. Mar. Biol. Biotechnol.* **1**, 78-88.
- Seilacher, A. (1984). Construction morphology of bivalves: evolutionary pathways in primary versus secondary soft-bottom dwellers. *Palaeontology* **27**, 207-237.
- Sigurdsson, J. B., Titman, C. W. and Davies, P. A. (1976). Dispersal of young post-larval bivalve mollusks by byssus threads. *Nature* **262**, 386-387.

- Skelton, P. W., Crame, J. A., Morris, N. J. and Harper, E. M.** (1990). Adaptive divergence and taxonomic radiation in post-Palaeozoic bivalves. In *Major Evolutionary Radiations* (ed. P. D. Taylor and G. P. Larwood), pp. 91-117. Oxford: Clarendon Press.
- Smeathers, J. E. and Vincent, J. F. V.** (1979). Mechanical properties of mussel byssus threads. *J. Molluscan Stud.* **45**, 219-230.
- Stanley, S. M.** (1970). *Relation of Shell Form to Life Habits of the Bivalvia (Mollusca)*. Boulder, CO: Geological Society of America.
- Stanley, S. M.** (1972). Functional morphology and evolution of byssally attached bivalve mollusks. *J. Paleontol.* **46**, 165-212.
- Stanley, S. M.** (1977). Trends, rates, and patterns of evolution in the Bivalvia. In *Patterns of Evolution, As Illustrated by the Fossil Record* (ed. A. Hallam), pp. 209-250. Amsterdam: Elsevier.
- Swanson, B. O., Blackledge, T. A., Summers, A. P. and Hayashi, C. Y.** (2006). Spider dragline silk: correlated and mosaic evolution in high-performance biological materials. *Evolution* **60**, 2539-2551.
- Todd, J.** (2001). Neogene marine biota of tropical America: bivalve life habits database. Iowa City, IA: University of Iowa.
- Vaccaro, E. and Waite, J. H.** (2001). Yield and post-yield behavior of mussel byssal thread: a self-healing biomolecular material. *Biomacromolecules* **2**, 906-911.
- Vermeij, G. J.** (1983). Traces and trends of predation, with special reference to bivalved animals. *Palaeontology* **26**, 455-465.
- Vogel, S.** (2003). *Comparative Biomechanics: Life's Physical World*. Princeton, NJ: Princeton University Press.
- Yonge, C. M.** (1962). On the primitive significance of the byssus in Bivalvia and its effects in evolution. *J. Mar. Biolog. Assoc. UK* **42**, 112-125.

Table S1. Complete database of byssal thread properties

Species	Thread length (mm)	Thread diameter (mm)	Thread area (mm ²)	Maximum force (N)	Total displacement (mm)	Maximum engineering stress (MPa)	Maximum true stress (MPa)	Yield stress (MPa)	Initial stiffness (MPa)	Final stiffness (MPa)	Toughness (MJ m ³)	Engineering failure strain	True failure strain	Yield strain
<i>Mytilus edulis</i>	5.95	123.27	0.01193	1.12923	4.84909	94.619	171.731		308.57	732.51	34.91	0.8150	0.5961	
<i>Mytilus edulis</i>	6.37	106.44	0.00890	1.36117	6.14978	152.973	300.657		485.75	1303.30	69.89	0.9654	0.6757	
<i>Mytilus edulis</i>	5.91	95.24	0.00712											
<i>Mytilus edulis</i>	3.99	114.36	0.01027	1.23440	5.22537	120.176	277.560		241.25	1242.45	78.02	1.3096	0.8371	
<i>Mytilus edulis</i>	2.97	119.55	0.01123	1.34394	4.11769	119.727	285.719		405.22	911.79	94.26	1.3864	0.8698	
<i>Mytilus edulis</i>	4.35	76.49	0.00460	0.97601	5.17023	212.399	464.848		861.21	1485.28	158.31	1.1886	0.7832	
<i>Mytilus edulis</i>	5.00	149.01	0.01744	1.71933	4.53773	98.591	188.067		307.07	624.54	46.53	0.9075	0.6458	
<i>Mytilus edulis</i>	4.31	146.27	0.01680											
<i>Mytilus edulis</i>	5.74	101.85	0.00815	1.26691	5.27240	155.501	298.334		231.10	948.29	71.41	0.9185	0.6516	
<i>Mytilus edulis</i>	13.00	221.85	0.03866											
<i>Mytilus edulis</i>	4.59	168.81	0.02238											
<i>Mytilus edulis</i>	7.54	109.90	0.00949	1.26169	7.12932	133.005	258.765		453.78	1201.72	56.76	0.9455	0.6655	
<i>Mytilus edulis</i>	5.51	129.46	0.01316	0.97097	4.69504	73.764	136.618		286.78	641.68	28.82	0.8521	0.6163	
<i>Mytilus edulis</i>	3.29	137.87	0.01493	1.26458	2.72134	84.706	154.771		319.45	600.46	35.51	0.8272	0.6028	
<i>Mytilus edulis</i>	2.43	170.30	0.02278											
<i>Mytilus edulis</i>	1.99	162.38	0.02071	1.41659	1.30066	68.405	113.115		231.46	577.57	23.21	0.6536	0.5030	
<i>Mytilus edulis</i>	2.93	126.73	0.01261	0.78030	1.96721	61.860	103.394		271.02	483.36	21.18	0.6714	0.5137	
<i>Mytilus edulis</i>	2.43	122.77	0.01184	1.36462	1.94775	115.275	207.673		341.66	834.55	45.67	0.8015	0.5886	
<i>Mytilus edulis</i>	7.05	132.02	0.01369	1.18339	6.81169	86.449	169.976	28.29	252.15	744.32	40.85	0.9662	0.6761	0.1161
<i>Mytilus edulis</i>	4.89	86.15	0.00583	0.95015	4.55899	163.001	314.968	68.65	560.19	1058.42	87.26	0.9323	0.6587	0.1231
<i>Mytilus edulis</i>	5.08	167.34	0.02199	1.01060	4.66764	45.950	88.171	20.92	150.94	336.84	24.09	0.9188	0.6517	0.1409
<i>Mytilus edulis</i>	3.44	106.93	0.00898	1.07627	4.07404	119.848	261.787	57.90	328.83	770.03	82.72	1.1843	0.7813	0.1771
<i>Mytilus edulis</i>	3.88	141.04	0.01562	1.26328	3.87312	80.859	161.574	30.26	204.81	597.35	41.80	0.9982	0.6923	0.1503
<i>Mytilus edulis</i>	4.30	153.62	0.01853											
<i>Mytilus edulis</i>	4.02	136.14	0.01456											
<i>Mytilus edulis</i>	3.31	136.61	0.01466											
<i>Mytilus edulis</i>	3.07	153.42	0.01849											
<i>Mytilus edulis</i>	2.86	78.13	0.00479	0.90012	2.78469	187.748	370.552	53.56	405.31	1111.43	96.81	0.9737	0.6799	0.1266
<i>Mytilus edulis</i>	3.16	127.28	0.01272											
<i>Mytilus edulis</i>	5.70	62.66	0.00308	0.36525	5.65701	118.446	235.998	44.22	394.88	897.39	61.20	0.9925	0.6894	0.1210
<i>Mytilus edulis</i>	3.95	60.40	0.00287	0.28216	3.87623	98.476	195.112	40.41	373.49	482.10	55.18	0.9813	0.6838	0.1267
<i>Mytilus edulis</i>	3.86	73.76	0.00427											
<i>Mytilus edulis</i>	3.58	67.69	0.00360	0.47135	3.52749	130.979	260.036	92.24	291.18	697.31	69.08	0.9853	0.6858	0.3076
<i>Mytilus edulis</i>	3.51	69.64	0.00381	0.39888	3.47887	104.721	208.513	60.37	154.41	614.77	52.44	0.9911	0.6887	0.3037
<i>Mytilus edulis</i>	5.03	168.82	0.02238	0.84694	4.61254	37.837	72.534	14.91	106.47	244.04	18.52	0.9170	0.6508	0.1439
<i>Mytilus edulis</i>	4.74	109.41	0.00940											
<i>Mytilus edulis</i>	5.42	109.07	0.00934	0.61135	4.73575	65.432	122.604	21.55	246.86	469.40	28.85	0.8738	0.6279	0.1098
<i>Mytilus edulis</i>	15.68	176.24	0.02439											
<i>Mytilus edulis</i>	10.48	118.30	0.01099											
<i>Mytilus edulis</i>	12.74	201.91	0.03202											
<i>Mytilus edulis</i>	12.32	211.72	0.03521											
<i>Mytilus edulis</i>	11.36	223.29	0.03916											
<i>Mytilus edulis</i>	9.84	202.97	0.03236											
<i>Mytilus edulis</i>	11.64	216.39	0.03678											
<i>Mytilus edulis</i>	9.68	132.92	0.01388											
<i>Mytilus edulis</i>	9.65	114.94	0.01038											
<i>Mytilus edulis</i>	8.90	129.22	0.01311											
<i>Mytilus edulis</i>	7.58	138.62	0.01509											
<i>Mytilus edulis</i>	10.09	139.18	0.01521											
<i>Mytilus edulis</i>	9.83	127.73	0.01281											
<i>Mytilus edulis</i>	7.58	169.90	0.02267											
<i>Mytilus edulis</i>	9.84	224.77	0.03968											
<i>Mytilus edulis</i>	8.74	57.15	0.00257											
<i>Mytilus edulis</i>	8.15	73.44	0.00424											
<i>Mytilus edulis</i>	9.38	95.85	0.00722											
<i>Mytilus californianus</i>	11.03	162.87	0.02083	1.51435	8.53079	72.687	128.904	38.67	512.59	672.20	31.96	0.7734	0.5729	0.0948
<i>Mytilus californianus</i>	12.89	172.03	0.02324	2.32569	14.24610	100.059	210.644	30.47	427.59	800.96	50.47	1.1052	0.7444	0.0962
<i>Mytilus californianus</i>	8.03	165.84	0.02160											
<i>Mytilus californianus</i>	8.53	159.02	0.01986	1.42642	6.76300	71.821	128.765	30.04	486.45	592.14	29.12	0.7928	0.5838	0.0913
<i>Mytilus californianus</i>	17.20	142.77	0.01601	1.67643	21.03828	104.718	232.805	24.91	378.30	1086.22	52.82	1.2232	0.7989	0.0970
<i>Mytilus californianus</i>	7.94	85.89	0.00579	1.51519	9.32712	261.512	568.710	37.63	408.12	2135.38	129.46	1.1747	0.7769	0.0980
<i>Mytilus californianus</i>	12.15	155.83	0.01907											
<i>Mytilus californianus</i>	9.57	87.46	0.00601											
<i>Mytilus californianus</i>	8.17	115.63	0.01050	1.16941	8.50809	111.362	227.333		118.41	929.87	46.60	1.0414	0.7136	
<i>Mytilus californianus</i>	14.62	130.25	0.01332	1.36402	11.21990	102.371	180.933	30.73	540.69	663.40	41.76	0.7674	0.5695	0.0752
<i>Mytilus californianus</i>	15.00	131.93	0.01367	1.88715	12.97468	138.048	257.457	29.12	810.09	883.07	63.00	0.8650	0.6232	0.0372
<i>Mytilus californianus</i>	12.11	151.53	0.01803	1.37417	8.74170	76.200	131.205	31.80	410.73	409.54	31.69	0.7219	0.5434	0.0938
<i>Mytilus californianus</i>	12.19	128.05	0.01288	2.07095	11.31396	160.813	310.069	54.00	814.48	1185.47	74.78	0.9281	0.6566	0.0861
<i>Mytilus californianus</i>	9.96	135.99	0.01452	1.42830	7.60157	98.336	173.388	46.54	581.01	834.48	43.73	0.7632	0.5671	0.0908
<i>Mytilus californianus</i>	13.85	131.37	0.01355											
<i>Mytilus californianus</i>	14.84	244.57	0.04698											
<i>Mytilus californianus</i>	10.36	157.92	0.01959											
<i>Mytilus californianus</i>	10.06	122.80	0.01184	1.26453	9.17638	106.769	204.159	37.17	497.27	636.54	50.88	0.9122	0.6482	0.0901
<i>Mytilus californianus</i>	17.40	215.35	0.03642											
<i>Mytilus californianus</i>	11.98	170.06	0.02271	1.84328	11.15336	81.152	156.704	22.16	158.11	612.99	37.99	0.9310	0.6580	0.1412
<i>Mytilus californianus</i>	9.46	122.59	0.01180	1.24228	8.21469	105.249	196.644	24.66	381.29	818.15	49.43	0.8684	0.6251	0.0707
<i>Mytilus californianus</i>	10.44	171.26	0.02304											
<i>Mytilus californianus</i>	9.87	144.72	0.01645	2.16596	9.67913	131.675	260.803	30.80	351.75	1010.34	61.90	0.9807	0.6834	0.1007
<i>Mytilus californianus</i>	15.90	147.03	0.01698											
<i>Mytilus californianus</i>	10.99	183.83	0.02654	0.50302	8.10432	18.952	32.928	5.21	69.18	138.85	7.94	0.7374	0.5524	0.0737
<i>Mytilus californianus</i>	9.40	226.49	0.04029	2.46425	7.79949	61.164	111.914	24.50	244.54	303.00	29.46	0.8297	0.6042	0.1071
<i>Mytilus californianus</i>	13.35	100.26	0.00789	1.48839	13.39975	188.526	377.755	43.05	548.48	1361.02	92.08	1.0037	0.6950	0.0929
<i>Mytilus californianus</i>	9.95	132.38	0.01376	0.56842	7.92276	41.298	74.182	13.15	116.60	242.94	18.47	0.7963	0.5857	0.1017
<i>Mytilus californianus</i>	9.16	150.64	0.01782	3.01471	8.49207	169.151	325.968	50.75	589.82	927.76	84.34	0.9271	0.6560	0.0887
<i>Mytilus californianus</i>	9.48	141.58	0.01574	2.04114	7.30638	129.652	229.576	54.76	632.99	765.60	57.49	0.7707	0.5714	0.1037
<i>Modiolus modiolus</i>	4.95	57.67	0.00261	0.26603	3.43978	101.845	172.618		328.14	1024.56	40.05	0.6949	0.5276	
<i>Modiolus modiolus</i>	4.91	60.40	0.00287	0.23352	2.55916	81.501	123.980	24.75	516.51	522.77	24.90	0.5212	0.4195	0.0502
<i>Modiolus modiolus</i>	2.79	40.84	0.00131	0.25411	1.99964	193.979	333.008	37.47	704.09	1105.60	76.00	0.7167	0.5404	0.0504
<i>Modiolus modiolus</i>	2.25</													

<i>Modiolus modiolus</i>	3.73	53.85	0.00228	0.27515	4.25220	120.813	258.539	15.54	176.05	999.66	72.09	1.1400	0.7608	0.0590
<i>Modiolus modiolus</i>	4.45	69.64	0.00381											
<i>Modiolus modiolus</i>	3.59	41.24	0.00134	0.15807	4.40951	118.336	263.686	20.13	288.35	892.26	76.67	1.2283	0.8012	0.0707
<i>Modiolus modiolus</i>	2.54	44.53	0.00156											
<i>Modiolus modiolus</i>	5.51	49.23	0.00190	0.19552	4.27677	102.719	182.448	22.01	462.78	502.65	41.70	0.7762	0.5745	0.0426
<i>Modiolus modiolus</i>	8.39	31.54	0.00078	0.23893	7.18146	305.817	567.583	69.98	1213.39	2114.16	133.77	0.8560	0.6184	0.0560
<i>Modiolus modiolus</i>	9.47	40.76	0.00130	0.21844	6.98035	167.407	290.802	39.85	937.22	988.91	65.19	0.7371	0.5522	0.0426
<i>Modiolus modiolus</i>	8.50	45.85	0.00165	0.14327	5.03259	86.772	138.147	24.41	588.79	579.52	29.02	0.5921	0.4650	0.0419
<i>Modiolus modiolus</i>	8.03	28.18	0.00062	0.21537	6.55875	345.309	627.351	78.98	1772.94	2082.24	150.15	0.8168	0.5971	0.0441
<i>Modiolus modiolus</i>	5.57	44.64	0.00157											
<i>Modiolus modiolus</i>	5.43	28.96	0.00066	0.18593	4.39358	282.271	510.664	53.16	1237.41	2292.00	118.89	0.8091	0.5928	0.0404
<i>Modiolus modiolus</i>	7.77	50.37	0.00199											
<i>Modiolus modiolus</i>	7.00	64.22	0.00324	0.20018	5.41858	61.801	109.641	13.22	322.72	352.77	24.86	0.7741	0.5733	0.0508
<i>Modiolus modiolus</i>	4.95	37.38	0.00110											
<i>Perna canaliculus</i>	6.07	61.88	0.00301											
<i>Perna canaliculus</i>	6.51	95.58	0.00718											
<i>Perna canaliculus</i>	7.25	116.83	0.01072											
<i>Perna canaliculus</i>	6.34	123.43	0.01197											
<i>Perna canaliculus</i>	8.61	101.67	0.00812											
<i>Perna canaliculus</i>	10.74	88.66	0.00617											
<i>Perna canaliculus</i>	15.17	116.58	0.01067											
<i>Perna canaliculus</i>	6.07	169.06	0.02245											
<i>Perna canaliculus</i>	12.57	147.32	0.01705											
<i>Perna canaliculus</i>	9.40	91.93	0.00664											
<i>Perna canaliculus</i>	5.94	75.25	0.00445											
<i>Perna canaliculus</i>	8.61	134.62	0.01423											
<i>Perna canaliculus</i>	6.23	175.99	0.02433											
<i>Perna canaliculus</i>	10.26	243.75	0.04666											
<i>Perna canaliculus</i>	6.88	202.24	0.03212											
<i>Perna canaliculus</i>	8.20	97.91	0.00753											
<i>Perna canaliculus</i>	10.78	191.71	0.02887											
<i>Perna canaliculus</i>	6.72	230.70	0.04180											
<i>Perna canaliculus</i>	12.50	158.17	0.01965											
<i>Perna canaliculus</i>	9.35	110.25	0.00955											
<i>Perna canaliculus</i>	12.07	140.60	0.01553											
<i>Perna canaliculus</i>	11.05	82.51	0.00535											
<i>Perna canaliculus</i>	14.03	171.91	0.02321											
<i>Perna canaliculus</i>	11.54	85.02	0.00568											
<i>Perna canaliculus</i>	15.33	97.53	0.00747											
<i>Perna canaliculus</i>	14.38	176.37	0.02443											
<i>Perna canaliculus</i>	9.04	178.73	0.02509											
<i>Perna canaliculus</i>	10.45	79.32	0.00494											
<i>Perna canaliculus</i>	16.97	172.61	0.02340											
<i>Perna canaliculus</i>	10.90	133.17	0.01393											
<i>Perna canaliculus</i>	12.95	77.74	0.00475											
<i>Perna canaliculus</i>	10.68	101.73	0.00813											
<i>Perna canaliculus</i>	9.11	75.50	0.00448											
<i>Perna canaliculus</i>	11.69	103.52	0.00842											
<i>Ctenoides mitis</i>	4.35	54.54	0.00234											
<i>Ctenoides mitis</i>	2.83	84.42	0.00560	0.09278	4.99854	16.576	45.853	3.96	30.85	61.94	17.31	1.7663	1.0175	0.1116
<i>Ctenoides mitis</i>	4.80	36.45	0.00104											
<i>Ctenoides mitis</i>	6.65	26.03	0.00053											
<i>Ctenoides mitis</i>	2.98	32.08	0.00081											
<i>Ctenoides mitis</i>	2.73	92.08	0.00666	0.08766	4.59307	13.163	35.310	1.39	22.37	51.52	11.23	1.6824	0.9867	0.0627
<i>Ctenoides mitis</i>	3.56	41.00	0.00132											
<i>Ctenoides mitis</i>	6.76	91.65	0.00660	0.10368	5.57104	15.716	28.667	5.99	120.29	51.88	8.63	0.8241	0.6011	0.0471
<i>Ctenoides mitis</i>	5.20	72.74	0.00416	0.11104	6.51333	26.720	60.188	4.47	105.98	196.19	17.56	1.2526	0.8121	0.0424
<i>Ctenoides mitis</i>	4.94	61.96	0.00302	0.11719	6.94312	38.865	93.490	8.40	138.59	410.56	27.25	1.4055	0.8778	0.0636
<i>Ctenoides mitis</i>	10.03	32.39	0.00082	0.04033	9.64350	48.952	96.017	12.46	301.32	279.85	27.34	0.9615	0.6737	0.0390
<i>Ctenoides mitis</i>	9.28	83.17	0.00543	0.17475	9.43428	32.166	64.868	7.60	140.72	310.90	15.80	1.0166	0.7014	0.0533
<i>Ctenoides mitis</i>	7.38	76.24	0.00457	0.08505	8.30710	18.630	39.600	3.04	49.84	187.18	10.15	1.1256	0.7541	0.0562
<i>Ctenoides mitis</i>	5.93	85.05	0.00568	0.15817	11.79245	27.841	83.207	4.27	101.16	149.38	28.52	1.9886	1.0948	0.0405
<i>Ctenoides mitis</i>	7.16	137.66	0.01488	0.52612	10.56309	35.349	84.329	9.23	242.69	133.71	33.37	1.4753	0.9064	0.0382
<i>Ctenoides mitis</i>	4.67	84.30	0.00558											
<i>Ctenoides mitis</i>	5.71	41.29	0.00134											
<i>Ctenoides mitis</i>	6.61	99.01	0.00770	0.17922	7.48807	23.278	49.649	4.45	78.90	288.53	11.75	1.1328	0.7575	0.0500
<i>Ctenoides mitis</i>	6.19	98.52	0.00762	0.20233	7.39886	26.541	58.265	5.80	72.28	266.62	14.76	1.1953	0.7863	0.0774
<i>Ctenoides mitis</i>	5.82	285.26	0.06391	1.34939	5.95052	21.114	42.701	4.19	168.87	231.45	10.03	1.0224	0.7043	0.0624
<i>Ctenoides mitis</i>	5.36	215.19	0.03637	0.48672	5.24346	13.383	26.475	2.43	32.10	157.66	6.20	0.9783	0.6822	0.0642
<i>Ctenoides mitis</i>	6.48	109.41	0.00940											
<i>Ctenoides mitis</i>	4.93	107.11	0.00901	0.38611	5.09742	42.851	87.158	9.52	126.37	506.30	21.10	1.0340	0.7100	0.0699
<i>Ctenoides mitis</i>	7.74	80.02	0.00503											
<i>Ctenoides mitis</i>	7.04	284.95	0.06377											
<i>Ctenoides mitis</i>	4.92	42.13	0.00139											
<i>Ctenoides mitis</i>	4.64	253.85	0.05061											
<i>Ctenoides mitis</i>	4.36	200.91	0.03170	0.07396	5.13960	2.333	5.083	0.58	7.79	9.20	1.67	1.1788	0.7788	0.0742
<i>Ctenoides mitis</i>	3.94	97.33	0.00744	0.14755	5.31483	19.832	46.584	3.42	52.71	262.85	12.88	1.3489	0.8540	0.0646
<i>Ctenoides mitis</i>	6.53	98.01	0.00754	0.15398	7.81563	20.409	44.837	2.46	36.47	225.12	10.92	1.1969	0.7870	0.0616
<i>Geukensia demissa</i>	5.38	24.75	0.00048											
<i>Geukensia demissa</i>	10.17	41.68	0.00136	0.06636	8.86675	48.635	91.037	14.26	191.04	205.12	29.09	0.8719	0.6269	0.0758
<i>Geukensia demissa</i>	4.69	37.16	0.00108											
<i>Geukensia demissa</i>	5.40	44.89	0.00158											
<i>Geukensia demissa</i>	7.38	21.43	0.00036	0.04007	7.99906	111.091	231.501	39.35	393.88	483.85	79.80	1.0839	0.7342	0.0961
<i>Geukensia demissa</i>	6.36	20.24	0.00032											
<i>Geukensia demissa</i>	6.40	37.25	0.00109	0.06005	5.12026	55.099	99.180	12.86	213.76	231.32	27.23	0.8000	0.5878	0.1002
<i>Geukensia demissa</i>	6.19	30.02	0.00071	0.05819	4.65152	82.209	135.433	16.96	365.27	296.25	45.01	0.7515	0.5604	0.0426
<i>Geukensia demissa</i>	6.14	25.40	0.00051	0.03535	4.86074	69.759	124.983	17.76	250.38	201.87	39.43	0.7917	0.5831	0.0639
<i>Geukensia demissa</i>	6.78	25.98	0.00053											
<i>Geukensia demissa</i>	6.09	48.23	0.00183	0.05203	5.74303	28.478	55.333	10.92	87.40	144.22	17.23	0.9430	0.6642	0.1182
<i>Geukensia demissa</i>	9.73	21.62	0.00037											
<i>Geukensia demissa</i>	7.13	21.00	0.00035	0.03024	6.63993	87.305	168.610	20.00	279.30	407.72	52.41	0.9313	0.6582	0.0605
<i>Geukensia demissa</i>	7.37	39.83	0.00125	0.04639	8.02338	37.234	77.770	10.04	70.27	208.11	25.01	1.0887	0.7365	0.1087
<i>Geukensia demissa</i>	5.58	28.39	0.00063	0.09530	4.12927	150.546	261.952	50.45	1050.87	803.19	71.75	0.7400	0.5539	0.0674
<i>Geukensia demissa</i>	4.47	47.69	0.00179	0.06539	2.83660	36.605	59.835	20.49	175.61	147.25	15.39	0.6346	0.4914	0.1224
<i>Geukensia demissa</i>	9.58	45.83	0.00165											
<i>Geukensia demissa</i>	8.68	65.99	0.00342	0.08637	7.77845	25.253	47.883	9.06	134.74	113.16	14.89	0.8961	0.6398	0.0954
<i>Geukensia demissa</i>	7.10	38.28	0.00115	0.06699	5.99106	58.205	107.319	20.47	291.92	264.40	30.81	0.8438	0.6118	0.0864
<i>Geukensia demissa</i>	7.12	53.12	0.00222	0.06066	6.09648	27.373	50.811	8.13	211.48	135.21	15.80	0.8562	0.6186	0.0650
<i>Geukensia demissa</i>														

<i>Geukensia demissa</i>	5.38	58.28	0.00267											
<i>Geukensia demissa</i>	4.34	50.01	0.00196											
<i>Geukensia demissa</i>	5.02	30.04	0.00071											
<i>Geukensia demissa</i>	6.79	46.28	0.00168	0.13816	6.87994	82.132	165.352	23.41	384.91	317.78	56.56	1.0132	0.6997	0.0661
<i>Geukensia demissa</i>	3.50	42.72	0.00143	0.13497	4.10649	94.163	204.643	18.53	229.20	376.36	65.61	1.1733	0.7762	0.0741
<i>Geukensia demissa</i>	6.88	55.40	0.00241	0.13980	7.66804	57.994	122.632	19.13	201.50	261.36	39.96	1.1145	0.7488	0.1150
<i>Atrina rigida</i>	7.33	27.60	0.00060											
<i>Atrina rigida</i>	11.51	69.97	0.00385											
<i>Atrina rigida</i>	7.13	76.30	0.00457	0.10470	3.61183	22.899	34.499	11.15	225.94	39.81	8.35	0.5066	0.4098	0.0534
<i>Atrina rigida</i>	9.65	46.54	0.00170	0.11420	6.70146	67.134	113.755	29.64	505.31	217.15	34.30	0.6945	0.5274	0.0674
<i>Atrina rigida</i>	6.49	43.57	0.00149	0.14252	3.06528	95.591	140.739	38.54	1048.31	70.04	30.01	0.4723	0.3868	0.0582
<i>Atrina rigida</i>	9.98	49.37	0.00191	0.08328	3.74969	43.502	59.847	27.04	721.84	139.35	13.94	0.3757	0.3190	0.0434
<i>Atrina rigida</i>	7.56	86.92	0.00593											
<i>Atrina rigida</i>	7.02	54.14	0.00230	0.10256	3.81781	44.550	68.779	23.81	620.98	111.07	18.94	0.5438	0.4343	0.0478
<i>Atrina rigida</i>	14.42	39.78	0.00124	0.12650	5.83862	101.782	142.993	42.33	1168.09	379.98	27.78	0.4049	0.3400	0.0450
<i>Atrina rigida</i>	7.89	68.82	0.00372	0.08961	3.20962	24.091	33.890	10.61	108.92	50.28	5.67	0.4068	0.3413	0.0875
<i>Atrina rigida</i>	8.06	51.49	0.00208	0.12846	4.17625	61.691	93.656	23.44	549.82	147.01	22.45	0.5181	0.4175	0.0410
<i>Atrina rigida</i>	6.93	57.66	0.00261											
<i>Atrina rigida</i>	7.84	40.11	0.00126											
<i>Atrina rigida</i>	8.36	70.58	0.00391											
<i>Atrina rigida</i>	12.30	49.18	0.00190	0.08747	3.94593	46.046	60.817	22.79	713.64	89.14	10.72	0.3208	0.2782	0.0389
<i>Atrina rigida</i>	7.95	64.24	0.00324	0.06502	6.25867	20.061	35.853	9.11	182.66	65.44	12.20	0.7873	0.5807	0.0682
<i>Atrina rigida</i>	8.91	49.67	0.00194											
<i>Atrina rigida</i>	9.47	47.53	0.00177	0.11327	6.52627	63.841	107.837	26.68	608.94	228.75	32.92	0.6892	0.5242	0.0509
<i>Atrina rigida</i>	10.18	38.69	0.00118	0.07210	6.86849	61.326	102.703	24.28	707.30	227.26	31.91	0.6747	0.5156	0.0381
<i>Atrina rigida</i>	15.30	52.76	0.00219	0.19236	15.59884	87.985	177.689	29.12	758.39	415.74	64.23	1.0195	0.7029	0.0455

Material properties of the byssal threads of selected bivalve molluscs. Empty cells represent missing values. 'Engineering' stress and strain (σ_E , ϵ_E) are defined as $\sigma_E = F/A_0$ =force/unit initial area, $\epsilon_E = \Delta L/L_0$ =change in length/initial length; 'true' stress and strain (σ_T , ϵ_T) are defined respectively as $\sigma_T = \sigma_E \exp(\epsilon_T)$ (assuming constant volume, i.e. a Poisson's ratio of 0.5) and $\epsilon_T = \ln(L/L_0)$. Yield stress and strain were defined as a sharp drop in stiffness at a characteristic stress and strain level; thread stiffness was determined both for the initial loading of the thread and at thread failure. The area under the stress-strain curve (the energy absorbed per unit volume, or the toughness of the material) was determined by fitting a polynomial to the curve and integrating over the total strain. A Microsoft® Excel® version of this file is available from the authors upon request.