

# Interspecific Comparison of the Mechanical Properties of Mussel Byssus

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**Abstract.** Byssally tethered mussels are found in a variety of habitats, including rocky intertidal, salt marsh, subtidal, and hydrothermal vents. One key to the survival of mussels in these communities is a secure attachment, achieved by the production of byssal threads. Although many studies have detailed the unique biomechanical properties of byssal threads, only a few prevalent species have been examined. This study assesses the variation in the mechanical properties of byssus in a broad range of mussel species from diverse environments, including intertidal and subtidal *Mytilus edulis*, *Modiolus modiolus*, *Geukensia demissa*, *Bathymodiolus thermophilus*, and *Dreissena polymorpha*. A tensometer was used to measure quasi-static and dynamic mechanical properties of individual threads, and several aspects of morphology were quantified. The results indicate that thread mechanical properties vary among mussel species, and several novel properties were observed. For example, of the species examined, *D. polymorpha* threads were the strongest, stiffest, least resilient, and fastest to recover after partial deformation. Threads of *M. modiolus* were characterized by the presence of two distinct yield regions prior to tensile failure. This comparative study not only provides insight into the ecological limitations and evolution of mussels, but also suggests new models for the design of novel biomimetic polymers.

## Introduction

In their postlarval state, most bivalve molluscs create a fibrous, extracellular complex known as byssus to secure them to the substrate while they undergo metamorphosis (Yonge, 1962). In some species of mussels, the ability to

produce byssal threads is retained through the adult stage, a form of neotenuous evolution that allowed mussel species to diversify and make the transition from infaunal to epibenthic dwellers (Yonge, 1962; Stanley, 1972). This habitat shift can be seen clearly in the Mytilidae, a wide-ranging mussel family that contains species that exhibit a mixture of lifestyles from the ancestral endobysate to the epibenthic dweller. Examples include *Geukensia demissa*, which buries about 75% of its shell into the sediment (Stanley, 1972), and *Mytilus edulis* and *Bathymodiolus thermophilus*, which live entirely exposed on hard substrate. Transitional species, such as *Modiolus modiolus* (hereafter referred to as *Modiolus*), can live partially buried in areas of soft sediment (Stanley, 1972) or survive as an epibysate on hard substrate (Holt *et al.*, 1998). This evolutionary shift from burrowing to epibenthic dwelling also occurred in the Dreissenidae mussels, separately from the Mytilidae (Morton, 1993). Extant species of this family, such as *Dreissena polymorpha*, exploit hard substrates in freshwater systems.

Mussels of the Mytilidae, which includes all marine species, have a byssal complex with three main components: a root that is attached to the byssal retractor muscles; a stem that extends from the root and consists of a series of overlapping cuffs; and fibrous byssal threads that individually project off of each cuff (Bell and Gosline, 1996). Individual byssal threads are made in the mussel foot through a process similar to polymer-injection molding (Waite, 1992). A central groove is formed when the edges of the mussel foot touch together and the opening is secured with sealant mucus. This groove is then filled with proteins by numerous glands also found within the foot. These proteins are massaged into highly oriented fibers and then coated with a tough lacquer cuticle to inhibit chemical and bacterial degradation (Rzepecki and Waite, 1993). Upon completion of the process which takes about 2 to 5 min, the edges of the foot part and the new thread is released, which elongates the stem distally (Waite, 1992).

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Abbreviations:  $\epsilon$ , strain;  $\sigma$ , stress;  $E_1$ , material stiffness.

The dreissenid byssus evolved independently from the byssus of the mytilids (Morton, 1993), and although the thread-making process is similar in the two taxa, the byssal structure is different. In dreissenid species such as *D. polymorpha*, threads branch from a single location on the byssus (Eckroat and Steele, 1993). In contrast to mytilids, dreissenids add new threads to the stem circumferentially, thereby increasing stem thickness instead of stem length (Eckroat and Steele, 1993).

Brown (1952) first described the morphology of the byssal structure and noted that the threads of *M. edulis* were composed of two distinct regions, a corrugated proximal region and a smooth distal region. Although the whole thread is mainly collagenous, the distal region has supplementary components, such as alanine and glycine, that make it similar to silk fibroin, and the proximal region has additional components similar to those found in elastin (Coyné *et al.*, 1997; Waite *et al.*, 2002). Not all species of mussels have the composite thread structure of *M. edulis*, however; some species have morphologically homogeneous threads.

The proximal and distal regions of *Mytilus* byssal threads also differ in mechanical properties: the distal region is stronger, stiffer, and less extensible than the proximal region (Smeathers and Vincent, 1979; Bell and Gosline, 1996). The distal region also yields (becomes less stiff) when extended beyond a critical length. The whole-thread tensile behavior of many *Mytilus* species is therefore triphasic: threads are initially stiff, then yield, and finally stiffen again before structural failure (Bell and Gosline, 1996).

In relation to other biological fibers, the material properties of these byssal regions are exceptional in several ways (Gosline *et al.*, 2002). The proximal section is similar in extensibility to other rubber-like proteins such as resilin and elastin, but is an order of magnitude higher in strength and stiffness. The distal region has stiffness and strength values close to those of collagen but is 10 times more extensible. These two regions join together to create a thread that, pound for pound, is comparable to Kevlar in toughness.

When subjected to cyclical tensile tests, the threads of *Mytilus* species exhibit some remarkable dynamic properties, such as resilience and their unusual ability to recover over time following deformation. Resilience is the elastic efficiency of a material, or the percentage of the strain energy stored during deformation that is recovered after stress has been removed. In byssal threads, resilience is highly dependent on the amount of strain applied—larger extensions produce lower resilience values (Smeathers and Vincent, 1979; Carrington and Gosline, 2004). Byssal resilience for *Mytilus* species ranged from 35%–68%, which is lower than for rubber-like proteins (about 90%; collagen and elastin, Gosline *et al.*, 2002). Lower resilience is usually associated with enhanced toughness in polymeric materials (Gosline *et al.*, 2002). Smeathers and Vincent (1979) noted that byssal threads recover stiffness, or “self-heal,” after

deformation. This recovery process was later quantified by Carrington and Gosline (2004): *M. californianus* was able to recover roughly 60% of its lost energy dissipation structure in 30 min, while *M. edulis* recovered only 45% in the same time.

A number of studies have detailed the mechanical abilities of the byssal threads of *M. edulis* and other prevalent *Mytilus* mussels (*M. galloprovincialis*, *M. trossulus*, and *M. californianus*; McDonald and Koehn, 1988; Bell and Gosline, 1996; Vaccaro and Waite, 2001; Lucas *et al.*, 2002; Waite *et al.*, 2002; Carrington and Gosline, 2004). Of these species, *M. californianus* threads appear superior in strength, extensibility, stiffness, and also resiliency and recovery after deformation. Bell and Gosline (1997) also found that *M. californianus* byssal threads are thicker than the threads of equally sized *M. trossulus*. Together, the mechanical and morphometric differences seen in the byssal threads of *M. californianus* may contribute to its strong attachment and ability to dominate wave-exposed shores. It is not known, however, how other mussel species compare, because no studies have included mussels from a variety of genera and habitats.

This study assesses the variation in mechanical properties of byssus from a range of mussels, including *M. edulis*, *Modiolus*, *G. demissa*, *B. thermophilus*, and *D. polymorpha*. Specifically, this study focuses on the material properties of extensibility, strength, and stiffness of the byssal threads. Also, dynamic mechanical tests assess the resilience of the threads of each species and their ability to recover initial material properties after partial deformation. Because morphometrics can help to place biomechanical data in an ecological context, relationships between mussel and thread dimensions were also measured and compared.

## Materials and Methods

Samples of five mussel species were collected from various locations: *Mytilus edulis* Linnaeus (1758) and *Geukensia demissa* Dillwyn (1817) from Narragansett Bay, Rhode Island; *Modiolus modiolus* Linnaeus (1758) from Nahant, Massachusetts; *Dreissena polymorpha* Pallas (1771) from Salisbury, Connecticut; and *Bathymodiolus thermophilus* Kenk and Wilson (1985) from the Eastern Pacific Rise (See Brazee, 2004, for more details). In the case of *M. edulis*, intertidal and subtidal populations were tested separately to examine whether habitat affected either morphometrics or biomechanics. Upon collection, all *M. edulis*, *G. demissa*, and *Modiolus* were placed in a recirculating aquarium (flow rate  $<5 \text{ cm s}^{-1}$ ) containing water of ambient temperature ( $\pm 2^\circ\text{C}$ ; Brazee, 2004) at the University of Rhode Island and maintained for about 3 to 5 days, which allowed time for new byssal threads to be produced. Thread complexes of *D. polymorpha* were dissected off in the field, and only the most recently made threads were used in testing. For *B.*

*thermophilus* threads, which were collected on a voyage of the submersible DSV *Alvin*, byssal complexes were removed from the mussel body on the surface ship. Byssal threads from all species were tested after their collection except for those of *B. thermophilus*, which were dehydrated for later use. A previous study indicated that this dehydration did not significantly affect the properties of the byssal threads (Brazee, 2004).

#### Material testing

**Quasi-static testing.** Quasi-static material testing followed the general protocol of Bell and Gosline (1996) and Carrington and Gosline (2004). Twenty threads for each species were pulled to failure using an Instron-5565 tensometer (Canton, MA) equipped with a computer interface. Each end of a single thread was fixed with cyanoacrylate glue between two small squares of cardstock, placed into grips, and extended at a rate of  $10 \text{ mm min}^{-1}$ . Threads that broke at the grips were not used, as it is likely that they broke from clamp damage. During all tensile experiments, the threads of marine species were submerged in seawater, whereas *D. polymorpha* threads were submerged in distilled freshwater. All water was maintained at  $15^\circ\text{C}$ , a temperature common to all the species being tested.

During each tensile test, the computer collected continuous load and extension values, which were then normalized to the size of the sample, producing a stress-strain curve. Load was converted into stress ( $\sigma$ , in pascals) by dividing by the cross-sectional area of each thread. Thread cross-sectional areas were assumed to be circular, and the diameter was measured at the break point. In cases where the threads did not appear cylindrical, the largest axis length was used to calculate cross-sectional area to maintain consistency within this and other studies. Thread diameter ( $\pm 0.01 \text{ mm}$ ) was calculated from digital micrographs, using image analysis software (Sigma Scan 5.0; SPSS, Inc.). Extension was converted into engineer's strain ( $\epsilon$ ) by dividing the change in length by the initial length of the thread. Note that this measure of strain is equivalent to "true" strain ( $\epsilon_t$ , calculated as the natural log of the ratio of length to initial length) only for small strains ( $< 5\%$ ; Denny, 1988). Engineer's strain can be converted to true strain by the formula  $\epsilon_t = \ln(\epsilon + 1)$ . The initial modulus ( $E_i$ , in pascals), or material stiffness, was calculated from the slope of the initial portion of the stress-strain curve. The average yield stress and yield strain were also calculated for those species whose threads exhibited a distinct yield region. Ultimate values were recorded at failure.

In addition, tensile material tests were conducted on the isolated proximal and distal sections from the threads of *Modiolus* and intertidal *M. edulis*. The remaining species had no discernible morphological regions and were therefore excluded from these tests. Despite the observations of

Eckroat and Steele (1993), no distinct topographical differences in regions of *D. polymorpha* threads were detectable, even under the magnification of a scanning electron microscope (Brazee, 2004).

**Dynamic testing.** Cyclical loading was used to examine dynamic properties of the byssal threads—specifically, the resilience and the ability to recover after deformation—following the methods of Carrington and Gosline (2004). A strain of 0.35 was chosen for cyclical tests because previous studies have established this value to be within the yield region for *Mytilus* threads (Carrington and Gosline, 2004). Ten threads of each species were cycled in tension twice and then left unstressed for 30 min. Data from the first cycle were used to compute resilience, or the amount of elastic energy retained after deformation (Smeathers and Vincent, 1979; Denny, 1988). Resilience was calculated by dividing the area under the returning curve by the area under the initial extension curve, expressed as a percentage. After 30 min, the threads were cycled again to estimate recovery of the initial material properties. Recovery was measured as the area enclosed by the time-delayed force-extension cycle, expressed as a percentage of the area within the initial cycle (Carrington and Gosline, 2004). Cyclical testing was also conducted on the isolated proximal and distal regions of *Modiolus* threads.

#### Mussel and thread morphometry

The shell length of each species was measured (to the nearest millimeter) using calipers. The morphometry of threads was quantified for each species, including length, diameter, shape, and plaque area. Thread length ( $\pm 0.02 \text{ mm}$ ) was obtained from the initial length measurements of successful tensile test data (where failure did not occur at the grips). Thread length was then expressed as a percentage of total shell length for comparisons among species ( $n = 12\text{--}16$ , except for *B. thermophilus*, where  $n = 5$ ). For *M. edulis* and *Modiolus*, the length of proximal and distal regions was also measured under a dissecting microscope (to the nearest  $0.1 \text{ mm}$ ,  $n = 9\text{--}10$ ). For comparisons among species, proximal length was expressed as a percentage of total length for each thread, and characteristic thread diameter was measured about  $2 \text{ mm}$  from the distal end as in Bell and Gosline (1996).

Scanning electron microscopy (SEM) was used to examine the surface topography of proximal and distal ends of representative byssal threads from each species. Three threads from each species were cross-sectioned at the proximal end and photographed using SEM to evaluate the overall thread shape of each species. The major and minor axes were measured (to the nearest  $0.1 \mu\text{m}$ ), and a ratio of major to minor axes was calculated for each species.

To estimate plaque area, 10 representatively sized mus-

sels for each species were allowed to produce threads on clear plastic. Two plaques were haphazardly selected from each mussel, and image analysis software was used to calculate the plaque area (in square millimeters) from digital micrographs. *Dreissena polymorpha* and *B. thermophilus* could not be maintained in the laboratory; plaques from these species were instead carefully extracted from their natural shell substrate ( $n = 20$  and  $n = 5$ , respectively). It was assumed that these methodological differences in thread production (environment, substrate) did not affect plaque area (but see Crisp *et al.*, 1985; Bell and Gosline, 1996). Mechanical and morphometric data were analyzed separately using analysis of variance with species as the independent factor. All statistical tests were performed using SYSTAT 10.0 (SPSS Inc; Richmond, CA) and assume a significance level ( $P$ ) of 0.05. *Post hoc* tests were done using the Tukey method of multiple pairwise comparisons.

## Results

### Material testing

**Quasi-static testing.** The stress-strain curves generated for whole threads during tensile tests differ among species (Fig. 1). *Mytilus edulis* curves exhibited three distinctive phases, as previously shown by Bell and Gosline (1996). In the initial phase, the thread acted as an elastic solid, producing a linear, stiff section of the stress-strain curve. A second phase began at about 30% strain, where there was a temporary decreased stiffness, or yield region. In the third phase, the thread stiffened once more before structural failure. In 84% of the tests, the failure occurred in the proximal region of the thread. Tensile curves of *Dreissena polymorpha* threads also exhibited three phases. The first phase of the curve was much like that of *M. edulis*, where the thread

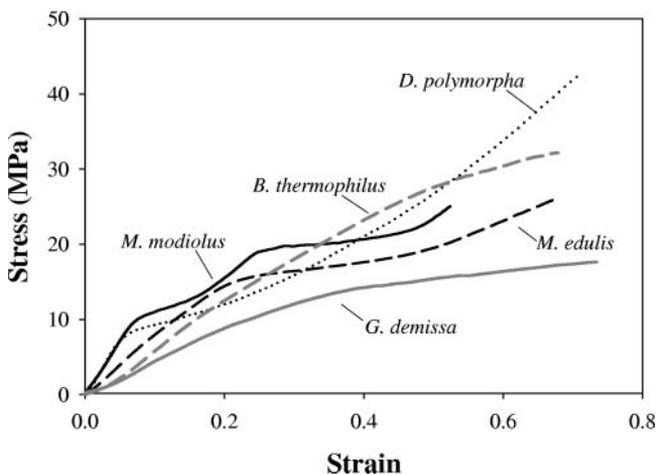
performed as an elastic solid. This phase typically ended at 5%–10% strain, and a yield region of decreased stiffness followed (Fig. 1). At about 20%–30% strain, the thread gradually regained stiffness similar to the first phase. Fifty-six percent of the breaks occurred at the proximal end of the thread. In contrast, the stress-strain curves for the whole threads of *Bathymodiolus thermophilus* and *Geukensia demissa* were stiffest initially and gradually lost stiffness as the threads were extended to failure (Fig. 1). Although *G. demissa* and *B. thermophilus* threads were homogeneous structures, the location of thread failure was still recorded; proximal end failures were observed in 29% and 47% of the samples for *G. demissa* and *B. thermophilus*, respectively.

The most unusual stress-strain curves were those of *Modiolus*, with two distinct yield regions. Threads were initially stiff, then yielded (reduced stiffness) at about 10% strain (Fig. 1). With increasing extension, the threads stiffened, and then yielded again at about 25%–30% strain. The slope once again increased before structural failure occurred. The proximal region was the most common area of failure, accounting for 75% of the breaks.

The ultimate stress (or strength) of whole threads varied within the suite of species examined ( $P < 0.001$ ; Table 1). Whereas the threads of marine species broke at similar stresses, ranging from 13–26 MPa, *D. polymorpha* threads were twice as strong, with a mean strength of 48 MPa. Ultimate strain (or extensibility), which ranged from 0.5 to 0.8, did not vary among species ( $P = 0.14$ ), but the initial modulus did ( $P < 0.001$ ; Table 1). The threads of intertidal and subtidal *M. edulis*, *G. demissa*, and *B. thermophilus* were less stiff, ranging 35–79 MPa. The stiffest threads were those of *D. polymorpha* (136.9 MPa), and *Modiolus modiolus* threads were intermediate.

Tensile testing illustrates that the biomechanical properties of *Modiolus* and *M. edulis* threads were dependent upon the region sampled (Fig. 2; Table 2). The stiffness (slope) of the proximal region of both species was relatively constant, whereas the distal regions had distinct yields, similar to those observed in whole-thread tests. Distal regions of *M. edulis* yielded once; those of *Modiolus* yielded twice. For both *Mytilus edulis* and *Modiolus* threads, the distal portion was 4 to 5 times stronger, 8 to 9 times stiffer, but roughly half as extensible as the proximal region ( $P < 0.05$ ; Table 2).

The proximal regions of *Modiolus* and *M. edulis* were comparable in strength ( $P = 0.87$ ), and stiffness ( $P = 0.50$ ), but the proximal region of *M. edulis* was twice as extensible as that of *Modiolus* ( $P < 0.01$ ; Table 2). The distal regions of the two species were similar in strength, extensibility, and stiffness ( $P = 0.56, 0.11, \text{ and } 0.69$ , respectively). In comparison to these isolated regions, the ultimate properties of the homogeneous threads were intermediate (*B. thermophilus* and *G. demissa*; Fig. 1; Table 1).



**Figure 1.** Representative tensile tests of whole threads for each species. Tests were performed in 15°C seawater at an extension rate of 10 mm/min.

Table 1

Material properties of whole byssal threads

Species	Strength (MPa)	Extensibility	Modulus (MPa)	Resilience (%)	Recovery (%)
Current Study					
<i>Bathymodiolus thermophilus</i>	26.19 ± 14.14 (15) <sup>a</sup>	0.60 ± 0.27 (15)	78.61 ± 33.15 (15) <sup>a</sup>	39.43 ± 1.88 (9) <sup>a</sup>	59.12 ± 5.25 (9) <sup>a,b</sup>
<i>Guekensia demissa</i>	20.52 ± 10.36 (14) <sup>a</sup>	0.71 ± 0.39 (14)	73.04 ± 38.89 (14) <sup>a</sup>	37.72 ± 4.69 (5) <sup>a,b</sup>	56.08 ± 11.32 (5) <sup>a,b</sup>
<i>Mytilus edulis</i>					
Intertidal	13.06 ± 10.20 (15) <sup>a</sup>	0.65 ± 0.23 (15)	35.29 ± 27.33 (15) <sup>a</sup>	33.52 ± 5.92 (8) <sup>b</sup>	58.85 ± 13.89 (8) <sup>a,b</sup>
Subtidal	20.46 ± 13.56 (16) <sup>a</sup>	0.76 ± 0.32 (16)	47.87 ± 25.23 (16) <sup>a</sup>	37.93 ± 4.00 (8) <sup>a,b</sup>	59.30 ± 7.04 (8) <sup>a,b</sup>
<i>Modiolus modiolus</i>	19.11 ± 18.76 (12) <sup>a</sup>	0.50 ± 0.14 (12)	83.83 ± 88.51 (12) <sup>a,b</sup>	34.20 ± 2.80 (7) <sup>a,b</sup>	44.89 ± 6.20 (7) <sup>a</sup>
<i>Dreissena polymorpha</i>	48.40 ± 26.02 (16) <sup>b</sup>	0.61 ± 0.23 (16)	136.91 ± 63.34 (16) <sup>b</sup>	24.81 ± 3.51 (9) <sup>c</sup>	67.55 ± 17.53 (9) <sup>b</sup>
<b>P Value</b>	<b>&lt;0.001</b>	<b>0.18</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.05</b>
Previous Studies					
<i>Mytilus californianus</i>	34.6 ± 16.41 (8) <sup>1</sup>	0.94 ± 0.39 (15) <sup>1</sup>	ND	35 <sup>2</sup>	65 <sup>2</sup>
<i>Mytilus trossulus</i>	20.3 ± 10.94 (11) <sup>1</sup>	0.66 ± 0.31 (12) <sup>1</sup>	ND	ND	ND
<i>Mytilis galloprovincialis</i>	17.1 ± 7.79 (3) <sup>1</sup>	0.62 ± 0.17 (3) <sup>1</sup>	ND	28 <sup>3</sup>	ND
<i>Mytilus edulis</i>	21 <sup>4</sup>	0.44 <sup>4</sup>	32–85 <sup>4</sup>	ND	45 <sup>2</sup>

Ultimate values were calculated using the major axis diameter at the location of failure. Letters indicate significantly different groups (Tukey pairwise comparisons;  $P < 0.05$ ). Values are mean ± SD ( $n$ ). ND indicates that no data were available.

<sup>1</sup> Data from Bell and Gosline (1996) (proximal region values, where failure most often occurred, were used for strength).

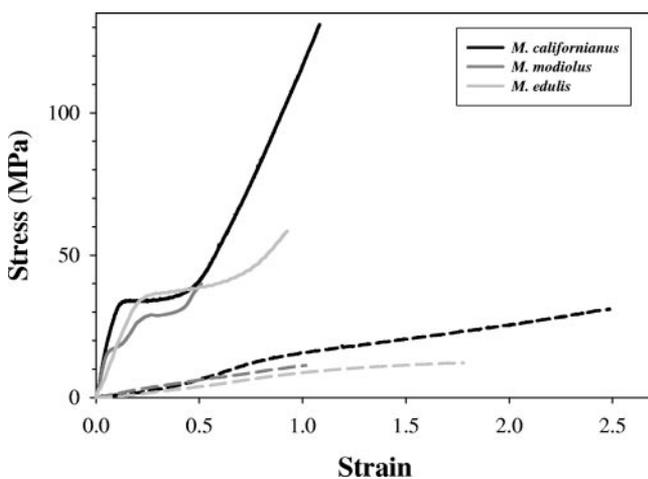
<sup>2</sup> Data from Carrington and Gosline (2004) (extended to 0.44 strain at an extension rate of 5 mm/min).

<sup>3</sup> Data from Waite *et al.* (2002) (extended to 0.5 strain at 24–28 mm/min).

<sup>4</sup> Data from Smeathers and Vincent (1979) (wet threads at an extension rate of 5 mm/min).

**Dynamic testing.** Species varied in the amount of elastic energy restored following deformation ( $P < 0.001$ ; Table 1). *Dreissena polymorpha* threads dissipated the greatest amount of energy during extension, with a resilience of about 25%. This was significantly lower than all other species tested (Table 1). The remaining species were statistically similar, with the exception of intertidal *M. edulis*, which had lower resilience than *B. thermophilus*.

Consecutive stress-strain curves of *D. polymorpha* were



**Figure 2.** Representative stress-strain curves of isolated distal and proximal regions. The distal strength of *Mytilus californianus* and *M. edulis* may be slightly underestimated due to grip failure. Data for *M. californianus* are from Bell and Gosline (1996).

also different from those of the mytilids (Fig. 3). When the threads of *D. polymorpha* were extended in a second tensile cycle, they remained slack until reaching the strain at which the yield occurred during the first cycle. After this point, a positive slope (stiffness) developed that was less steep than that of the first extension. Conversely, the mytilid threads were never completely slack. During the second cycle, a positive slope was observed immediately, although this slope was not nearly as steep (stiff) as that of first cycle. In all species tested, this second cycle never exhibited a yield. The amount of recovery, or the percentage of energy-dissipating ability regained in 30 min after deformation, was between 45% and 68% for all species. *D. polymorpha* threads had the highest recovery; they were significantly different only from *Modiolus* ( $P < 0.05$ ). After resting for 30 min, *D. polymorpha* threads still had some slack but recovered stiffness and yield typical of threads that had not been previously tested (Fig. 3A). Note that the return curves for all cycles overlap, indicating that the slack is the result of reversible plastic deformation and not grip slippage. For the mytilids, only a modest recovery of stiffness was observed after 30 min, and the yield region did not return.

Isolated regions of the *Modiolus* threads also differed in resilience and recovery (Fig. 4). The proximal region was more resilient than the distal region ( $P < 0.05$ ), and the two sections differed in their ability to recover ( $P < 0.001$ ). The proximal portion recovered about 83% of the ability to dissipate energy within a 30-min interval, whereas the distal region recovered only about 37%. The most striking differ-

Table 2

Material properties of isolated regions of byssal thread

Species	Strength (MPa)	Extensibility	Modulus (MPa)	Resilience (%)	Recovery (%)
Proximal Region					
<i>Modiolus modiolus</i>	12.87 ± 4.87 (5)	0.84 ± 0.26 (5)	21.79 ± 6.86 (5)	42.45 ± 5.78 (7)	82.69 ± 11.20 (7)
<i>Mytilus edulis</i> (intertidal)	12.42 ± 5.10 (10)	1.89 ± 0.71 (10)	17.06 ± 14.83 (10)	ND	ND
<i>Mytilus edulis</i>	ND	1.22 ± 0.13 (10) <sup>1</sup>	ND	ND	ND
<i>Mytilus californianus</i>	34.6 ± 16.40 (8) <sup>2</sup>	1.97 ± 0.47 (5) <sup>2</sup>	15.6 ± 2.90 (5) <sup>2</sup>	42 <sup>3</sup>	ND
<i>Mytilus trossulus</i>	20.3 ± 10.94 (11) <sup>2</sup>	1.65 ± 0.51 (5) <sup>2</sup>	20.6 ± 11.63 (5) <sup>2</sup>	ND	ND
<i>Mytilus galloprovincialis</i>	17.1 ± 7.79 (3) <sup>2</sup>	1.64 ± 0.71 (3) <sup>2</sup>	26.4 ± 19.9 (3) <sup>2</sup>	53 <sup>4</sup>	ND
Distal Region					
<i>Modiolus modiolus</i>	50.36 ± 29.96 (7)	0.58 ± 0.07 (7)	168.6 ± 121.0 (7)	35.32 ± 2.59 (7)	36.05 ± 6.79 (7)
<i>Mytilus edulis</i> (intertidal)	58.77 ± 21.38 (7)	0.89 ± 0.46 (7)	148.3 ± 46.3 (7)	ND	ND
<i>Mytilus edulis</i>	83 ± 42 (8) <sup>5</sup>	0.66 ± 0.03 <sup>1</sup>	ND	ND	ND
<i>Mytilus californianus</i>	73.3 ± 31.74 (31) <sup>2</sup>	1.09 ± 0.16 (28) <sup>2</sup>	868.6 ± 405.2 (5) <sup>2</sup>	34 <sup>3</sup>	47 <sup>3</sup>
<i>Mytilus trossulus</i>	56.2 ± 37.52 (17) <sup>2</sup>	0.79 ± 0.19 (14) <sup>2</sup>	109.3 ± 19.2 (5) <sup>2</sup>	ND	ND
<i>Mytilus galloprovincialis</i>	ND	ND	300.4 ± 156.1 (3) <sup>2</sup>	28 <sup>4</sup>	72 <sup>6</sup>

For all species, the proximal region is characterized by its high extensibility and low strength. In contrast, the distal region has high strength and is an order of magnitude stiffer than the proximal region. The proximal region is also more capable of recovering strain-dissipating energy. Values are mean ± SD (*n*). ND indicates that no data were available.

<sup>1</sup> Data from Smeathers and Vincent (1979) (wet threads at an extension rate of 5 mm/min).

<sup>2</sup> Data from Bell and Gosline (1996).

<sup>3</sup> Data from Carrington and Gosline (2004) (pulled to 0.35 strain at an extension rate of 10 mm/min).

<sup>4</sup> Data from Waite *et al.* (2002) (pulled to 0.5 strain).

<sup>5</sup> Data from Price (1981) (pulled at an extension rate 25 mm/min).

<sup>6</sup> Data from Vaccaro and Waite (2001) (recovery period of 1 h).

ence between the two regions was the mechanical behavior following deformation. Seventy percent of the proximal regions stiffened in the second cycle, but the distal region always softened (became less stiff). Of the proximal regions that exhibited this behavior, increased stiffness ranged from 1% to 15%.

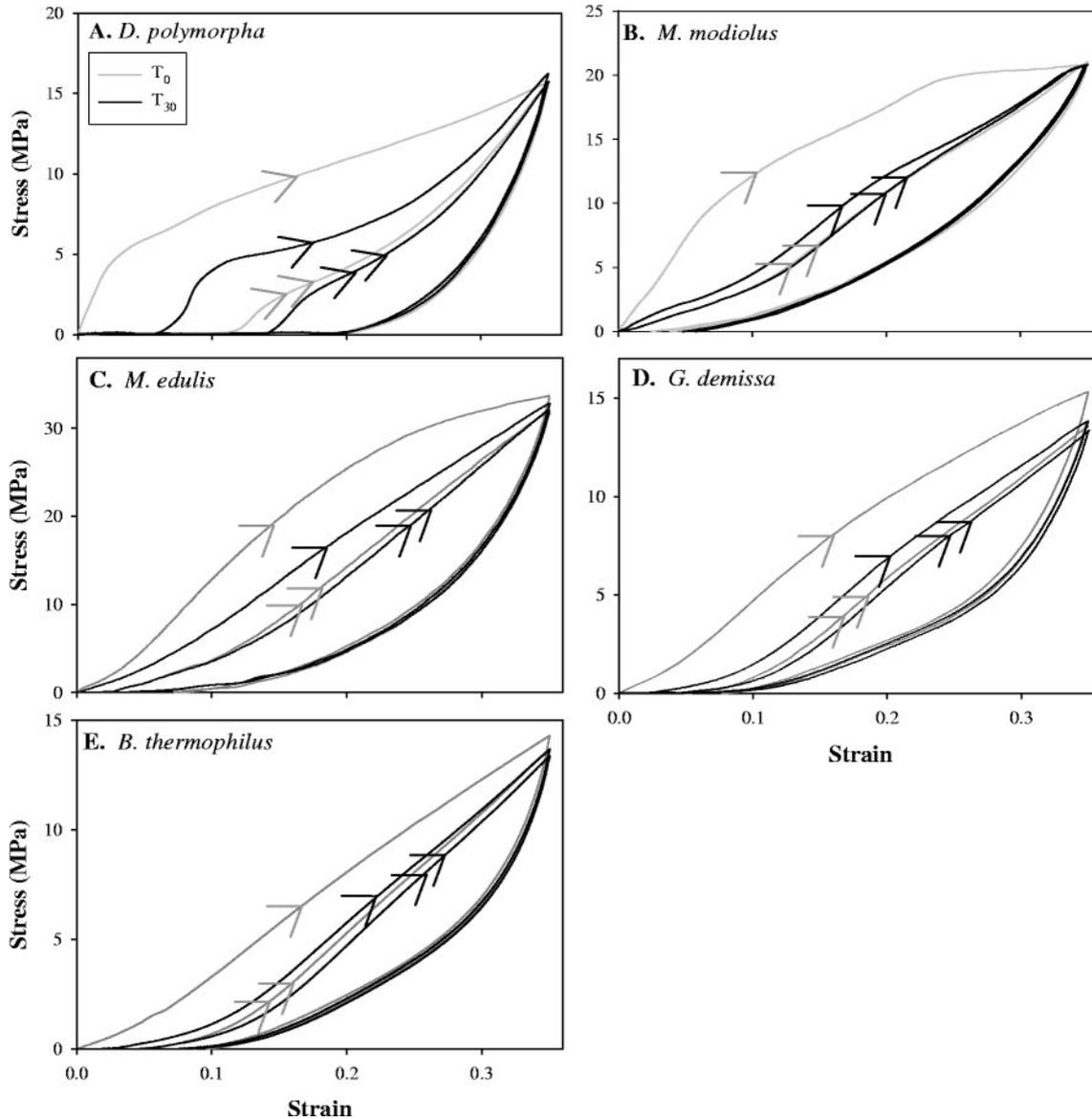
#### Mussel and thread morphometry

Mussel morphometry was not consistent among species. Species differed significantly in dry body weight and shell length ( $P < 0.001$ ; Table 3), and thread lengths were highly variable, ranging from 6 to 31 mm (Table 3). When thread lengths were normalized to shell lengths, mussels could be categorized into two groups: (1) *G. demissa* and *D. polymorpha* with relatively short threads, about one-fifth of their total shell length; and (2) *Modiolus modiolus*, *M. edulis*, and *B. thermophilus* with proportionately longer threads, which were around one-third of their total shell length. The proportional length of the proximal region of a byssal thread was different for the two species examined ( $P < 0.001$ ; Table 3). In both subtidal and intertidal *M. edulis*, the proximal region accounted for about 40% of total thread length, but only 21% of the total thread was proximal in *Modiolus modiolus*. Thread diameter and plaque areas were significantly larger in *B. thermophilus* ( $P < 0.001$ ); ranging 3 to 10 times values for the other species examined (Table 3).

Scanning electron micrographs confirmed that the cross-sectional shapes of threads of all species were elliptical (Fig. 5). The major axis was more than twice the minor axis and did not differ among species ( $P = 0.49$ ; Table 4). Scanning electron micrographs also illustrated differences in surface topography of the threads (Fig. 6). *Bathymodiolus thermophilus* threads were generally smooth throughout the length of the thread and appeared homogenous. The threads of *G. demissa* were uniform and furrowed lengthwise. Although the distal ends of both *Modiolus* and *M. edulis* threads were smooth, their proximal ends were corrugated circumferentially. The *Modiolus* proximal region, however, had more longitudinal grooves than the *M. edulis* proximal region, which appeared more crimped. The proximal and distal regions of *D. polymorpha* threads appeared untextured and were not morphologically distinguishable.

#### Discussion

The biomechanics and morphometry of the byssal threads combine to define the mechanical limitations of mussel attachment. Mussel tenacity, or attachment strength, can be increased in several ways; by enhancing the material properties of threads, increasing the diameter of individual threads, or increasing the number of threads in the byssus complex (Bell and Gosline, 1997; Moeser *et al.*, 2006; Moeser and Carrington, 2006). Even though seasonal changes in thread material properties have been observed in



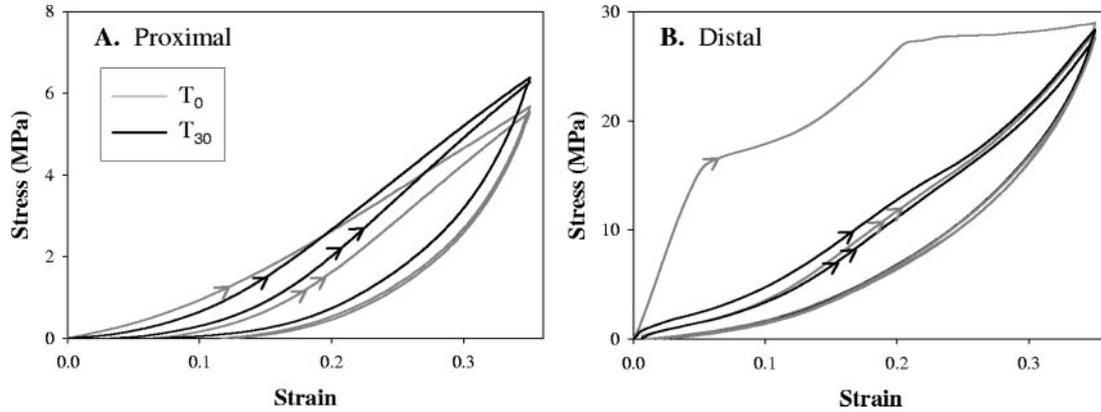
**Figure 3.** Cyclical loading of whole threads extended to 35% strain. Threads were initially cycled twice, held slack for 30 min at the initial thread length and then cycled twice again. Note different recovery patterns between the threads of (A) *D. polymorpha* and the Mytilidae: (B) *Modiolus modiolus*, (C) *Mytilus edulis*, (D) *Geukensia demissa*, and (E) *Bathymodiolus thermophilus*.

*M. edulis* (Mooser and Carrington, 2006), the material properties were similar among mytilids despite the broad range of habitats represented. Comparing these results to those of Bell and Gosline (1996), threads of *M. californianus* rank among the strongest and most extensible threads of the marine mussels (Table 1). These superior threads may contribute to the dominance of *M. californianus* on even the most exposed coastlines, areas where other species would be dislodged.

However, threads of the freshwater mussel, *Dreissena polymorpha*, were stronger, slightly stiffer, and equally as extensible as threads of marine species (Table 1; Bell and

Gosline, 1996). The superior strength of *D. polymorpha* was a surprising result, considering that marine habitats are typically characterized by fast-moving, irregular water motion, whereas most freshwater environments are hydrodynamically benign in comparison.

The high strength of the *D. polymorpha* threads may be due to their unique molecular structure. *Dreissena polymorpha* threads are composed mainly of polyphenolic proteins and contain no collagen (Rzepecki and Waite, 1995), whereas collagen is the dominant protein in mytilid byssus (Waite *et al.*, 2002). Specific environmental conditions of the freshwater and marine systems could also be influencing



**Figure 4.** Cyclical loading of isolated regions of *Modiolus modiolus* threads, with a 30-min time delay. Regions were cycled to 35% strain: (A) proximal region and (B) distal region. After 30 min, the proximal region stiffens and recovers 83% of its energy-dissipating ability, whereas the distal region recovers only 37%.

the mechanical ability of the threads. Recent evidence has shown that changes in water chemistry result in variation of the molecular interactions in a byssal thread, which leads to changes in its mechanical behavior (Sun *et al.*, 2001). For example, the modulus of the proximal region of *M. galloprovincialis* decreased when nitrogen was added to seawater and increased when phenoloxidase was added (Sun *et al.*, 2001). Also, tests have shown that dithiothreitol abolished the yield in the distal region of a *M. galloprovincialis* thread; it effectively reduced disulfide cross-links (Vaccaro and Waite, 2001). Perhaps chemical variations between freshwater and seawater habitats, such as the presence or absence of certain ions, play a role in the material differences seen in *D. polymorpha*. It is also possible that water quality changes the composition of a thread. The lacquer cuticle that coats the threads of both mussel families becomes metal-enriched in polluted areas (Rzepecki and Waite, 1995). Although the extent of the lacquer's contribution to the overall thread mechanics is unknown (Rzepecki and Waite, 1991), differences between environments could affect material comparisons.

The shape of the stress-strain curve also varied among species. Although homogeneous threads had ultimate mechanical properties similar to those of composite threads, their tensile behavior was less complex, with curves that steadily decreased slope and had no obvious yield. In contrast, all composite threads examined had at least one distinct yield region that, for the mytilid threads, was due to a yield in the distal portion. The yield enhances the attachment strength of the multi-thread byssus by two mechanisms (Bell and Gosline, 1996): (1) by providing the extension necessary for the alignment of the mussel to the applied load and (2) by allowing the recruitment of other byssal threads, which are radially oriented around the mussel. Therefore, the yield region of the thread improves the function of the byssus complex, which enhances the mussel's ability to inhabit hydrodynamically challenging environments.

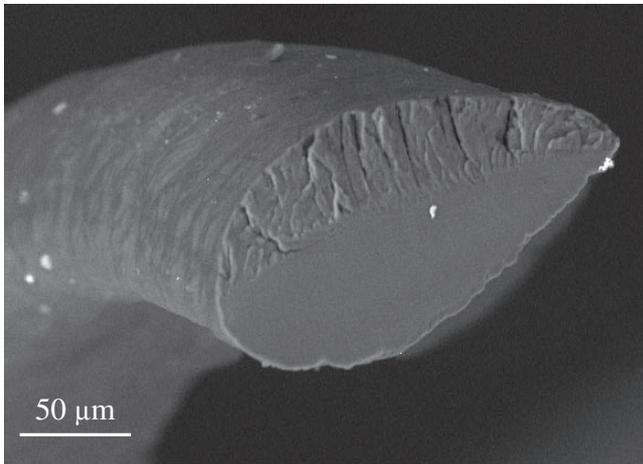
One novel mechanical behavior demonstrated in this study was the double yield of *Modiolus* threads, which is due to the behavior of their distal component. The initial steep slope of the *Modiolus* threads yielded at about 10%

**Table 3**

*Byssal thread morphometrics for the five species examined*

Species	Thread length (mm)	Thread diameter (mm)	Plaque area (mm <sup>2</sup> )	Percent proximal	Shell length (mm)
<i>Bathymodiolus thermophilus</i>	30.03 ± 13.40(5) <sup>a</sup>	0.54 ± 0.16(10) <sup>a</sup>	1.80 ± 0.56(5) <sup>a</sup>	NA	89.0 ± 21.8(5) <sup>a,b</sup>
<i>Geukensia demissa</i>	14.72 ± 5.31(19) <sup>b</sup>	0.08 ± 0.02(7) <sup>b</sup>	0.25 ± 0.11(20) <sup>b</sup>	NA	78.4 ± 10.9(14) <sup>b</sup>
<i>Mytilus edulis</i>					
intertidal	11.58 ± 2.71(23) <sup>b</sup>	0.08 ± 0.01(8) <sup>b</sup>	0.44 ± 0.24(20) <sup>b</sup>	0.42 ± 0.05(10) <sup>a</sup>	37.4 ± 3.8(15) <sup>c</sup>
subtidal	16.15 ± 6.87(24) <sup>b</sup>	0.13 ± 0.03(10) <sup>b</sup>	0.57 ± 0.17(20) <sup>b</sup>	0.41 ± 0.08(10) <sup>a</sup>	61.4 ± 8.5(16) <sup>d</sup>
<i>Modiolus modiolus</i>	31.11 ± 7.77(19) <sup>a</sup>	0.11 ± 0.03(10) <sup>b</sup>	0.07 ± 0.03(20) <sup>b</sup>	0.21 ± 0.04(18) <sup>b</sup>	99.0 ± 19.0(12) <sup>a</sup>
<i>Dreissena polymorpha</i>	5.96 ± 1.59(25) <sup>c</sup>	0.06 ± 0.01(13) <sup>b</sup>	0.17 ± 0.07(20) <sup>b</sup>	NA	26.3 ± 3.0(15) <sup>d</sup>
<b>P values</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>

Values are means ± SD (*n*). Letters indicate significantly different groups (Tukey pairwise comparisons *P* < 0.05). NA indicates not applicable.



**Figure 5.** Scanning electron micrograph of a *Bathymodiolus thermophilus* byssal thread, cross-sectioned in the proximal region. The cross-section is elliptical and the major axis is at least twice the length of the minor axis.

strain, and was followed by a second yield near 30% strain, where other *Mytilus* species typically yield. One consequence of the double-yielding performance is that the comparative toughness of the thread is enhanced at low strain (<30%), which has the effect of increasing the overall attachment strength of the byssus complex (Bell and Gosline, 1996). This presence of an additional yield suggests an alternative molecular structure for the *Modiolus* distal region, compared to other species.

As has been observed in other *Mytilus* species (Bell and Gosline, 1996), the distal region of *Modiolus* is stiffer, stronger, and less extensible than the proximal region. However, some of these ultimate material properties differ between species. Although the distal region of *Modiolus* thread is similar to that of *Mytilus* spp., its proximal region is less extensible than those of *M. edulis* and *M. californianus*, which stretch to almost twice their original lengths (Bell and Gosline, 1996). Greater extensibility, which enhances attachment strength, may be an adaptation of *Mytilus* species to environments commonly exposed to wave action.

The resilience of *D. polymorpha* threads, 25%, was lower than in all mytilids, which exhibited a resilience of about 35%. Lower resilience in a material is due to a greater amount of molecular friction while dissipating strain energy (Gosline *et al.*, 2002). The specific molecular basis for the differences in resilience is unknown. Interestingly, it is the *D. polymorpha* threads that most rapidly recover their energy-dissipating behavior after a 30-min resting phase. The faster recovery of *D. polymorpha* threads is due to the rapid restoration of thread stiffness with only a modest restoration of initial thread length (removing slack). In comparison, mytilid threads restore stiffness gradually. This range of mechanical behaviors has been observed in other elastomeric proteins, such as spider silk and hagfish slime threads (Denny, 1976; Fudge *et al.*, 2003). Tests of isolated regions of *Modiolus* thread showed that the proximal region most likely provides the whole thread with most of the newly recovered energy; recovery was 83% and 36% for the proximal and distal regions, respectively. Distal recovery in *Modiolus* is lower than in *M. californianus* (Carrington and Gosline, 2004). The faster recovery rate of *M. californianus* is potentially advantageous in habitats with heavy wave action, where repeated forces are common.

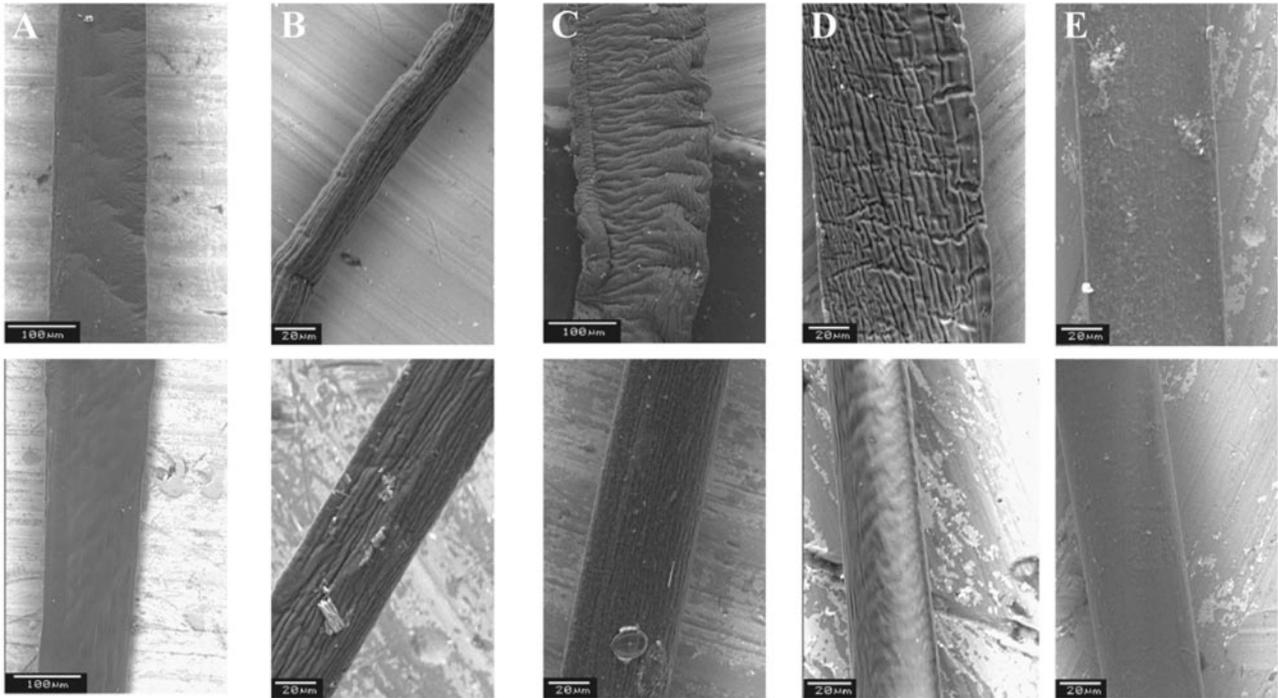
Given the diversity of habitats and environmental conditions of the mussel species examined, the uniformity of certain byssal features was remarkable. Thread diameters and plaque sizes were similar for all species, with the exception of *B. thermophilus*. Even the smallest mussel, *D. polymorpha*, which was about 26 mm in length, had byssal threads of the same thickness and plaques with the same area as the largest mussel, *Modiolus*, which is about 10 cm long. This is surprising, considering that threads and plaques are molded by the mussel foot, which varies in size, and that increased thread diameter enhances attachment strength because thicker threads are able to sustain higher loads. The size constraint of threads may be due to a number of physical or environmental factors. For example, although *Modiolus* is larger than *M. edulis*, it appears to have a much smaller foot (Stanley, 1972; S. Brazee, pers. obs.). Also, plaque size has been positively correlated to sediment size

**Table 4**

*Byssal thread cross-sectional dimensions measured under a scanning electron microscope*

Species	Major axis ( $\mu\text{m}$ )	Minor axis ( $\mu\text{m}$ )	Ratio (major/minor axis)
<i>Bathymodiolus thermophilus</i>	227.67 $\pm$ 30.04	72.00 $\pm$ 20.59	3.39 $\pm$ 1.19
<i>Geukensia demissa</i>	64.87 $\pm$ 7.19	21.67 $\pm$ 0.81	3.00 $\pm$ 0.35
<i>Mytilus edulis</i>	103.23 $\pm$ 43.97	38.63 $\pm$ 4.61	2.62 $\pm$ 0.80
<i>Modiolus modiolus</i>	89.53 $\pm$ 37.73	41.50 $\pm$ 27.47	2.37 $\pm$ 0.53
<i>Dreissena polymorpha</i>	67.87 $\pm$ 27.43	26.27 $\pm$ 4.72	2.52 $\pm$ 0.54

All threads were measured in the proximal region, which may be more elliptical than the distal region for composite mytilid threads. Values are mean  $\pm$  SD;  $n = 3$ .



**Figure 6.** Scanning electron micrographs of surfaces of proximal (top) and distal (bottom) regions of each species: (A) *Bathymodiolus thermophilus*, (B) *Geukensia demissa*, (C) *Mytilus edulis*, (D) *Modiolus modiolus*, and (E) *Dreissena polymorpha*. Scale bars: 100  $\mu\text{m}$  in A and top image of C; 20  $\mu\text{m}$  in B, D, E, and bottom image of C.

(Meadows and Shand, 1989) and negatively correlated to attachment strength (Allen *et al.*, 1976). It is also possible that thread diameter is constrained by the process of thread formation (*e.g.*, gland secretion, kinetics). A complete analysis of interspecific variation in attachment strength should also consider many other factors that influence byssogenesis, such as predator presence, food availability, water motion, season, salinity, and sediment size (Denny, 1995; Leonard *et al.*, 1999; Pelc and Alexander, 1999; Hunt and Scheibling, 2001; Moeser *et al.*, 2006; Moeser and Carrington, 2006).

Thread shape was also similar among species in this study, supporting many previous observations that byssal threads are ovate in cross-section (Smeathers and Vincent, 1979; Price, 1981; Eckroat and Steele, 1993). All threads were measured in the proximal region, which may be more elliptical than the distal region for composite mytilid threads. In previous mechanical studies, calculations converting load into stress assumed a circular cross-sectional area.

However, because thread cross-sections are instead elliptical, with a major axis more than twice as long as the minor axis, these area calculations could be in error by a factor of 2. As a result, thread strength and stiffness estimates based on a single major axis measurement would underestimate true values.

*Dreissena polymorpha* and *G. demissa* had byssal threads

that were one-fifth of the shell length, while the remaining species had threads that were one-third of the shell length. The reduced length of the *D. polymorpha* threads is likely due to the length of the pedal groove of the mussel. Eckroat and Steele (1993) noted that the groove of *M. edulis* stretches the entire length of the foot, whereas the groove of *D. polymorpha* extends only about half of the length of the foot. The shorter length of the *G. demissa* thread may be more attributable to habitat, as it is the only mytilid that displays a reduced thread length. *Geukensia demissa* always lives infaunally, and like the primitive endobyssate mussels, it relies on a longer shell to increase its stability in soft sediments, while byssal thread production is supplementary (Stanley, 1972). Longer threads may be more important in epifaunal mussels where threads work collectively to counter hydrodynamic forces.

Marked by low stiffness and high extensibility, the corrugated proximal region found in *Modiolus* and *Mytilus* spp. aids in thread realignment and recruitment because it is so extensible (Bell and Gosline, 1996). The proximal region lengths varied between *Modiolus* and *M. edulis*, which suggests physical differences in the ventral grooves of the foot of the mussel species. Although a longer proximal region may increase thread extension and therefore tenacity, no obvious trend in proximal length is apparent in the epibenthic mytilids. *Mytilus californianus* threads are 20% proximal, and *M. trossulus* and *M. galloprovincialis* have

proximal regions that are 26% and 35% of the thread, respectively (Bell and Gosline, 1996).

Thread surface topography varied among species, despite many similarities in mechanics and morphometrics. *Mytilus edulis* and *Modiolus* were most similar, with a corrugated proximal region and a smooth distal region. *Geukensia demissa* and *B. thermophilus* both appear homogeneous, but *B. thermophilus* has distinctly less topographic lineation. Although *D. polymorpha* threads appeared uniform in this study, Eckroat and Steele (1993) reported dissimilar proximal and distal sections in scanning electron images under similar magnification. This discrepancy may be due to differences in thread preparation; Eckroat and Steele (1993) fixed threads in sodium phosphate buffer and dehydrated them with ethanol. Threads in their study were also coated with gold-palladium, which accentuates topographical variations.

Together, biomechanics and the morphometry of the byssal threads and shell determine the attachment strength of a mussel. An understanding of environmental loading (by moving water or predator) is necessary to adequately evaluate the performance of a byssus in nature. Thus, although this suite of mussels seem to have developed several strategies for dealing with their unique habitat challenges, many environmental complexities make it difficult to identify which modifications may be strictly related to tenacity. For example, *B. thermophilus* has material properties equivalent to those of the other marine mussels, yet its thread diameters and plaques are much larger than in all other species, even when adjusted to the shell size. This magnification of *B. thermophilus* threads implies increased attachment strength, but it is unknown whether the number of byssal threads is similar among species.

While this study only begins to uncover relationships between biomechanics, habitat, and phylogeny, several unique material properties were discovered. For example, the unusual double-yielding behavior of *Modiolus* threads has not been described previously; further research on the molecular mechanisms responsible for this behavior could provide insight into the evolution of mussel attachment as well as the design of novel biomimetic materials. Additionally, the threads of *D. polymorpha* have a slightly higher strength than even those of *M. californianus*, and they are equally extensible as well. The threads of *D. polymorpha*, like the composite mytilid threads, have stress-strain curves with a distinct yield, and they also exhibit the most rapid recovery of all species tested. Dreissenidae threads may therefore serve as an important model for the design of self-healing polymers, which has become a high research priority (Vaccaro and Waite, 2001). Overall, future research on the molecular underpinnings of the material performance of mussel byssus that employs a broad comparative approach, beyond the genus *Mytilus*, will provide valuable

insight into the evolution of mussel attachment as well as into the design of novel bio-inspired materials.

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### Literature Cited

- Allen, J. A., M. Cook, D. J. Jackson, S. Preston, and E. M. Worth. 1976. Observations on the rate of production and mechanical properties of byssus threads of *Mytilus edulis* L. *J. Molluscan Stud.* **42**: 279–289.
- Bell, E. C., and J. M. Gosline. 1996. Mechanical design of mussel byssus: material yield enhances attachment strength. *J. Exp. Biol.* **199**: 1005–1017.
- Bell, E. C., and J. M. Gosline. 1997. Strategies for life in flow: tenacity, morphometry, and probability of dislodgment of two *Mytilus* species. *Mar. Ecol. Prog. Ser.* **159**: 197–208.
- Braze, S. L. 2004. An interspecific comparison of biomechanical properties and morphometrics of mussel byssal threads. M.S. Thesis. University of Rhode Island.
- Brown, C. H. 1952. Some structural proteins of *Mytilus edulis* L. *Q. J. Microsc. Sci.* **93**: 487–501.
- Carrington, E., and J. M. Gosline. 2004. Mechanical design of mussel byssus: load cycle and strain rate dependence. *Am. Malacol. Bull.* **18**: 135–142.
- Coyne, K. J., X. Qin, and J. H. Waite. 1997. Extensible collagen in mussel byssus: a natural block copolymer. *Science* **277**: 1830–1832.
- Crisp, D. J., G. Walker, G. A. Young, and A. B. Yule. 1985. Adhesion and substrate choice in mussels and barnacles. *J. Colloid Interface Sci.* **104**: 40–50.
- Denny, M. W. 1976. The physical properties of spider's silk and their role in the design of orb-webs. *J. Exp. Biol.* **65**: 483–506.
- Denny, M. W. 1988. *Biology and the Mechanics of the Wave-Swept Environment*. Princeton University Press, Princeton.
- Denny, M. W. 1995. Predicting physical disturbance: mechanistic approaches to the study of survivorship on wave-swept shores. *Ecol. Monogr.* **65**: 371–418.
- Eckroat, L. R. and L. M. Steele. 1993. Comparative morphology of the byssi of *Dreissena polymorpha* and *Mytilus edulis*. *Am. Malacol. Bull.* **10**: 103–108.
- Fudge, D. S., K. H. Gardner, V. T. Forsyth, C. Riekel, and J. M. Gosline. 2003. The mechanical properties of hydrated intermediate filaments: insights from hagfish slime threads. *Biophys. J.* **85**: 2015–2027.
- Gosline, J., M. Lillie, E. Carrington, P. Guerette, C. Ortlepp, and K.

- Savage, 2002.** Elastic proteins: biological roles and mechanical proteins. Pp. 15–38 in *Elastomeric Proteins: Structures, Biomechanical Properties, and Biological Roles*, P. J. Shrewry, A. S. Tatham, and A. J. Bailey, eds. Cambridge University Press, Cambridge.
- Holt, T. J., E. I. Rees, S. J. Hawkins, and R. Seed. 1998.** *Biogenic Reefs*, Vol. 9: *An Overview of Dynamic and Sensitivity Characteristics for Conservation Management of Marine SACs*. Scottish Association of Marine Sciences/UK Marine SACs Project, Oban, Scotland.
- Hunt, H. L., and R. E. Scheibling. 2001.** Predicting wave dislodgment of mussels: variation in attachment strength with body size, habitat, and season. *Mar. Ecol. Prog. Ser.* **213**: 157–164.
- Leonard, G. H., M. D. Bertness, and P. O. Yund. 1999.** Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. *Ecology* **80**: 1–14.
- Lucas, J. M., E. Vaccaro, and J. H. Waite. 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.
- McDonald, K. H., and R. K. Koehn. 1988.** The mussels *Mytilus galloprovincialis* and *Mytilus trossulus* on the Pacific coast of North America. *Mar. Biol.* **99**: 111–118.
- Meadows, P. S., and P. Shand. 1989.** Experimental analysis of byssus thread production by *Mytilus edulis* and *Modiolus modiolus* in sediments. *Mar. Biol.* **101**: 219–226.
- Moeser, G. M., and E. Carrington. 2006.** Seasonal variation in mussel byssal thread mechanics. *J. Exp. Biol.* **209**: 1996–2003.
- Moeser, G. M., H. Leba, and E. Carrington. 2006.** Seasonal influence of wave action on thread production in *Mytilus edulis*. *J. Exp. Biol.* **209**: 881–890.
- Morton, B. 1993.** The anatomy of *Dreissena polymorpha* and the evolution and success of the heteromyarian form in the Dreissenidae. Pp. 185–215 in *Zebra Mussels: Biology, Impacts, and Control*, T. F. Nalepa and D. W. Schloesser, eds. CRC Press, Boca Raton, FL.
- Pelc, M., and R. R. Alexander. 1999.** Salinity and sediment-mediated byssal thread production by *Mytilus edulis* Linnaeus and *Geukensia demissa* Dillwyn from New Jersey salt marshes. *J. Shellfish Res.* **18**: 375–384.
- Price, H. A. 1981.** Byssus thread strength in the mussel, *Mytilus edulis* L. *J. Zool. (Lond)* **194**: 245–255.
- Rzepecki, L. M., and J. H. Waite. 1991.** DOPA proteins: versatile varnishes and adhesives from marine fauna. *Bioorg. Mar. Chem.* **4**: 119–148.
- Rzepecki, L. M., and J. H. Waite. 1993.** The byssus of the zebra mussel, *Dreissena polymorpha*. I. The morphology and *in situ* protein processing during maturation. *Mol. Mar. Biol. Biotechnol.* **2**: 255–266.
- Rzepecki, L. M., and J. H. Waite. 1995.** Wrestling the muscle from mussel beards: research and applications. *Mol. Mar. Biol. Biotechnol.* **3**: 313–322.
- Smeathers, J. E., and J. F. V. Vincent. 1979.** Mechanical properties of mussel byssus threads. *J. Molluscan Stud.* **45**: 219–230.
- Stanley, S. M. 1972.** Functional morphology and evolution of byssally attached bivalve mollusks. *J. Paleontol.* **46**: 165–212.
- Sun, C., E. Vaccaro, and J. H. Waite. 2001.** Oxidative stress and the mechanical properties of naturally occurring chimeric collagen-containing fibers. *Biophys. J.* **81**: 3590–3595.
- Vaccaro, E., and J. H. Waite. 2001.** Yield and post-yield behavior of mussel byssal thread: a self-healing biomolecular material. *Biomacromolecules* **2**: 906–911.
- Waite, J. H. 1992.** The formation of mussel byssus: anatomy of a natural manufacturing process. Pp. 27–54 in *Results and Problems in Cell Differentiation*, Vol. 19: *Biopolymers*, S.T. Case, ed. Springer-Verlag, Berlin.
- Waite, J. H., E. Vaccaro, C. Sun, and K. J. Lucas. 2002.** Collagens with elastin- and silk-like domains. Pp. 189–212 in *Elastomeric Proteins: Structures, Biomechanical Properties, and Biological Roles*, P. R. Shrewry, A. S. Tatham, and A. J. Bailey, eds. Cambridge University Press, Cambridge.
- Yonge, C. M. 1962.** On the primitive significance of the byssus in the Bivalvia and its effects in evolution. *J. Mar. Biol. Assoc. UK* **42**: 113–125.